Human Genetic and Nasal Morphological Responses to Climate

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Honors Thesis in the Department of Anthropology
University of Michigan
24 April 2017

Acknowledgements

First and foremost, I would like to thank my thesis advisor, Dr. Abigail Bigham, who took my interest in genetics and biological anthropology and guided me along the path of independent research.

My gratitude is also extended to the various faculty, staff, and students of the Department of Anthropology at the University of Michigan, both those who have shaped my anthropological endeavors at this university as well as those who played a more direct role in shaping this thesis. This includes the graduate students of Dr. Bigham's lab, Ainash Childabayeva, Vincent Battista, and Elizabeth Werren. Dr. Erik Mueggler must also be acknowledged for his input on my project.

I would also like to acknowledge Dr. Mark Shriver of Pennsylvania State University and Dr. Peter Claes of the University of Leuven for their contributions, especially data.

I am incredibly grateful for the funding I was awarded by donors through the University of Michigan Department of Anthropology.

Most importantly, I would like to thank the participants who volunteered to provide their DNA and/or facial morphological data that were used in this research.

Abstract

Humans are able to thrive in many environments around the world, including the extremes, ranging from hot and humid to cold and dry. To make this possible, various internal structures of the nose regulate the temperature and humidity of inhaled air before it reaches the bronchi and lungs, preventing damage to delicate pulmonary structures in harsh environments. Therefore, the shape of the nose reflects morphological changes caused by climatic pressures. It has been observed that external nasal morphology correlates with a cline from hot-humid climates to cold-dry climates: the closer to the equator, the lower and wider the noses, and the closer to the poles, the higher and narrower the noses. However, relatively little research has been conducted on the links between genetics and the phenotypic patterns in nasal morphology related to climate adaptation.

This study examines the correlation between genes and single nucleotide polymorphisms (SNPs) that have been previously linked to the nose (*C5orf64* SNP rs11738462, *PAX1* SNP rs2424399, and *PAX3* SNP rs7559271), with phenotypic nasal morphology, which is quantitatively measured by 3D facial landmarking. These SNPs were isolated within DNA collected from Maya individuals residing in Palenque, Chiapas, Mexico, a population which has historically inhabited this constant warm and humid climate. Genotype data were then analyzed and compared to nasal phenotype data collected from the same cohort. Phenotype data from a cohort of Northern European individuals whom have historically inhabited a colder and drier climate were also compared to phenotype data collected from the Maya cohort in order to see if climate has a significant impact on nasal morphology. The results of this study show no genotype-phenotype correlation within the Maya cohort, but significant differences in nasal phenotype were found between the two cohorts.

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Introduction

Humans have inhabited a wide range of climates around the world throughout our evolutionary history, ranging across extremes from hot and humid to cold and dry. Cultural and behavioral adaptations have allowed for success in these climates, including the production and use of insulated clothing and shelter. Yet these environmental pressures can cause physiological and genetic adaptations within bodies as well.

Many organisms, including humans, follow general ecogeographical rules to maximize thermoregulatory efficiency, including Bergmann's and Allen's rules. Allen's rule states that body shape tends to me more compact and rounded in cold climates and more elongated in warm climates. Bergmann's rule states that body size increases as environmental temperature decreases. These rules reflect how surface area to volume ratios impact the thermogenic properties of an organism: the lower the surface area to volume ratio an organism has, the more heat will be retained. These rules have been found to apply to humans, as body mass varies inversely with annual temperature, and the surface area to volume ratio is positively correlated with temperature (Katzmarzyk and Leonard 1998; Savell et al. 2016). These trends act to most efficiently preserve or expel heat in a given environment.

Similar to general body form, the shape of the nose also reflects morphological changes caused by climatic pressures. Various internal structures of the nose regulate the temperature and humidity of inhaled air before it reaches the bronchi and lungs. This regulation prevents damage to delicate pulmonary structures in harsh environments, where inhaled air must be very close to body temperature and highly saturated for optimal gas exchange (Negus 1954). The nasal cavity is particularly important for this regulation, as this is where most of the heat and moisture exchange of inhaled air takes place, and the nose can provide approximately 90% of the

temperature and humidity changes required in the air for proper gas exchange (Naftali et al. 2005). This is achieved via contact between the air and the mucosal tissues within the nasal cavity, which can be altered by changing the shape of the nasal cavity. The degree to which inhaled air is warmed and humidified depends on the amount of contact created between the air and the nasal mucosal tissues (Clement and Gordts 2005, Mowbray and Gannon 2001; Seren and Seren 2009). Surface area to volume ratio, residence time, and degree of turbulence each impact the degree that air contacts the mucosal tissues within the nasal cavity, and thus the degree to which air is warmed and humidified (Churchill et al. 2004; Clement and Gordts 2005; Naftali et al. 2005; Noback et al., 2011; Seren and Seren 2009).

Both the variation in nasal cavity shape and external shape of the nose correlates with a cline from hot-humid climates to cold-dry climates — the closer to the equator, the lower and wider the noses, and the closer to the poles, the higher and narrower the noses. This is because high and narrow noses correspond to greater contact between the air and mucosal tissues within the nasal cavity via increased turbulence and a greater surface-to-volume ratio in the cavity (Noback 2011; Seren and Seren 2009). These morphological differences have been recorded among human populations (Evteev et al. 2014; Fusake et al. 2015, Hernandez et al. 1997, Wolpoff 1968, Yokley 2009; Zaidi et al. 2017), as well as in non-human primate groups like macaques (Rae et al. 2003) and non-primate groups like rats (Rae et al. 2006).

Research has also been conducted on maxillary sinus shape and variation within this structure relating to climate (Butaric and Maddux 2016; Shea 1977). Results of these studies are similar to those produced by studies conducted on correlation between external nose shape and nasal cavity shape with climate. Though measurements of the maxillary sinus are independent from external nose shape, studies have revealed how important internal nasal structures, like the

maxillary sinus, are for regulating air conditions. Individuals with narrower nasal cavities have wider maxillary sinuses, and tend to be from colder environments; those with wider nasal cavities have narrower maxillary sinuses, and tend to be from hotter climates (Butaric and Maddux 2016). These results support the idea that the internal nasal structures, including the maxillary sinus, are correlated with climate due to their air conditioning capacities.

It is challenging to determine if these differences between human populations in nasal morphology have been caused by natural selection or genetic drift. Several studies have found that the human skull, particularly regions surrounding and including the nose, are differentiated to a greater degree among human populations than would be expected under genetic drift alone (Evteev et al. 2014; Guo et al. 2014; Hubbe et al. 2009; Roseman 2004; Roseman and Weaver 2004; Zaidi et al. 2017). One study found that the width of the nares and the alar base width diverge from the expectations for variance caused exclusively by genetic drift (Zaidi et al. 2017). Therefore, differences in these measurements of the nose between different populations can be attributed to divergent selection. However, nares width was only found to be weakly correlated with temperature, so there are other factors contributing to nasal shape divergence as well (Zaidi et al. 2017).

Several genome-wide association studies (GWAS) have been conducted in the past in order to find locations within the genome that have some correlation with mid-facial morphology (Claes et al. 2014, Liu et al. 2012, Paternoster et al. 2012, Shaffer et al. 2016). Several locations within the genome have been suggested to impact nasal morphology, like four genes of interest identified by Adhikari et al. (2016): *DCHS2*, *RUNX2, GLI3*, and *PAX1*. Studies have also repeatedly found significant association between single nucleotide polymorphisms (SNPs) in

PAX3 and nasion position (the point on the nose between the eyes) (Adhikari et al. 2016, Liu et al. 2012, Paternoster et al. 2012).

Here, an exploration was carried out on the correlation between external nasal morphology and variation in genotype at various locations in the genome. A population from a climate that is warm and humid consistently throughout the year (a cohort of Maya ancestry) was compared to a population from a colder, less humid environment (a cohort of Northern European ancestry). As air conditions in hot and humid environments are very close to optimal for gas exchange within the lungs without much alteration, very little stress is placed on nasal morphology by the environment, and thus pressure for natural selection to act on genes controlling nasal morphology is expected to be minimal. However, there is a higher likelihood for natural selection to have had an impact on Northern European populations, where the cold and dry climate favors a higher and narrower nose for efficient air conditioning.

Methods

Participant recruitment and population samples

Research participants for this study were recruited from Palenque, Chiapas, Mexico, a city with a significant indigenous Maya population. Unrelated adult participants between the ages of 18 and 35 were recruited to participate. Language was used as a proxy to control for Maya ancestry, as recruited individuals were required to either speak a Maya language or have both parents and both sets of grandparents speak a Maya language. All participants were in good health, without cardiovascular, pulmonary or metabolic problems, and were not smokers.

A total of 101 male and female individuals between the ages of 18 and 35 years old were recruited and consented to the study. Height was measured using a stadiometer and weight was measured using a scale. Six mls of blood were drawn from an antecubital vein by a local health professional for anemia testing and DNA extraction; saliva samples were also collected for DNA extraction. DNA was stabilized in the field and hand transported to the University of Michigan. DNA extraction was performed using the Puregene protocol (*Qiangen, Hilden, Germany*) according to the manufacturer's instructions. Mid-facial morphology measurements were taken by collecting 3D facial photographs (*3dMD, Atlanta, GA*). Three photos were taken of each participant while standing. The three images were then stitched together using the 3dMDface system. All participants provided informed, written consent and the study was approved by the University of Michigan IRB and by the ethics committee at the Centro de Investigación y Docencia Económica (CIDE), Mexico City, Mexico.

Northern European data were taken from a dataset published by Zaidi et al. (2017), which is part of a larger dataset for studies at the Pennsylvania State University and the University of Illinois at Urbana-Champaign. A total of 236 male and female individuals between the ages of 18

and 59 were recruited from Ireland (N = 151) and Poland (N=85). Each participant reported that all four of their grandparents were from the sampling region. Standing height and body weight measurements were collected, and 3D facial photographs were taken (*3dMD, Atlanta, GA*). Genotype data for the SNPs of interest were unavailable for this population.

Candidate gene and SNP selection

A set of candidate genes potentially associated with craniofacial variation centered around nasal morphology was identified. A preliminary list of genes was put together by searching the Online Inheritance of Man (OMIM) database (https://www.omim.org), using keywords including "craniofacial" and "nasal morphology". These searches generally yielded genes that have previously been linked to disorders that result in craniofacial abnormalities. The Web of Science (https://apps.webofknowledge.com) was used to find published research on correlations between genes and SNPs within them, and external nasal features. SNPs that have been identified as having a correlation between external nasal features were added to the list of candidate SNPs. More data on those SNPs were collected using the UCSC Genome Browser (http://genome.ucsc.edu). The list of candidate SNPs was then narrowed down by looking at the human genomic population data on the 1000 Genomes Project Browser (http://phase3browser.1000genomes.org), and SNPs with higher allelic frequency differences between Indigenous American populations and European populations were favored. Using these methods, three SNPs were chosen to be tested for associations with variation in nasal morphology: rs11738462 in *C5orf64*, rs2424399 in *PAX1*, and rs7559271 in *PAX3*. A summary of methods used for SNP selection is provided in Figure 1.

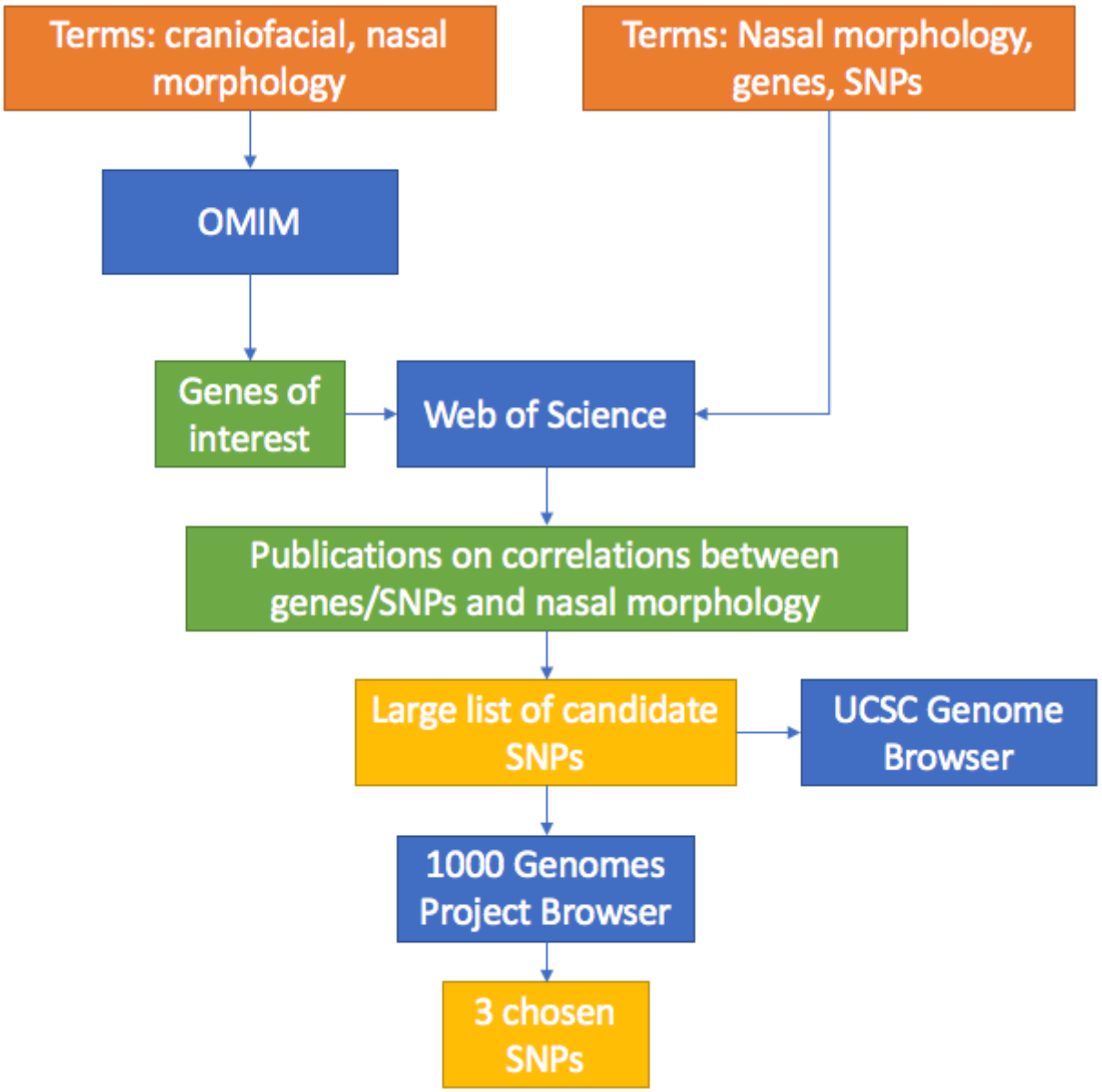

Figure 1: Summary of methods used to select the three SNPs (rs11738462 in *C5orf64*, rs2424399 in *PAX1*, and rs7559271 in *PAX3*) used in this study.

The three chosen SNPs, rs11738462 in *C5orf64*, rs2424399 in *PAX1*, and rs7559271 in *PAX*, were ultimately chosen for their previously identified associations with measurements on and around the nose. The SNP in *C5orf64* was found to have an association with the pronasale to alare distance as well as nasal ala length (Paternoster et al. 2012); the SNP in *PAX1* was found to

have an association with nasal width (Shaffer et al. 2016); and the SNP in *PAX3* was found to have an association with the nasion to mid-endocanthion point (Adhikari et al. 2016, Liu et al. 2012, Paternoster et al. 2012) (Table 1, Figure 3). However, in a study by Shaffer et al. (2016), the associations between the SNPs in *C5orf64* and *PAX3* with nasal morphology were not found.

Gene	Chromosome	SNP ID	Derived/ Ancestral Allele	Function	Literature Findings	1000 Genomes Project Findings (derived/ancestral allele)							
						All American	Medellin, Columbia	Los Angeles, California	Lima, Peru	All European	Northern and Western European ancestry in Utah	Finnish in Finland	British in England and Scotland
C5orf64	chr5	rs11738462	A/G	intron	Association with pronasale to alare distance (tip of nose to widest part of the nostril) and nasal ala length (Paternoster et al. 2012) No evidence for this association found by Shaffer et al. (2016)	0.18/0.82	0.25/0.75	0.19/0.81	0.06/0.94	0.19/0.81	0.24/0.76	0.25/ 0.75	0.18/0.82
PAX1	chr20	rs2424399	A/C	intron	Association with nasal width (Shaffer et al. 2016)	0.59/0.42	0.60/0.40	0.57/0.43	0.50/0.50	0.76/0.24	0.73/0.27	079/0.21	0.72/0.28
PAX3	chr2	rs7559271	A/G	intron	Association with nasion to mid-endocanthion point (midpoint between the left and right endocanthi, the innermost corners of the eyes) (Paternoster et al. 2012) No evidence for these associations found by Shaffer et al. (2016)	0.42/0.58	0.52/0.48	0.31/0.69	0.28/0.72	0.60/0.40	0.61/0.39	0.63/ 0.37	0.60/0.40

Table 1: Summary of information on each of the three chosen SNPs, compiled from the UCSC Genome Browser, publications made available through the Web of Science, and population genetics data provided through the 1000 Genomes Project Browser.

SNP genotyping

Primer design was performed using Primer Design 3 Plus (http://primer3plus.com/cgi-bin/dev/primer3plus.cgi) and restriction enzymes were selected using NEB Cutter V2.0 (http://nc2.neb.com/NEBcutter2/). SNPs were genotyped using polymerase chain reaction (PCR) followed by restriction enzyme digestion. Genotyping was performed using agarose gel electrophoresis. The amplified fragments of DNA containing the *C5orf64* SNP rs11738462 and the *PAX1* SNP rs2424399 were digested with the restriction enzyme BsrDI and the fragment containing the *PAX3* SNP rs7559271 was digested with the restriction enzyme PstI. See Table 2 for a summary of primers and PCR conditions used, and Table 3 for a summary of restriction enzyme digestion information.

Before the DNA samples from the Maya cohort were genotyped, optimizations were performed to increase the sensitivity of the assays. To do this, several trials of PCR using control DNA were performed using varying amounts of $MgCl_2$, betaine, and annealing temperatures. By taking these PCR products and running them through gel electrophoresis, it was determined (i) if the desired segment of DNA was amplified by checking the length (bp) of the DNA fragments, and (ii) which conditions are optimal for visualizing genotype results on the agarose gels. The conditions that produced the brightest bands of the correct length on the agarose gel were chosen (Table 2).

After optimization, a practice PCR/digestion was performed under the optimized conditions for each SNP to ensure that the restriction enzymes cut at the appropriate site on the PCR product. Seven DNA samples including both lab control DNAs and DNA from the Maya cohort were used in this process. After digestion at the temperature suggested by the

manufacturer of the enzymes, gel electrophoresis was performed, and each enzyme was found to have cut at the appropriate location (Table 3). PCR and restriction enzyme digestion were then performed under optimized conditions on the entire Maya cohort. Electrophoresis was performed using 2% agarose gels with ethidium bromide staining. Genotypes were visually determined by examining the length of the DNA fragments in each sample, which corresponds to a particular genotype (Table 3).

Gene	SNP ID	Forward Primer Sequence (5'-3')	Reverse Primer Sequence (5'-3')	PCR product size	Annealing Temperature (°C)	Amount of 1.25 mM dNTPs	Amount of 10x PCR Buffer	Amount of MgCl2	Amount of Taq polymerase	Amount of Betaine	Amount of DNA
C5orf64	rs11738462	GCCAAGTCCCGATTCCTGTA	CCTTGGCCTTGGCATGTAA	308 bp	58°C	4.00 µL	5.00 µL	2.50 µL	0.25 µL	0 µL	2.00 µL
PAX1	rs2424399	GATGCATGCGTTCCTCCTCT	TTACTGAGAGGGGCCTCCAG	565 bp	63°C	4.00 µL	5.00 µL	3.00 µL	0.25 µL	5.00 µL	2.00 µL
PAX3	rs7559271	GTGGCAGGGTTCAAAAGCAAT	AAGCAAAACTAGACCCGCCC	345 bp	63°C	4.00 µL	5.00 µL	3.00 µL	0.25 µL	0 µL	2.00 µL

Table 2: A summary of the optimized PCR conditions for each SNP. Amount of reagent used refers to the amount needed to run one sample

Gene	SNP ID	Enzyme	Bands if Homozygous Ancestral	Bands if Heterozygous	Bands if Homozygous Derived
C5orf64	rs11738462	BsrDI	GG = 101, 207 bp	GA = 101, 207, 308 bp	AA = 308 bp
PAX1	rs2424399	BsrDI	AA = 349, 216 bp	AC = 349, 216, 565 bp	CC = 565 bp
PAX3	rs7559271	PstI	GG = 242, 103 bp	GA = 242, 103, 345 bp	AA = 345 bp

Table 3: Enzymes used for restriction enzyme digests for each of the SNPs, including the length of the DNA fragments expected based on the genotype of the individual.

Phenotyping with 3D facial images

The 3D images from the Maya cohort were taken, assembled, and processed using the 3dMDface system (*3dMD, Atlanta, Georgia*) at Dr. Mark Shriver's lab at Pennsylvania State University. An anthropometric mask was placed upon each of the photos, which were then imported into the MeshLab system (*Visual Computing Lab, ISTI – CNR, Pisa, Italy*) for landmarking and distance measuring. Four landmarks around the nasal region were placed by hand using visual estimation, including the left and right alares and the left and right alar curvatures. Linear distances between these points were measured in millimeters to give the alar base width and the nares width (Figure 3). These distances were also calculated for the Northern European cohort and were provided in the publication by Zaidi et al. (2017). These landmarks on each face were then compared within and between the Maya and Northern European cohorts and against the Maya genotype data to interrogate for links between nasal phenotype and genotype.

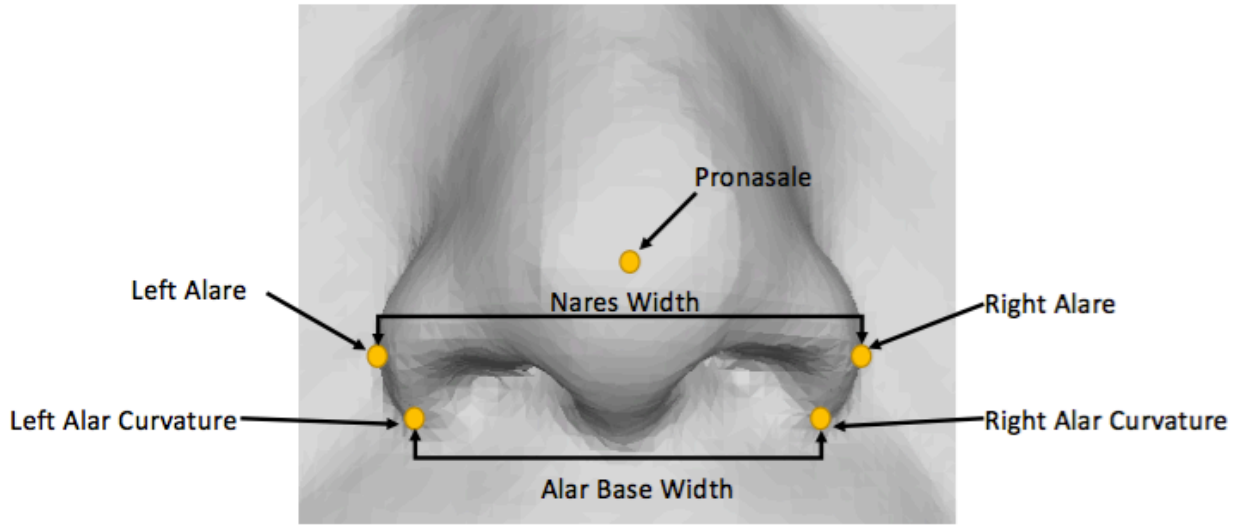

Figure 2: Measurements used in this study to quantify nasal morphology. Landmarked locations include the left and right alare and the left and right alar curvature. Linear distances were then measured between those points to give the alar base width and nares width.

Statistical analysis

For statistical analysis, SPSS version 22.0 (*IBM, Armonk, New York*) was primarily used, as well as Plotly (*Plotly, Montreal, Canada*) for graphical outputs. T-tests were utilized to determine if there were statistically significant differences within and between the Maya and Northern European cohorts regarding sex, nares width, and alar base width. Linear regression models were used to determine if there were statistically significant correlations between nasal phenotype and genotype independently for each of the three SNPs.

Results

Population data

Of the 101 Maya individuals recruited, 100 were used in this study due to the corruption of the 3D facial images of one individual. Of this cohort, 50 were female and 50 were male, and they ranged between the ages of 18 and 35 years of age. For males and females combined, the nares width ranged from 32.7152 to 44.5768 mm with a mean of 38.2787 ± 2.6179 mm; the alar base width ranged from 26.5229 to 39.4572 mm with a mean of 32.4445 ± 2.9440 mm (Table 4). Male and female nares width (male average = 39.4833 mm, female average = 37.0742 mm, $P = 3.087\times10^{-6}$) and alar base width (male average = 33.4243 mm, female average = 31.4648 mm, P = 0.0007156) differed significantly (Table 5, Figure 3).

Data from 236 Northern European individuals were available for this study. I removed all individuals above the age of 35, resulting in 215 individuals ranging in age between 18 and 35 years old. Of this cohort, 134 were female and 81 were male. The nares width ranged from 23.3690 to 39.8688 mm with a mean of 32.0755 ± 2.6608 mm; the alar base width ranged from 26.0144 to 40.7801 mm with a mean of 33.8606 ± 2.4384 mm (Table 4). Again, male and female nares width (male average = 34.1278 mm, female average = 30.8350 mm, $P = 1.308\times10^{-20}$) and alar base width (male average = 35.4397 mm, female average = 32.9061 mm, $P = 7.167\times10^{-14}$) were significantly different (Table 5, Figure 3).

		Nares Width Range	Mean Nares Width	Alar Base Width Range	Mean Alar Base Width
Maya Cohort	**All**	32.7152- 44.5768 mm	38.2787 ± 2.6179 mm	26.5229- 39.4572 mm	32.4445 ± 2.9440 mm
	Males	34.8129-44.5518 mm	39.4833 ± 32.0612 mm	26.9787-39.4572 mm	33.4243 ± 2.9358 mm
	Females	32.7152-44.5768 mm	37.0742 ± 2.603 mm	26.5229-39.1588 mm	31.4648 ± 2.6666 mm
Northern European Cohort	**All**	23.3690-39.8688 mm	32.0755 ± 2.6608 mm	26.0144-40.7801 mm	33.8606 ± 2.4384 mm
	Males	30.0819-39.8688 mm	34.1278 ± 2.2379 mm	30.1686-40.7801 mm	35.4397 ± 2.2872 mm
	Females	23.3690-35.0399 mm	30.8350 ± 2.0649 mm	26.0144-38.2964 mm	32.9061 ± 1.9960 mm

Table 4: Ranges and averages of nares width and alar base width measurements within the Maya and Northern European cohorts.

	Male Mean Nares Width	Female Mean Nares Width	p-value	Conclusion	Male Mean Alar Base Width	Female Mean Alar Base Width	p-value	Conclusion
Maya Cohort	39.4833 mm	37.0742 mm	3.087×10^{-6}	Significant difference	33.4243 mm	31.4648 mm	0.0007156	Significant difference
Northern European Cohort	34.1278 mm	30.8350 mm	1.308×10^{-20}	Significant difference	35.4397 mm	32.9061 mm	7.167×10^{-14}	Significant difference

Table 5: Results from two-sample t-tests to test for a significant difference in nares width and alar base width between males and females. Significance is considered if $p<0.05$.

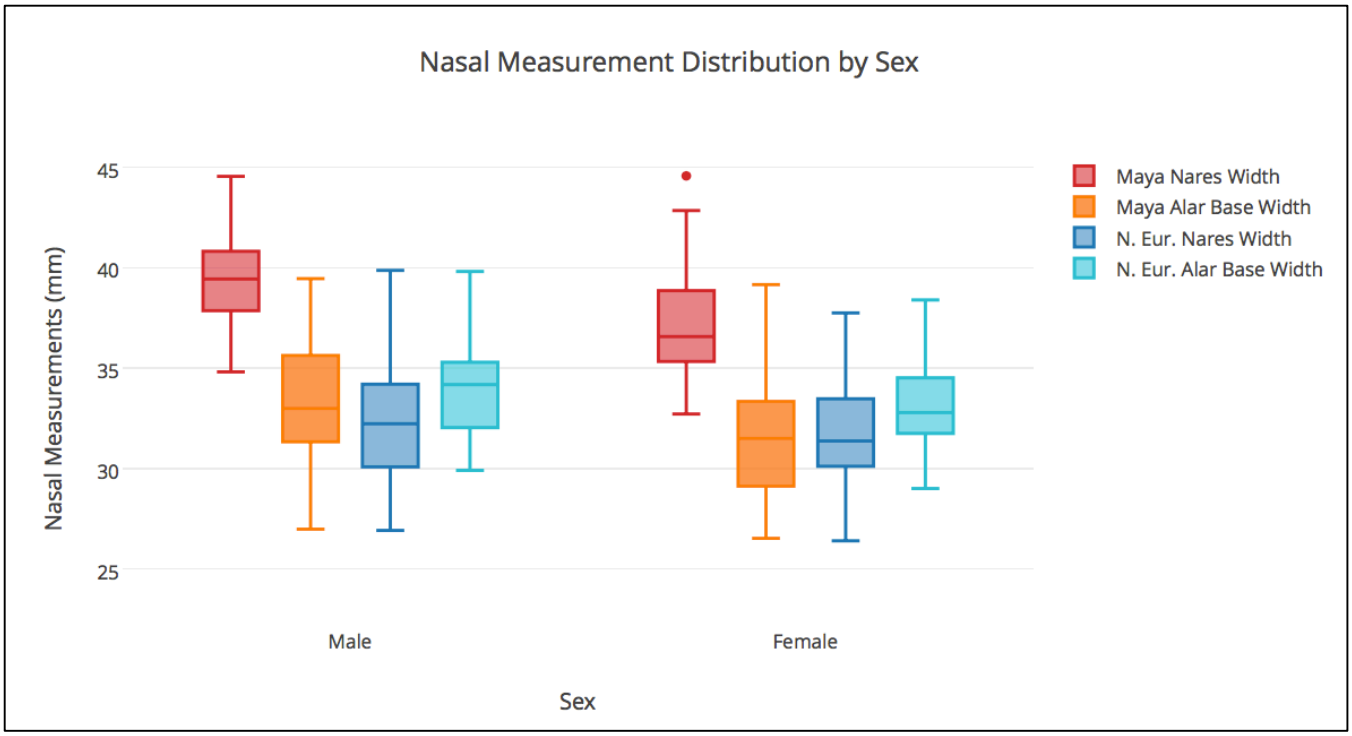

Figure 3: Boxplot comparing varying nares widths and alar base widths between the sexes among the two cohorts. Male and female nares width and alar base width were statistically significantly different within the Maya and Northern European cohorts ($p<0.05$).

Next, nasal measurements were compared between the Maya cohort and the Northern European cohort. These two populations significantly differed for both nares width (Maya average = 38.2787 mm, N. Eur. average = 32.0755 mm, $P = 2.0102\times10^{-47}$) and alar base width (Maya average = 32.4445 mm, N. Eur. average = 33.8606 mm, P = 0.000048831) (Table 6, Figure 4).

Mean Maya Nares Width	Mean N. Eur. Nares Width	p-value	Conclusion	Mean Maya Alar Base Width	Mean N. Eur. Alar Base Width	p-value	Conclusion
38.2787 mm	32.0755 mm	2.0102×10^{-47}	Significant difference	32.4445 mm	33.8606 mm	0.000048831	Significant difference

Table 6: Results from two-sample t-tests to test for significant difference in nares width and alar base width between the Maya cohort and the Northern European cohort. Significance is considered if $p<0.05$.

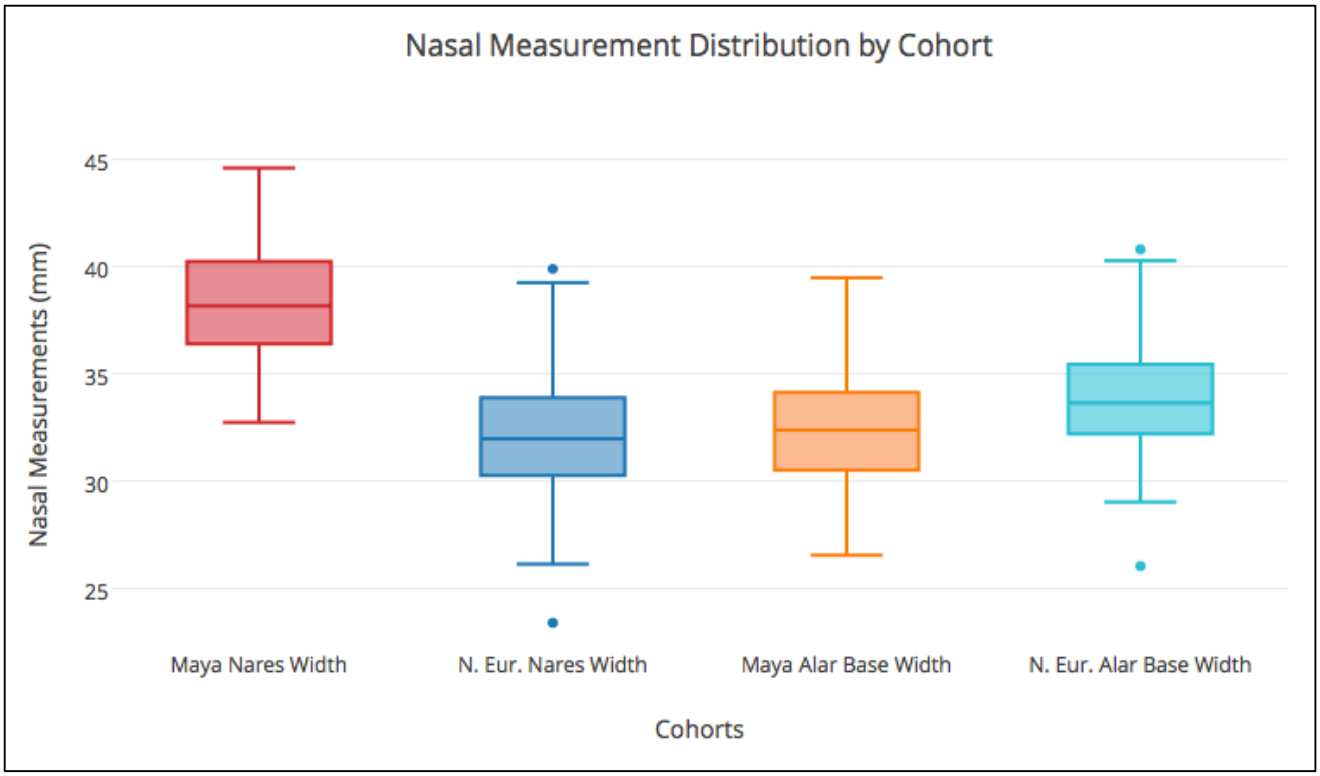

Figure 4: Boxplot comparing the varying the distribution of nares width and alar base width among the Maya and Northern European cohorts. Nares width and alar base width were statistically significantly different between the Maya and Northern European cohorts ($p<0.05$).

SNP Associations with nares width and alar base width

Genotype and allele frequencies were calculated for each polymorphism in 100 individuals from the Maya cohort. For the *C5orf64* SNP rs11738462 there were 78 GG homozygotes, 20 GA heterozygotes, and 2 AA homozygotes (78%, 20%, and 2% of the population, respectively); for the *PAX1* SNP rs2424399 there were 19 AA homozygotes, 29 AC heterozygotes, and 52 CC homozygotes (19%, 29%, and 52% of the population, respectively); for the *PAX3* SNP rs7559271 there were 69 GG homozygotes, 25 GA heterozygotes, and 6 AA homozygotes (69%, 25%, and 6% of the population, respectively) (Table 7, Figure 5).

Gene	SNP ID	Genotype Frequencies	Allele Frequencies
C5orf64	rs11738462	GG: 78%	G: 88%
		GA: 20%	A: 12%
		AA: 2%	
PAX1	rs2424399	AA: 19%	A = 33.5%
		AC: 29%	C = 66.5%
		CC: 52%	
PAX3	rs7559271	GG: 69%	G: 81.5%
		GA: 25%	A: 18.5%
		AA: 6%	

Table 7: Summary of genotype and allele frequencies for each SNP from the Maya cohort.

Figure 5: Genotype and allele frequencies for each SNP, based on results collected from the Maya cohort.

For the Maya cohort, the mean nares width and mean alar base width were calculated for each genotype (Table 8). A linear regression model was used to assess the impact of genotype (minor allele frequency) on phenotype within the Maya cohort. This model was used to test each SNP separately against nares width and alar base width. All three SNPs, *C5orf64* SNP 11738462, *PAX1* SNP rs2424399, and *PAX3* SNP rs7559271, were found to be not significantly associated with nares width or alar base width (Table 9).

Gene	SNP ID	Genotype	Mean Nares Width	Mean Alar Base Width
C5orf64	rs11738462	GG	38.2017 mm	32.2876 mm
		GA	39.1289 mm	33.1804 mm
		AA	35.0081 mm	31.2090 mm
PAX1	rs2424399	CC	37.9138 mm	32.0059 mm
		CA	37.0330 mm	31.1382 mm
		AA	39.1263 mm	32.9119 mm
PAX3	rs7559271	GG	38.0905 mm	32.2124 mm
		GA	38.7768 mm	33.2158 mm
		AA	38.3681 mm	31.9005 mm

Table 8: Mean nares width and mean alar base width for each genotype of the Maya cohort.

					Nares Width		Alar Base Width	
Gene	SNP ID	Ancestral/Derived Allele	Minor Allele	Minor Allele Frequency	R^2	p-value	R^2	p-value
C5orf64	rs11738462	G/A	A	0.12	0	0.996	0.005	0.486
PAX1	rs2424399	C/A	A	0.335	0.03	0.085	0.019	0.168
PAX3	rs7559271	G/A	A	0.185	0.008	0.39	0.019	0.168

Table 9: Summary of results from the linear regression model used to test correlation between genotype (minor allele frequency) on nares width and alar base width. No significant association was found between genotype and phenotype. Significance is considered if $p<0.05$ and $p<0.016$ after a Bonferroni correction for multiple tests, $\alpha = 0.05$).

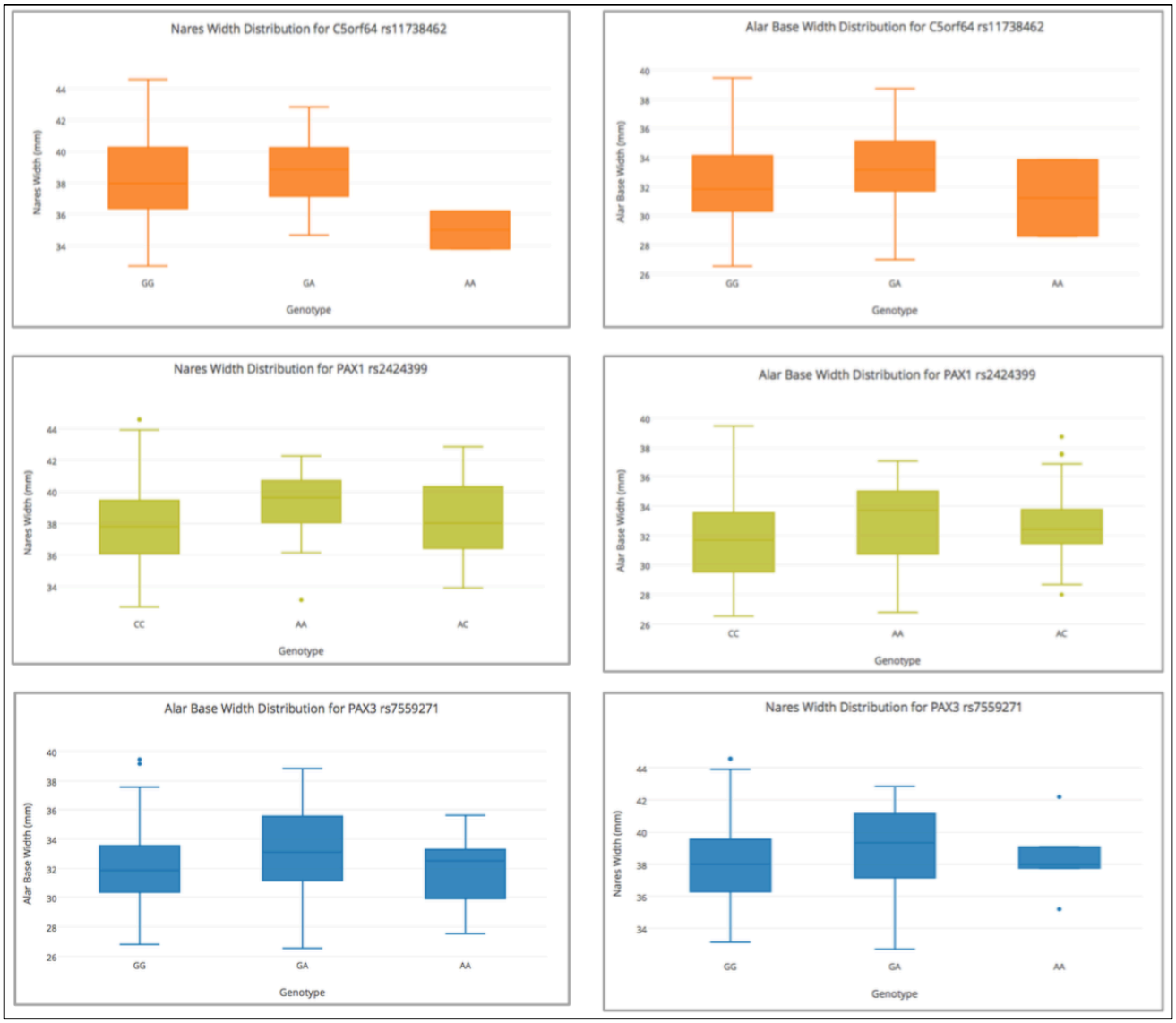

Figure 6: Distribution of nares width and alar base width for each genotype, based on results collected from the Maya cohort.

Discussion

Summary of findings

The goal of this study was identify markers of natural selection and genetic adaptation caused by climactic pressures placed upon nasal form and function. Inhaled air must be at or near body temperature and almost completely saturated before it hits the bronchi for optimal gas exchange. The nose plays an important role in altering the temperature and humidity of this inhaled air (Negus 1954). Therefore, nasal shape is particularly important where there is a drastic difference between external conditions and the air conditions necessary for optimal breathing and gas exchange. In cold-dry climates, the pressure placed upon nasal structure to optimize air contact with nasal mucosal tissues is high. In contrast, in warm-humid climates, where inhaled air is consistently near or at optimal temperature and humidity levels, climatic pressures are relieved as the nose should have very little need to influence air conditions.

The phenotypic comparisons between the Maya cohort and the Northern European cohort carried out in this study supports previous research (Farkas, Kolar and Munro 1986; Franciscus and Long 1991, Zaidi et al. 2017). Both nares width and alar base width were found to be significantly different between the cohorts ($P = 2.0102\times10^{-47}$ and $P = 0.000048831$, respectively) (Table 6, Figure 4). Nares width was significantly larger in the Maya cohort compared to the Northern European cohort, which supports previous data demonstrating that populations adapted to cold-dry climates (like the Northern Europeans) have narrower noses than those adapted to warm-humid climates (like the Maya) (Farkas, Kolar and Munro 1986; Franciscus and Long 1991, Zaidi et al. 2017). However, the Northern European cohort had a higher average alar base width (32.4445 mm) compared to the Maya cohort (33.8606 mm). This suggests that alar base width may be less directly tied to air conditioning capabilities of the nose and thus weakly, if at

all, correlated with climate. These data support findings by Zaidi et al. (2017) that suggested that it is not merely the width of the nose that is under climatic selective pressure, which would include the nares width and the alar base width, but it is the width of the nares that is specifically under selective pressure.

This study's results suggested that within the Maya cohort there is no association between the three SNPs (the *C5orf64* rs11738462, the *PAX1* rs2424399, and the *PAX3* rs7559271) and nares width and alar base width. These results, however, are not completely surprising when viewed from an evolutionary perspective, as it is unexpected that the Maya cohort experienced selective pressures that impacted nasal morphology. However, DNA was not available from the Northern European cohort for this study, and thus these data should serve as a comparative set to be used in future genetic investigations in cold-dry-adapted populations.

There was a high degree of sexual dimorphism seen in nose shape, as males in both cohorts had significantly larger nares width and alar base width measurements (Table 4, Table 5, Figure 3). Could this sexual dimorphism be a result of sexual selection, and if so, is it a result of an honest signal of adaption to the local environment, or merely a preferred aesthetic appearance? Could cultural differences in preferred nasal shape have influenced varying shapes across cultures and regions? Previous research has found that sexual selection and/or local adaptation could have played important roles in shaping human mid-facial soft-tissue morphology (Guo et al. 2014). Environmental selective pressures and sexual selection may have reinforced each other (Zaidi et al. 2017), but more research needs to be done to investigate these questions. Other research has suggested that the relatively larger noses seen in males compared to females is a result of differing activity levels and increased respiratory demands in males (Holton et al. 2016). This relates to previous findings that the size of the airway is a result of the

energetics of the organism, while the shape of the airway is more reflective of adaptation to climate (Bastir and Rosas 2013). However, the strength of the correlation between the two is unclear and further investigation is required.

Limitations and complications

Originally, the 3D facial images of the 101 recruited Maya participants were to be processed and landmarked by a research team at a laboratory external to the University of Michigan. However, they were unable to perform the promised analyses in time for the thesis. Therefore, I performed the landmarking by hand. I marked each of the four landmarks on each of the 100 facial images one time. For greater statistical accuracy, it would have been optimal to have two separate individuals place each landmark two to three times and average the results (Aldridge et al. 2005). Unfortunately, due to the limited time available to complete the landmarking and analysis, this was not possible. This may have caused inaccuracies in the data.

Furthermore, because of the limited time available to complete the landmarking, only four landmarks were placed (right and left alar curvature, right and left alares) to measure two distances (the alar base width and the nares width). I chose these landmarks and distances because they relate to external nasal width and were relatively easy to accurately mark and measure. However, the three SNPs that I chose had not previously been found to be explicitly associated with these distances. The *C5orf64* SNP rs11738462 and the *PAX1* SNP rs2424399 were found to be associated with nasal width (Shaffer 2016), but the *C5orf64* SNP rs11738462 was also found to be associated with the pronasale to left alare distance (tip of the nose to the widest part of the left nostril) (Paternoster et al. 2012), and the *PAX3* SNP rs7559271 was found to be associated with the nasion to mid-endocanthion point (point on the surface of the nose

between the eyes to the midpoint between the innermost corner of the eyes [not on the facial surface]) (Paternoster et al. 2012). Therefore, it was not expected that the *PAX3* SNP rs7559271 would correlate with nares width or alar base width, and there was only a small chance that the *C5orf64* SNP rs11738462 correlate with either of those measurements. This study would have been more powerful if it examined many more landmarks around the nose (including the nasion, pronasale, subnasale and left and right endocanthii, for example) and used Euclidian Distance Matrix Analysis (EDMA) (Lele and Richtsmeier 1991) to measure the distances between all of these landmarks.

This study would have been more powerful if it had larger sample sizes, particularly from the Maya cohort. Having only 100 participants is decent, but less than ideal and certainly not powerful enough when looking for significant phenotype-genotype associations. Principal components analysis (PCA) could have better controlled for significant principal components (PCs), which would reduce the data to only a few variables that have the most genetic variability information. More importantly, this study would have yielded more useful and powerful results if DNA samples from the cold-dry adapted population, the Northern European cohort, had been available. It is likely that evidence of natural selection would have been found if the genotypes of a hot-humid adapted population had been compared to a cold-dry adapted population.

Conclusion

Variation in nasal morphology seen around the world can be explained in large part by physiological adaptation to air conditions. This study confirmed previous findings that populations in cold-dry climates tend to have narrower noses than populations in warm-humid

environments. Though no significant genotype-phenotype relationships were identified, phenotype-phenotype trends observed between the Maya and Northern European cohorts suggest that genetic adaptation and thus evolution by natural selection has taken place in response to climatic pressures. Further study is required to test to see if there are genotype-phenotype correlations within cold-adapted populations.

Relatively little is known about how genotype controls phenotype. This research contributes to our general understanding of how DNA functions, its impact on phenotype, and how it can be altered by pressures from external climatic conditions. These pressures may change not only your phenotype and genotype, but those changes may be passed down to future generations as an adaptation that vies a survival advantage in a given environment.

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