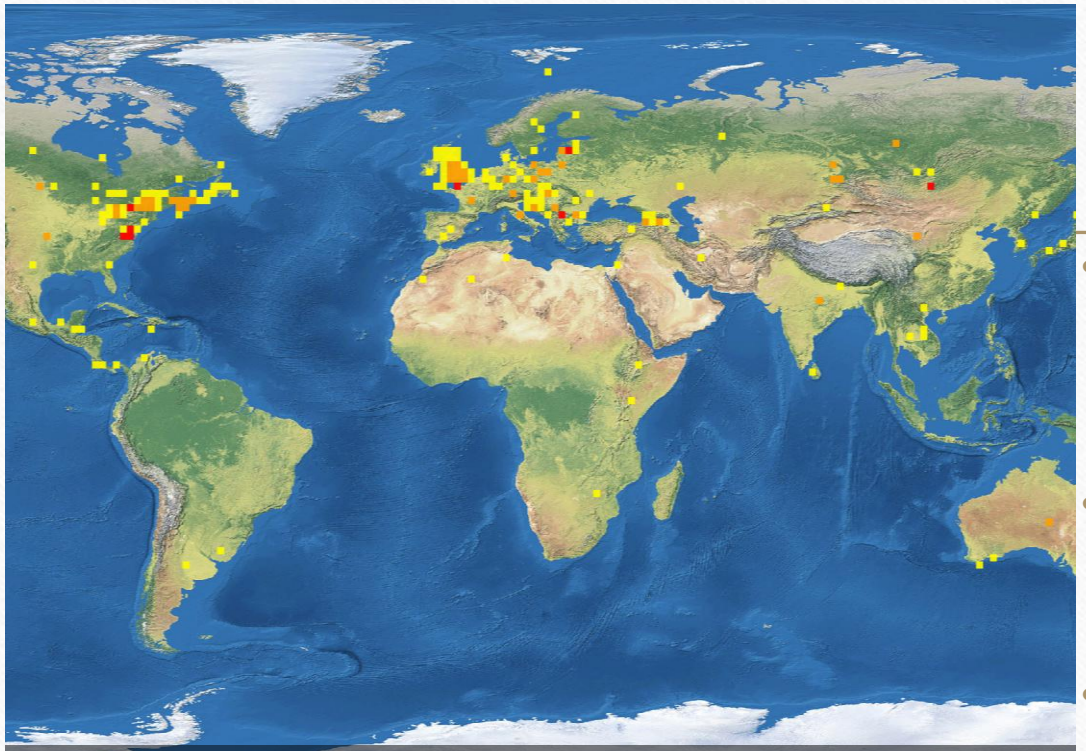
Comparing *Ixodes* Tick BIN Diversity Between North America and Europe Using DNA Barcodes

INTRODUCTION

Ixodes- genus of hard ticks

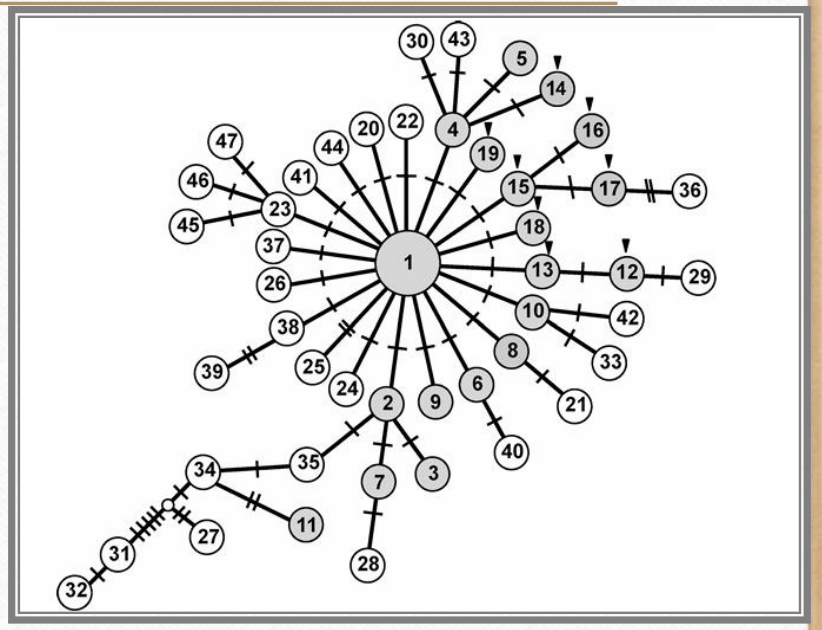


Retrieved from BOLD Taxonomy Browser: Ixodes

- Largest genus (over 240 species) in the subfamily *Ixodinae* (Estrada-Peña et al., 2017) and *Ixodes spp* have been sampled on almost every continent.
- *Ixodes spp* are clinically significant carriers of multiple vector-borne illnesses and they have a broad host range.
- Examples of diseases include Lyme disease, Anaplasmosis and Babesiosis.
- DNA barcoding of *Ixodes* species will allow for standardized comparisons of species-level diversity across large-geographical distributions.

Why compare *Ixodes* biodiversity across Canada and Europe?

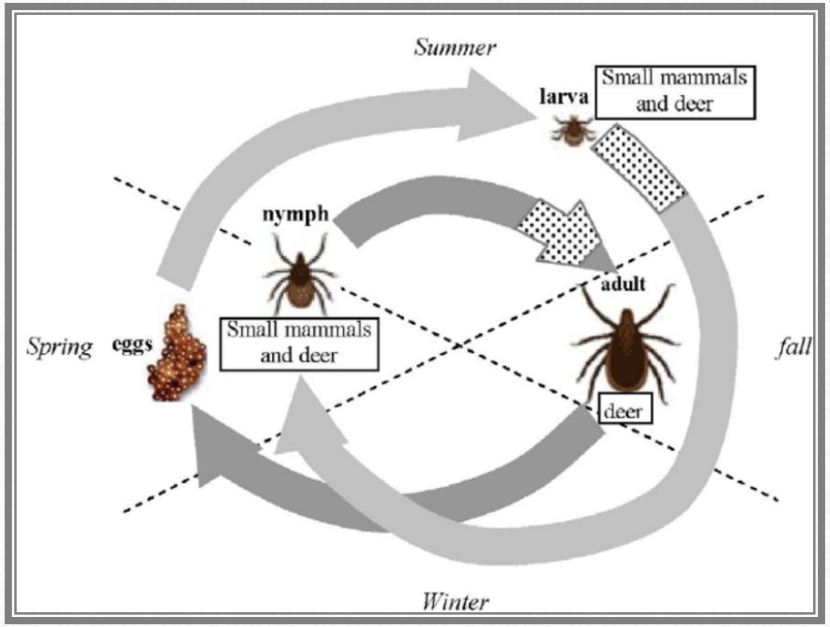
- *Ixodes spp* are genetically variable. In fact, a study on *Ixodes scapularis* (Krakowetz et al., 2011) showed 19 mitochondrial haplotypes, including novel ones (shaded circle 12, 14, 16, and 17).
- Both North America and Europe have extensively sampled *Ixodes spp* in the BOLD database allowing for large scale comparison and while many studies focus on single *Ixodes* species or regions, few compare the biodiversity across continents.
- The continents vary in climate, host species, patterns of land use, sampling frequency and tick management practices, all of which may contribute to the observed barcode diversity of *Ixodes* ticks.
- By comparing the BIN diversity of these 2 continents, sampling biases and community similarity can be identified.



(Krakowetz et al., 2011): Triangles over the shaded circles 12,14, 16 and 17 show haplotypes present in Canada and not the U.S.

Importance of studying BIN diversity with respect to latitude data across the 2 continents

- Climate change can also affect the biodiversity of ticks.
- Ticks are known for preferring high humidity areas and warm temperatures to accelerate the development between life stages (Estrada-Peña et al., 2017)
- A study by Medlock et al. in 2013 on *Ixodes ricinus* in Europe showed that they have been shifting to higher altitudes likely due to milder winters and another study in North America (Tardy et al., 2023) showed the movement of *Ixodes scapularis* north into Canada.
- Using BOLD data, we can explore whether *Ixodes* BINS occurring at higher latitude have broader geographic as this could be indicative that not only the two species mentioned above but possibly other Ixodes species are also migrating to higher altitudes.
- This project is entirely exploratory



(Nonaka et al., 2010)

METHODS: BOLD & BIODIVERSITY WEBSITE AND RSTUDIO

Pipeline for Comparing Ixodes BIN Diversity Across Continents

PART 1: ASSESSING NUMBER OF UNIQUE BINS IN EACH CONTINENT

Downloaded Ixodes tick (tsv) data from BOLD database.

Assigned countries to North America and Europe (dplyr package) and filtered dataset to retain only samples from selected countries and non-missing BINs.

BIN Diversity Analysis

Counted unique BINs per continent (after removing NAs) + visualized BIN counts via bar plot using ggplot2 (Figure 1).

Similarity of bins between continents

Constructed presence-absence matrix of BINs by continent and calculated Jaccard distance using vegdist in vegan package to assess BIN overlap.

PART 2: COMPARING BIN DIVERSITY WITH LATITUDE DATA

Latitude-Based Analysis

Extracted and cleaned latitude from coordinate data.

Grouped data into 5° latitude bins.

BIN Richness by Latitude

Counted unique BINs per latitude bin and visualized spatial BIN distribution using bar plots (Figure 2).

Geographic Range vs Latitude

Calculated mean latitude and latitude range per BIN.

Visualized with scatter plot and regression line (Figure 3).

Assessed correlation using Spearman's test.

FIGURES

Figure 1: Bar plot showing number of unique counts in North America and Europe

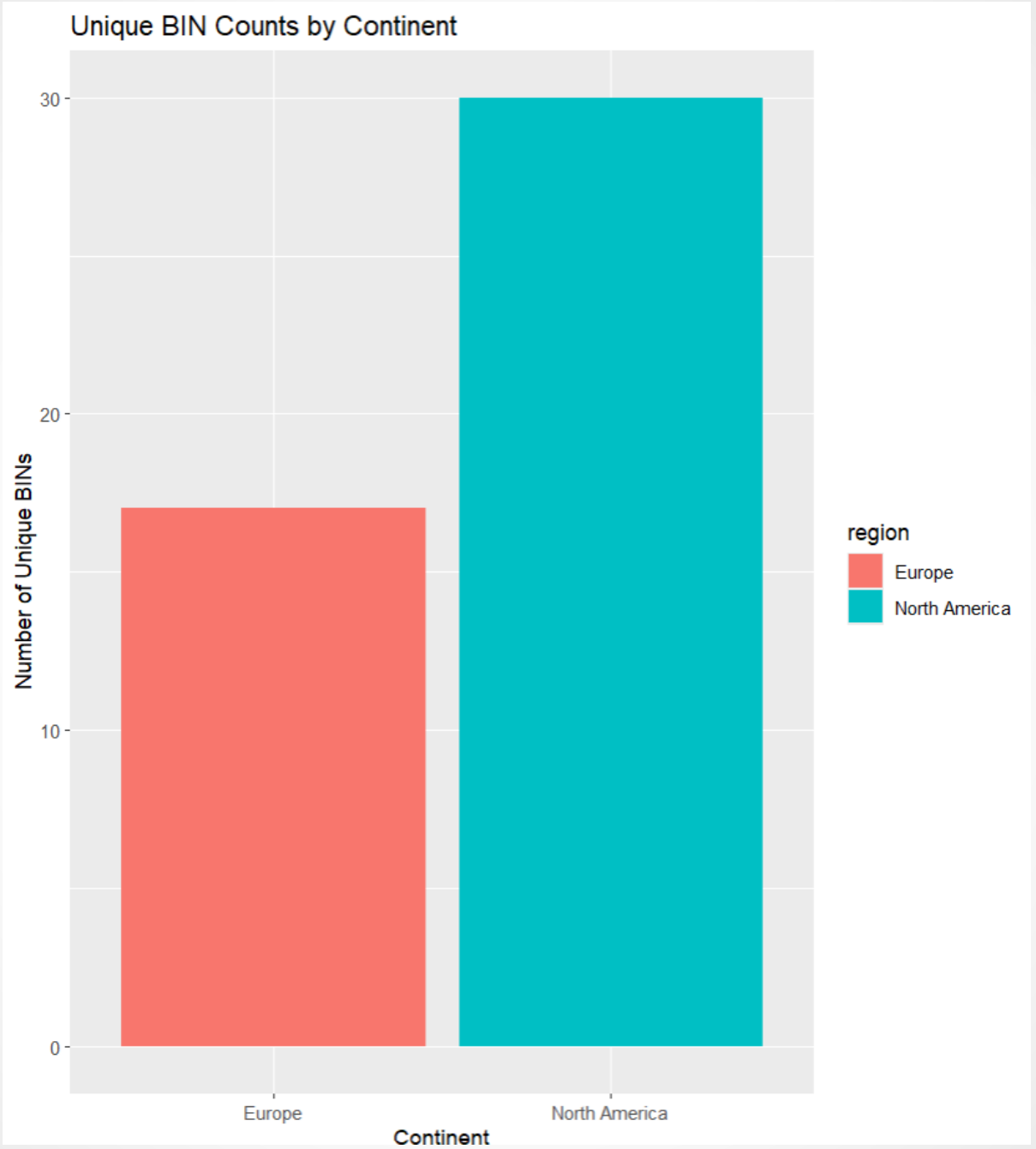


Figure 2: Bar plot showing the BIN diversity of North America and Europe across 5-degrees interval latitude bins

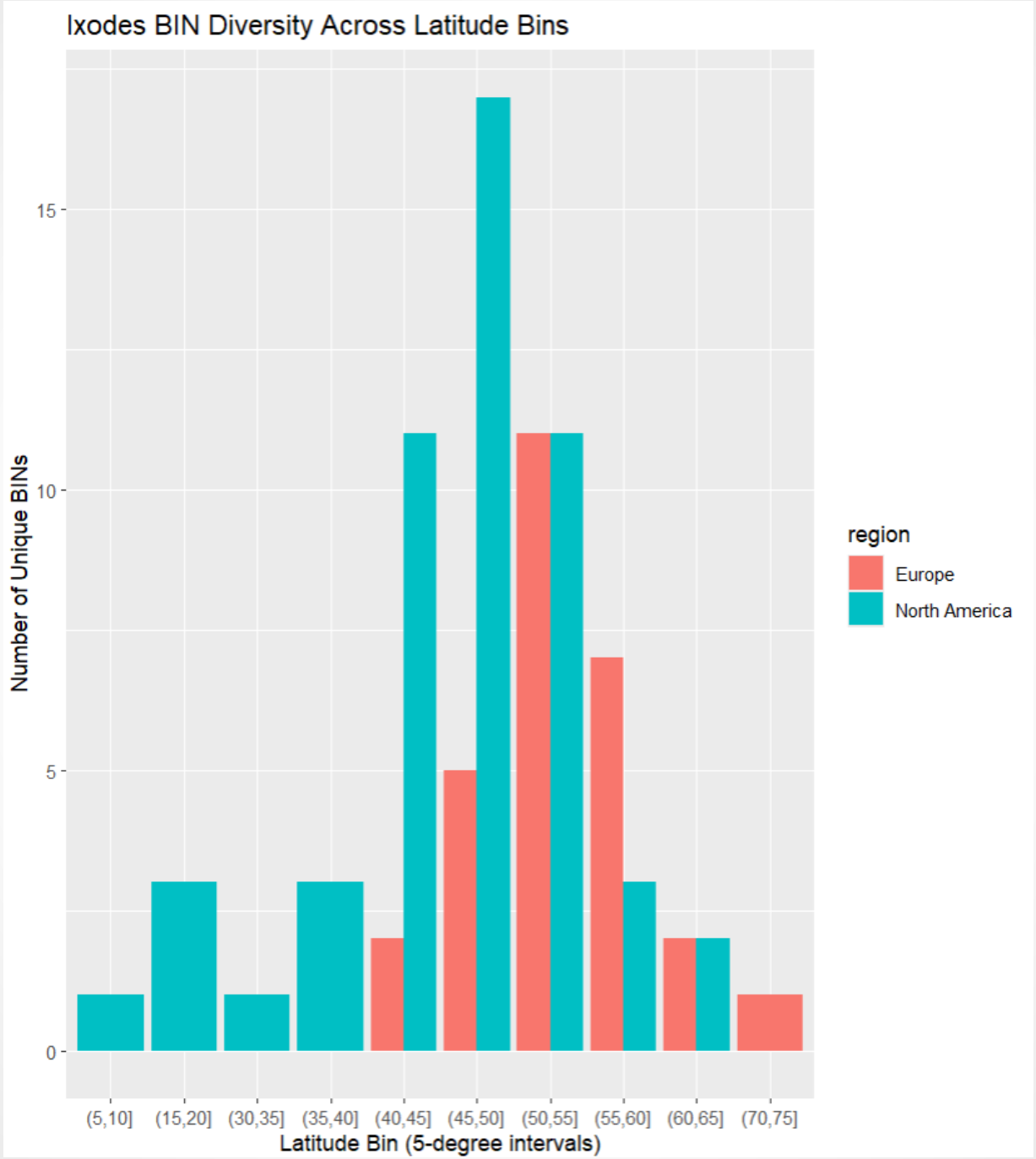
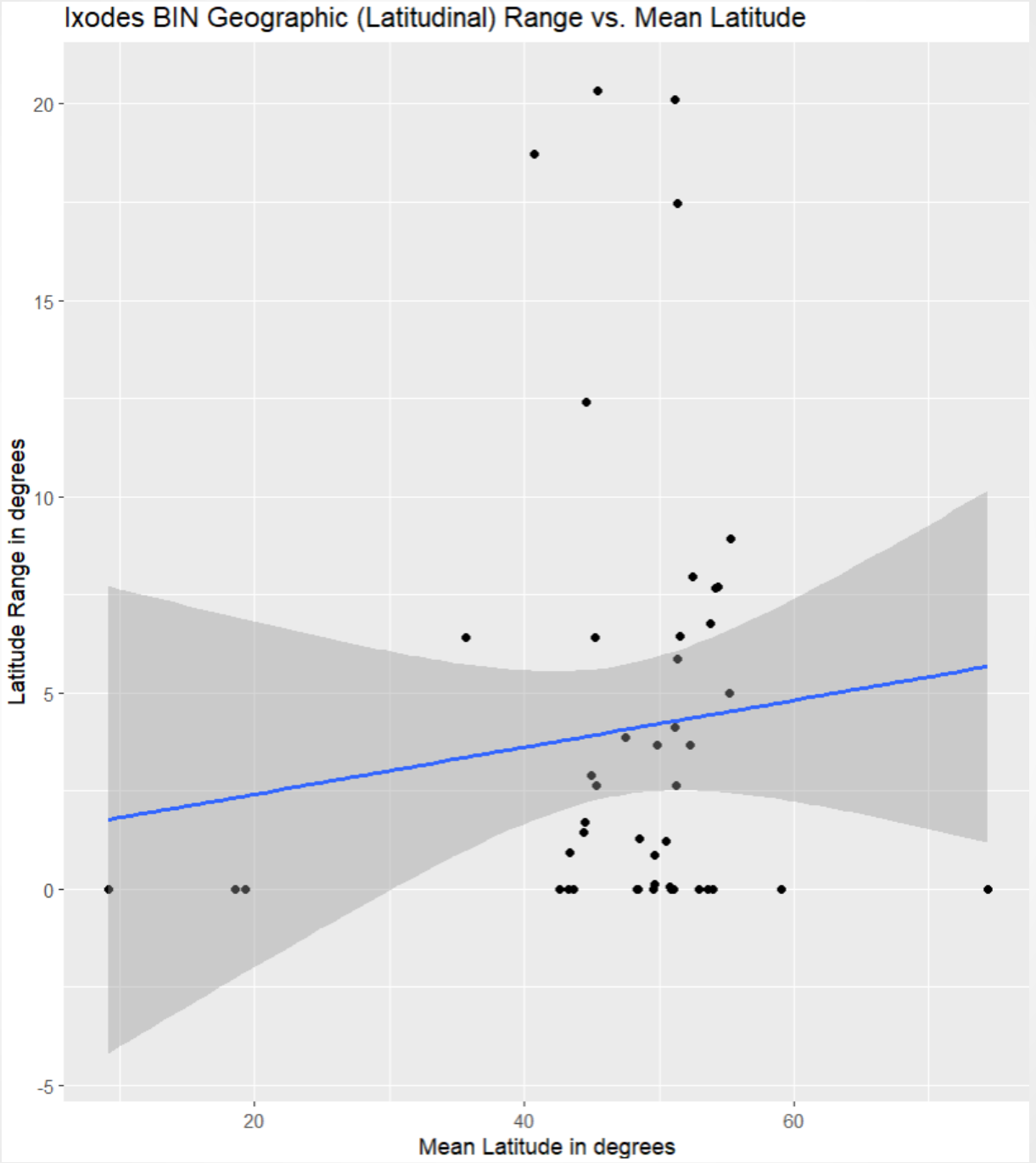


Figure 3: Scatter plot investigating whether geographic range (maximum latitude- minimum latitude) increase at high altitudes



RESULTS/DISCUSSION

BIN DIVERSITY ACROSS THE TWO CONTINENTS

- For this project, select countries where *Ixodes spp.* were sampled, were grouped by continent: North America or Europe.
- Diffeerence in number of unique BIN counts for North America (30) and Europe (17) could be due to:

1. Greater sampling effort in North America compared to Europe or there could have been a difference in the identification methods of *Ixodes spp.* between the 2 continents.
2. But if sampling was equal in both continents. *Ixodes spp.* could be more prevalent, widespread and abundant in North America due to climate change, land use or host species dynamics.

- To determine the presence/absence of different BINS across the 2 continents, a matrix was created.
- From the matrix, it seemed that different BINS exist between the 2 continents. To further confirm the dissimilarity of Ixodes BINS, the jaccard distance was calculated. 0.9976303 which is close to 1 was obtained hence, the BIN communities in Europe and North America are completely different.
- This could mean that tick species may have evolved separately due to geographical barriers and that environmental factors differ enough to support different *Ixodes* species. Therefore, the diseases caused by ticks could also be different in the continents.

EXAMINING BIN RICHNESS BY 5 DEGREES LATITUDE INTERVAL IN NORTH AMERICA AND EUROPE

- From Figure 2, it seems that both continents show maximum BIN richness between 45°–55°.
  - ✓ North America peaks sharply at 45°–50° with more than 15 BINs.
  - ✓ Europe peaks at 50°–55° with about 12 BINs.
- This might suggest that temperate regions support the highest *Ixodes* diversity in both continents.
- With this data, it can be interpretated that mid-latitude zones (30°–60° in North/South Hemispheres) might offer optimal environmental conditions (for *Ixodes* ticks to diversify and persist.
- In most latitude bins , North America has more unique bins than Europe and as mentioned earlier this could be a result of more extensive sampling in North America compared to Europe.
- Furthermore, the reason why there are no bins lower than 40° in Europe can be due to lack of sampling in southern Europe.
- From figure 2, it seems that both number of European and North American bins decreased above 60° suggesting that tick diversity decline at extreme climates (provided sampling is equal).

EXPLORING WHETHER BINS AT HIGH ALTITUDES HAVE GREATER GEOGRAPHICAL RANGE

- Figure 3 demonstrated a slight positive slope- indicating that BINs occurring at higher mean latitudes might tend to have broader latitudinal ranges. This could be an indication of migration of ticks to higher altitudes due to climate change.
- There are a lot of outliers, and the data are widely spread suggesting that the relationship between mean_latitude and latitude range might be weak.
- Therefore, a Spearman non-parametric statistical test was conducted to assess the correlation between mean_latitude and geographical range. A rho value of 0.212 was obtained indicating the weak association between these 2 variables. The p value obtained was greater than 0.05 indicating that the
- The results provided useful information about the weak positive trend even though the pattern was not strong for it to be statistically conclusive. This can hopefully help develop hypotheses for more targeted studies. For example, we can study whether individual BINS have expanded their latitudinal ranges over time by using historical and recent occurrence data.

Acknowledgments

- I would like to acknowledge a fellow classmate Hannah Glowacki on her suggestions of different taxonomies when I was having trouble choosing one. She also ran my coded R script on her computer to make sure that my codes run well on another computer before submission.
- I would like to acknowledge that I received guidance from our TA Saira Asif when it came to analysing figure 3 and choosing a statistical test to see if geographic ranges change with mean_latitude
- I would also like to acknowledge our professor Dr Karl Cottenie for posting examples R script on how to use packages such as vegan, tidyverse and ggplot2 along with answers in V2 for challenge questions as it really helped me when I had to figure out how to clean up my data and make the different plots.

Reference slide 1 (papers consulted)

- Estrada-Peña, A., D'Amico, G., Palomar, A. M., Dupraz, M., Fonville, M., Heylen, D., Habela, M. A., Hornok, S., Lempereur, L., Madder, M., Núncio, M. S., Otranto, D., Pfaffle, M., Plantard, O., Santos-Silva, M. M., Sprong, H., Vatansever, Z., Vial, L., & Mihalca, A. D. (2017). A comparative test of ixodid tick identification by a network of European researchers. *Ticks and Tick-Borne Diseases*, *8*(4), 540–546. https://doi.org/10.1016/j.ttbdis.2017.03.001
- Krakowetz, C. N., Lindsay, L. R., & Chilton, N. B. (2011). Genetic diversity in Ixodes scapularis (Acari: Ixodidae) from six established populations in Canada. *Ticks and Tick-Borne Diseases*, *2*(3), 143–150. https://doi.org/10.1016/j.ttbdis.2011.05.003
- Medlock, J. M., Hansford, K. M., Bormane, A., Derdakova, M., Estrada-Peña, A., George, J.-C., Golovljova, I., Jaenson, T. G. T., Jensen, J.-K., Jensen, P. M., Kazimirova, M., Oteo, J. A., Papa, A., Pfister, K., Plantard, O., Randolph, S. E., Rizzoli, A., Santos-Silva, M. M., Sprong, H., … Van Bortel, W. (2013). Driving forces for changes in geographical distribution of Ixodes ricinus ticks in Europe. *Parasites & Vectors*, *6*(1), 1. https://doi.org/10.1186/1756-3305-6-1
- Tardy, O., Acheson, E. S., Bouchard, C., Chamberland, É., Fortin, A., Ogden, N. H., & Leighton, P. A. (2023). Mechanistic movement models to predict geographic range expansions of ticks and tick-borne pathogens: Case studies with Ixodes scapularis and Amblyomma americanum in eastern North America. *Ticks and Tick-Borne Diseases*, *14*(4), 102161. https://doi.org/10.1016/j.ttbdis.2023.102161

Reference slide 2 (websites and videos)

Websites:

en:similarity [Analysis of community ecology data in R] For creating presence/absence matrix and measuring jaccard distance

Basic barplot with ggplot2 – the R Graph Gallery

Linear model and confidence interval in ggplot2 – the R Graph Gallery

BOLD Data Portal - Search Results

Correlation Test Between Two Variables in R - Easy Guides - Wiki - STHDA

YouTube videos:

Using the vegan R package to generate ecological distances (CC188)

Clean Coordinates Data from Same Column in R

Scatter plot and Line plot in R (using ggplot2)

Bar Charts and Pie Charts in R | R Tutorial 2.1 | MarinStatsLectures

Correlation in R | Pearson, Spearman, Robust, Bayesian | How to conduct, visualise and interpret

R programming for beginners. Manipulate data using the tidyverse: select, filter and mutate.