ABSTRACT

This study utilizes data obtained through dryad submitted by the article “High genomic diversity and candidate genes under selection associated with range expansion in eastern coyote (*Canis latrans*) populations.” This data was used to observe the relationship between the percentage of wolf introgression and coyote heterozygosity in the coyotes migrating in eastern North America. From the analyses, moderate correlation between these two variables was observed, suggesting that canid admixture along these fronts in the east has allowed the coyote population to genetically diversify. As of a result of their ability to interbreed with other canids, this coyote migration may be less limited by factors that genetically hinder other migrant species into novel areas, such as bottlenecking and inbredness.

INTRODUCTION

Recent coyote colonization of the eastern portion of North America from the western source population has led to much discussion and research concerning the genetics of these colonizers. Much of the discussion has primarily revolved around the debate of their taxonomical classification, and whether these migrants should be separate species from both the grey wolf and western coyote. Although classification is important for conservation efforts, little attention has been given to exactly how wolf introgression with coyotes has changed these eastern populations in terms of heterozygosity. Heterozygosity measurements are a useful tool in population genetics to determine factors such as degree of inbredness within subpopulations and deviation of populations from Hardy-Weinberg equilibria.

With the colonization of eastern North America occurring so recent, questions were raised as to how genetically diverse the migrant population is, and how that diversity compares to its source population. Other studies have shown that western coyotes and eastern coyotes Fst values differ significantly, however not many have shown the general direct relationship between canid introgression and coyote heterozygosity (Peppers et al. 1996). Some hypotheses suggest that wolf/dog admixture in this population has led to higher genetic diversity. Other theories suggest that since the colonizer number is low, genetic diversity is limited due to inbreeding and a low effective population size.

In this study, data from 425 coyote samples were used to observe how wolf introgression affected coyote heterozygosity at 60 SNPs (Monzón et al. 2014). This study gives an idea on how wolf admixture is affecting the genetics of these migrants. The original hypothesis is that despite the relatively low number of migrants that may lead to high inbreeding, heterozygosity within this population would still be high at these SNPs due to wolf introgression on the colonization fronts.

MATERIALS AND METHODS

*Data and Variable Calculations*

Data was obtained through the dryad database from the article, “Assessment of coyote–wolf–dog admixture using ancestry‐informative diagnostic SNPs.” In the study, 425 individual coyote samples and two wolf samples were collected from 10 locations across the northeastern part of North America. For our purposes, the genetic data for the two wolves were discarded and the remaining 425 coyote samples were analyzed on the basis of percentage of introgression from wolf, sample location, and relative heterozygosity. Wolf introgression percentage and sample locations were obtained directly from dryad for all samples.

Heterozygosity measurements were made analyzing nucleotide differences at 60 SNPs given in the data. The SNPs are autosomal and of western grey wolf, western coyote, eastern wolf, and dog origin (vonHoldt et al. 2010; vonHoldt et al. 2011). The accumulated number of heterozygous SNPs was calculated for each coyote sample by counting every SNP that had two differing nucleotides at their specific locus. This number was then divided by the total amount of SNPs (60) to attain a relative amount of heterozygosity for each coyote as a percentage. The samples were then organized from lowest percentage of wolf introgression to highest with their respective relative heterozygosity data and sample location. This data was then used for the following statistical tests.

*X-Y Scatter Plot and Linear Regression Analysis*

An X-Y scatter plot was generated for the 425 individual coyote samples in Rstudio statistical programming (Rstudio, 2015). The x-axis was the amount of wolf introgression as a percentage and the y-axis was the relative coyote heterozygosity. From this data, a linear regression analysis and best-fit line were generated to observe the general trend between introgression and heterozygosity.

*Pearson Correlation Test*

A Pearson coefficient correlation test was calculated between the wolf introgression (x-axis) and heterozygosity (y-axis) variables. The test was conducted in Rstudio statistical programming with the package “ggpubr” with a 95% confidence interval (Rstudio, 2015). A plot of this analysis was generated in Rstudio using the same package. A correlation coefficient of >0.30 was considered to show significant correlation between the variables.

*Multiple Regression Analysis*

A multiple regression analysis was conducted to detect if geographic location also influenced heterozygosity results. This test was ran in Rstudio using the lm() function (Rstudio, 2015). Wolf-introgression and the ten locations were adjusted individually to see the effect that each of the eleven variables had on heterozygosity. A table was produced to display these results along with a residual analysis to illustrate the effect that location and heterozygosity together have on location. An accepted p-value of <0.004 was calculated using the following equation. The common accepted p-value of 0.05 was divided by the number of variables being observed in the multiple regression analysis (intercept, wolf introgression, and ten different locations).

A screenshot of a computer

Description automatically generatedRESULTS

X-Y Scatter Plot

*Figure 1: X-Y Scatter Plot of Percent of Wolf DNA compared to Coyote heterozygosity for all 425 coyote samples. Each dot represents a separate individual. The calculated y-intercept of the best-fit line is 0.1838 with a slope of 0.6397.*

Pearson Correlation Test

A screenshot of a map

Description automatically generated

*Figure 2: Pearson Correlation Test between amount of wolf introgression and relative coyote heterozygosity for all 425 coyote samples with a 95% confidence interval. Each dot represents a separate individual. Calculated correlation value of 0.44 with a p-value of <2.2e-16.*

Results from the statistical tests displayed a positive relationship between wolf-coyote introgression and coyote heterozygosity for the 425 individuals represented in the data. From the X-Y Scatter plot, a slope of 0.6397 was calculated for the best-fit line. The correlation tests yielded a moderate correlation value of 0.44. Out of the 425 coyotes analyzed, the range for percentage of wolf introgression was approximately 17%-50%. The range for relative coyote heterozygosity was 8.3%-55% at the 60 SNPs.

Multiple Regression Analysis

|  |  |  |  |
| --- | --- | --- | --- |
| VARIABLE | COEFFICIENT | T-VALUE | P-VALUE |
| Intercept | 0.175 | 5.037 | 7.07e-7 |
| Wolf-Introgression | 0.65 | 9.893 | 2e-16 |
| Maine | -0.008 | -0.233 | 0.816 |
| Massachusetts | -0.046 | -1.129 | 0.260 |
| New Hampshire | 0.018 | 0.404 | 0.686 |
| New Jersey | 0.006 | 0.159 | 0.874 |
| New York | 0.007 | 0.219 | 0.827 |
| Ohio | 0.015 | 0.454 | 0.650 |
| Pennsylvania | 0.007 | 0.219 | 0.827 |
| Quebec | 0.020 | 0.598 | 0.550 |
| Rhode Island | -0.052 | -1.065 | 0.287 |
| Vermont | 0.0052 | 0.157 | 0.876 |

A map of a computer

Description automatically generated

*Figure 3: Residual Analysis of Wolf Introgression and Location on Coyote Heterozygosity. Each dot represents an individual. Red line represents nonlinear relationship between x (location/wolf introgression) and y (heterozygosity) variables.*

Results from the multiple regression analysis show that sample location had little to no influence in determining the coyote heterozygosity. All location t-values were considerably smaller than the wolf introgression t-value. All location p-values were above the accepted cut-off of p<0.004. The residual analysis showed that coyote heterozygosity was not a function of both location and wolf introgression.

DISCUSSION

This study suggests that admixture between grey wolves and other canids with the eastern migrating coyote front has led to higher heterozygosity in the migratory coyote population. This data supports the original hypothesis that despite there being a recent low colonizing number, canid introgression in migrating coyotes is positively correlated with coyote heterozygosity and is independent of location. Location may have less of an impact due to the high dispersal ability of coyotes and the absence of predators in much of their eastern range. Some locations had more influence than others but were trivial compared to the effect of wolf introgression. This may indicate that this migratory front has been able to avoid genetic limitations that other novel migratory species experience. This makes this migration unique in this aspect, as these populations have been introduced to alleles that may increase their success in a variety of new areas.

There are some possible sources of error and bias in this paper that need addressed to improve clarity. Data was limited to only the northeast portion of the United States and some parts of southeastern Canada. Although heterozygosity was shown to increase with wolf introgression, the lack of source population heterozygosity data makes it difficult to understand the overall net change in genetic diversity. Also, some of the SNPs used in this study were of domesticated dog origin, which could have affected the accuracy of conclusions constructed for sample location influence. It would have been beneficial to include a phylogenetic analysis of the individuals included in the research, however data was limited in this aspect.

The coyote migration that has occurred over the last century has given us the opportunity to study novel migration firsthand, and the impact that migration has on local wildlife and the colonizers. Genetically, these migrants have benefited from their ability to interbreed with other canids, implicating that they will proliferate in their new homes, which is something that has largely already been observed.

LITERATURE CITED

Monzón J, Kays R, Dykhuizen DE. 2014. Assessment of coyote–wolf–dog admixture using ancestry-informative diagnostic SNPs. Molecular Ecology. 23(1):182–197. doi:10.1111/mec.12570.

RStudio Team (2015). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com/.

Peppers JA, Kennedy P, Kennedy ML. 1996. Spatial genetic variability in the coyote (Canis latrans). The Southwestern Naturalist. 41(4):388–394.

vonHoldt BM, Pollinger JP, Earl DA, Knowles JC, Boyko AR, Parker H, Geffen E, Pilot M, Jedrzejewski W, Jedrzejewska B, et al. 2011. A genome-wide perspective on the evolutionary history of enigmatic wolf-like canids. Genome Res. 21(8):1294–1305. doi:10.1101/gr.116301.110.

vonHoldt BM, Pollinger JP, Lohmueller KE, Han E, Parker HG, Quignon P, Degenhardt JD, Boyko AR, Earl DA, Auton A, et al. 2010. Genome-wide SNP and haplotype analyses reveal a rich history underlying dog domestication. Nature. 464(7290):898–902. doi:10.1038/nature08837.