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POPULATION GENETICS OF BLACK BEAR (*URSUS AMERICANUS*)

by

Amanda K. Thibodeau

A Thesis Submitted in Partial Fulfillment
of the Requirements for a Degree with Honors
(Biology)

The Honors College

University of Maine

May 2007

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ABSTRACT

DNA analysis is a ubiquitous tool to identify variation within populations. By using microsatellites, highly variable genetic loci distributed throughout the entire nuclear genome, genetic characteristics can be identified in the population. Genetic variation in the black bear, *Ursus americanus,* was characterized through samples gathered from the Maine population. In total, five loci were characterized for analysis. In order to aid in wildlife forensic cases, I examined genetic variations in black bears. The range of observed heterozygosity for the population sample was 0.729-0.871; the number of alleles per locus ranged from 7 to 15. Sampling the population and determining the frequencies of the alleles can introduce information about the genetic characteristics of the population. The allele frequencies that have been recorded here can be used in cases to determine if two unknown samples are from a single individual, or to determine that samples came from different animals. Analysis of the information can also aid in the knowledge of population structure and genetic diversity within the population. With the characterization of individuals within a family, multiple paternity, a phenomenon seen in several different taxa, can be observed. In this population study, however, multiple paternity was not observed.

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INTRODUCTION

In the State of Maine, the black bear, *Ursus americanus*, is an important part of the state's ecosystem. With the Maine black bear population estimated at a healthy 23,000 animals today, the State has regulated the legal harvesting of this large mammal during specific periods in the fall (Martin et al., 2006). The legal hunting season for Maine black bear typically spans from late August to late November (MDIFW, 2007). When a person is suspected of hunting a Maine black bear outside of the legal season, an investigation is launched and evidence is gathered to address the accusation of illegal activity. Many times, an investigation can be greatly helped with the collection of bear DNA evidence, but lack of a reference database complicated interpretation of the evidence until recently.

A study of genetic variation was performed to create a reference database for allelic frequencies in the population. I used DNA profiling to examine variation among samples of DNA from a portion of the Maine black bear population. DNA profiling uses microsatellite loci as markers; they are sections of DNA that possess a repeat motif of two to four base pairs. Microsatellites are used because they are highly polymorphic within a population and have high mutation rates; they are thus regarded as a great tool for differentiating individuals within populations (Jarne and Lagoda, 1996).

Determining the frequency of alleles at a particular locus in a population helps in forensic investigations by assessing the inclusion of samples, the probability that two samples have the same multilocus genotype. Determining the likelihood of a genotype requires the use of an allelic database to aid in determination of inclusion. Determining

exclusion does not rely upon knowledge of population genetic structure but rather on the accurate processing and analysis of genetic information from the samples. Having the database assists in the prosecution of illegal activities especially when there is no other evidence or witnesses to corroborate the prosecution's speculations. In this study, allelic frequencies determined at five loci were used to establish a database. By creating a database of the frequency of alleles within a population, the probability that a specific multilocus profile can occur can be estimated. Knowing the probability of two individuals sharing the same alleles at a specific location can help aid in criminal forensic cases.

In addition, examining genotypes can be used to generate pedigrees and estimate multiple paternity or polyandry (multiple mating in females) in black bears to be studied. Analyzing loci can potentially reveal multiple paternity in a litter with a size >1 offspring in the population of black bears. During the careful collection of samples in the field, notation was made of known family kinship. Kinships were known for mother-offspring combinations, with spans of generations including current cubs, yearlings and offspring from previous years that now have their own families. Polyandry or multiple paternity has been documented in many taxa. Multiple paternity documented in other taxa include antelopes (Carling et al., 2003), snowshoe hare (Burton, 2002), white-tailed deer (DeYoung et al., 2002), mice (Baker et al. ,1999*b*), bats (Rossiter et al., 2000), birds (Griffith et al., 2002) and many others.

With the identification of polyandry or multiple paternity in families of the black bear population a dominant male is sometimes observed. A dominant or super male is a male in the population that has been found to sire a greater percentage of the population

than any other male that has been characterized and found to have offspring. The key to a super male is “multiple mating; their reproductive success is expected to increase as their number of mates rises” (DeWoody 2005, p1404). There are several different theories explaining why one male in the population dominates the breeding over others. One of the obvious answers is pure size. If one animal is larger than the rest, that individual may dominate others and have access to more breeding females (Kovach 2003). Another hypothesis is that the super male might be extremely fertile. It is well known that it is advantageous for the male to mate with as many females as possible to pass on their genotype to as many offspring as possible to secure their position in evolution.

This research is the first to examine genetic variation in Maine black bears. As such, it will be used in both forensic cases and will help in understanding the population biology of the species.

METHODS AND MATERIALS

Sample Collection

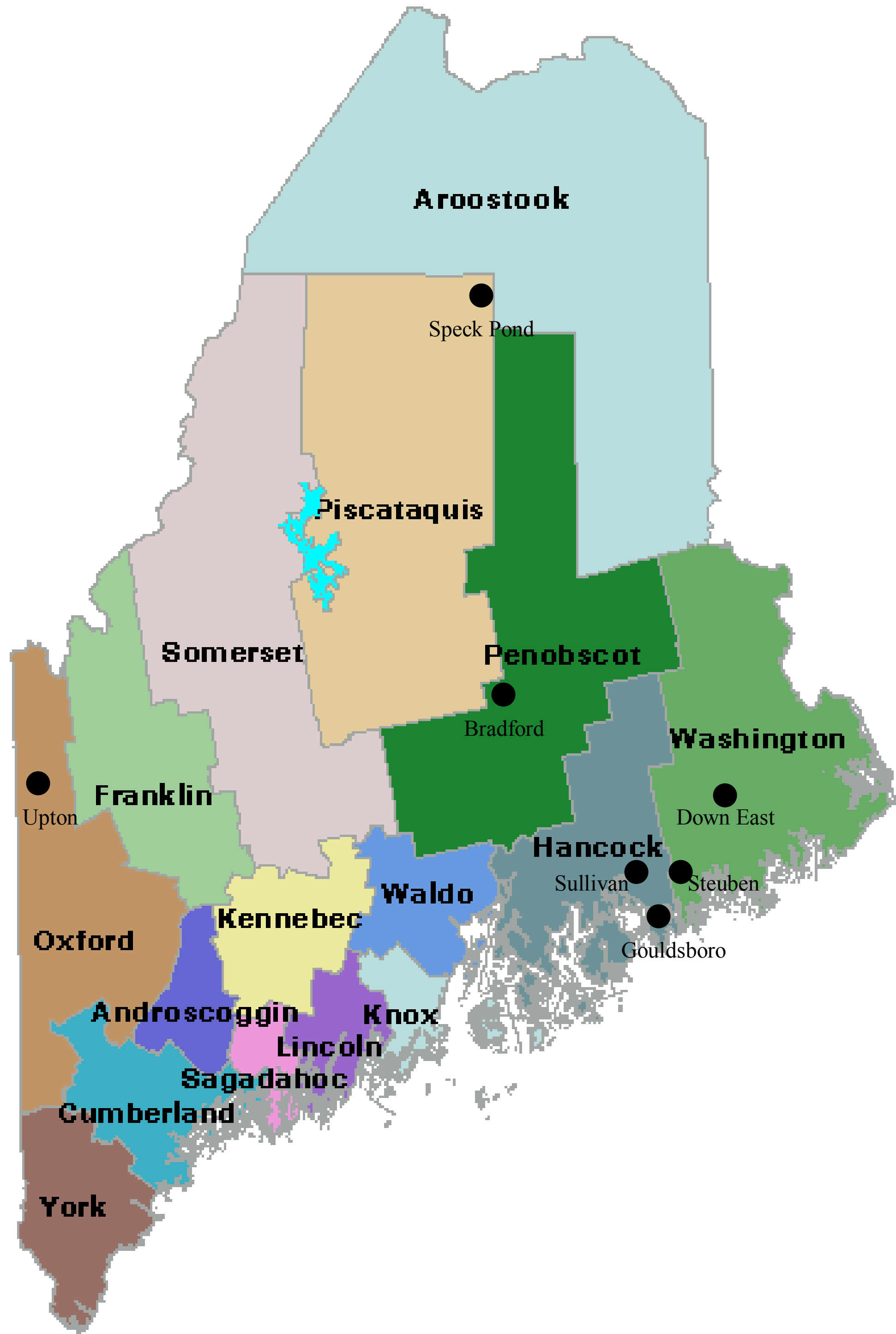
DNA was obtained via tissue (bear footpad as well as muscle tissue from bear hunts), hair, earplug, and buccal swab samples from black bears in Maine. Several groups collected samples using different techniques for the range of samples taken. The footpads were shed by the bear and collected from the den floor and placed into a manila envelope. If available a tuft of hair was taken from adults and then placed into the same manila envelope as footpad. The buccal swabs were taken by inserting a sterile cotton swab into the animal's mouth and rubbing against the cheek and molars, then placed into a sterile container. Plugs of tissue from cubs' ears were taken as tags were applied to the animals. The ear punches were then placed in a manila envelope and allowed to air dry. The pieces of muscle tissue were collected at tagging stations around the State and placed into labeled zipper-locked bags. Samples were collected by game wardens from locations indicated in Figure 1. In all, 162 samples were collected and given to the Molecular Forensics Laboratory at the University of Maine.

Extracting DNA from Samples

The tissue samples were stored at -25° C. Ear plug and buccal swab samples were stored at 5° C. The DNA was extracted from all of the samples using QIAamp® DNA Mini Kit (Qiagen, Valencia, CA) following the manufacturer's tissue extraction protocol. For each sample, about 25mg of the sample was obtained by cutting a piece of the sample with a sterile razor blade. Samples were placed into 1.5 ml tubes and 180 µl of Buffer ATL (Qiagen, Valencia, CA) was added to each tube. To digest tissue, 20 µl

Figure 1. Bear collection locations.

Each ● represents one population location.



of Proteinase K was added to each tube, and then mixed by vortexing. Tubes were incubated in a water bath at 56 °C overnight. After incubation, the tubes were briefly centrifuged at 7,000 rpm. 200 µl of Buffer AL (Qiagen, Valencia, CA) was added to each sample and mixed by vortexing for about 15 seconds, then incubated in a water bath at 70 °C for 10 minutes. After incubation, the tubes were centrifuged for 30 seconds at 7,000 rpm. 200 µl of 95-100% ethyl alcohol was added to each sample and vortexed for 30 seconds at 7,000 rpm.

The entire liquid contents of each tube was placed into the top of a QIAamp spin column placed in a 2.0 ml collection tube. The tubes were centrifuged at 7,000 rpm for 1 minute. The spin columns were then placed into new 2.0 ml collection tubes, and the filtrates in the old collection tubes was discarded.

500 µl Buffer AW1 (Qiagen, Valencia, CA) was added to each column and centrifuged for 1 minute at 7,000 rpm. The spin columns were placed again into new collection tubes and the old collection tubes that contained the filtrate was discarded. 500 µl of Buffer AW2 (Qiagen, Valencia, CA) was added to each column and then centrifuged at 14,000 rpm for 3 minutes. The spin columns were placed into new 2.0 ml collection tubes and centrifuged, without adding anything, at 14,000 rpm for 1 minute.

The spin columns were then placed into 1.5 ml microcentrifuge tubes, 75 µl of buffer AE was added to each tube, and tubes were incubated at room temperature for 3 minutes. The tubes were then centrifuged at 8,000 rpm for 1 minute. Resulting DNA samples were stored at 4°C until needed for the microsatellite PCR reactions.

PCR Protocol

PCR amplifications of the samples were completed in two different multiplex reactions. A 25 µl total volume consisted of 2 µl DNA, 2.5 µl 10X PCR Buffer, 1 µl of 50 mM DNTP, 0.3 µl of 10 mM stock primers, 0.2 µl Taq polymerase, and 19.4 µl (reaction 1) or 18.8 µl (reaction 2) distilled water. The primers used in reaction 1 were: G10BF, G10BR, G10LF, G10LR (Paetkau and Strobeck. 1994 and Paetkau et al. 1998). The primers used for reaction 2 were as follows: G10AF, G10AR, G10DF, G10DR, G10HF, G10HR (Table 1) (Paetkau and Strobeck. 1994 and Paetkau et al. 1998). The PCR conditions for reaction 1 (RXN 1): 90 seconds at 92° C, 15 seconds at 92° C, 30 seconds at 55° C, and 2 minutes at 72° C. The aforementioned conditions were then repeated for 34 cycles followed by extension for 10 minutes at 72° C. Once the ten-minute extension was completed, the cycler was put on hold and the temperature was set at 4° C until the samples were removed from the thermal cycler. For reaction 2 (RXN 2) the annealing temperature was 56° C. The PCR conditions for RXN 2 were: 90 seconds at 92° C, 15 seconds at 92° C, 30 seconds at 56° C, and 2 minutes at 72° C. The aforementioned condition was then repeated for 34 cycles and then extended for 10 minutes at 72° C. Once the ten-minute extension was complete the cycler was put on hold and the temperature was set at 4° C until the samples were removed.

Separation of microsatellite alleles

Amplified PCR products (1µl) were loaded into a well of a 96-well plate along with 0.5 µl ROX 350 size standard, and 8.5 µl Hi-Di Formamide. The prepared plate was

then given to the University of Maine DNA Sequencing Facility where the DNA microsatellite amplimers were separated.

Analysis of PCR Sequenced Products

GeneMapper v.4.0 was used to visualize the results of the separations by examining chromatograms. The chromatograms were analyzed and alleles at all loci were scored for each individual (Raymond and Rousset 1998).

Analytical Methods

After scoring of all the viable samples had been completed, a database of the information was created using Microsoft Excel. Multiple paternity was examined for families with known mothers that had families of three or more offspring from the same year. Once it was estimated which alleles from each locus the mother passed down to the offspring, hypothetical genotypes could be determined for the father. If offspring did not share the same alleles as the rest of the offspring from the same year, it would be concluded that a separate male sired that individual.

Statistical Methods

To conduct statistical tests, allele frequencies were calculated for the population at each locus using the computer program GENEPOP 3.4 (Raymond and Rouseset, 1998).

Estimates for the population parameter theta (θ) were calculated for each allele at each locus, and the overall θ estimate was found for the entire population. The formula

known as Equation 4.10 was used to calculate θ for match probabilities to adjust multilocus probabilities for population structuring (National Research Council, 1996).

For a Heterozygote:

$$\text{Probability} = 2[\theta + (1-\theta)p][\theta + (1-\theta)q]/ (1+ \theta)(1+20)$$

For Homozygote:

$$\text{Probability} = 2[\theta + (1-\theta)p][3\theta + (1-\theta)p]/ (1+ \theta)(1+20)$$

In addition to estimates of allele frequencies heterozygotes observed in the population (H_o) were calculated, along with the expected heterozygosities (H_e).

Table 1. Oligonucleotide primers used in amplification of dinucleotide repeat black bear microsatellite loci.

Locus	Reaction	Primers	Primer Sequence (5'-3')
G10B[a]	1	G10BF	GCC TTT TAA TGT TCT GTT GAA TTT G
		G10BR	56-FAM-GAC AAA TCA CAG AAA CCT CCA TCC
G10L[b]	1	G10LF	GTA CTG ATT TAA TTC ACA TTT CCC
		G10LR	NED-GAA GAT ACA GAA ACC TAC CCA TGC
G10A[b]	2	G10AF	GAC CCT GCA TAC TCT CCT CTG ATG
		G10AR	NED-GCA CTG TCC TTG CGT AGA AGT GAC
G10D[a]	2	G10DF	GAT CTG TGG GTT TAT AGG TTA CA
		G10DR	5HEX- CTA CTC TTC CTA CTC TTT AAG AG
G10H[b]	2	G10HF	CTC TTG CCT TAC TTA CAT GG
		G10HR	56-FAM- ATC AGA GAC CAC CAA GTA GG

[a] (Paetkau and Strobeck. 1994), [b](Paetkau et al. 1998)

RESULTS

Seventy individual bear samples were used to create the allelic frequency database from the population. The samples consisted of solitary individual bears and one member from each known family. Table 2 is a database that was compiled with frequencies of alleles included for the population. Table 3 is the raw data of scored loci within each individual analyzed for the database. Figure 3-9 show typical allelic patterns for each loci.

The number of alleles observed at loci G10B, G10L, G10A, G10D and G10H were 8, 15, 7, 8 and 13 respectively (Table 2).

Observed and expected numbers of heterozygosity are listed in Table 4; expected and observed heterozygosity are presented in Table 5.

Families were examined to identify multiple paternity. No evidence of multiple paternity in these families was found. Two pedigrees from the 5 families genotyped (Figure 3 and Figure 4) can be found in the appendix.

Table 2. Estimated allele frequencies for the Maine black bear population.

Locus	Allele	Frequency
G10B	147	0.0580
	148	0.0072
	149	0.4130
	151	0.1522
	153	0.1812
	155	0.0580
	175	0.1159
	159	0.0145
G10L	123	0.0072
	129	0.1957
	130	0.0072
	131	0.0942
	133	0.1667
	137	0.0145
	141	0.0072
	143	0.0290
	144	0.0652
	148	0.0942
	150	0.1232
	152	0.1232
	154	0.0290
	159	0.0217
	160	0.0217
G10A	168	0.0072
	179	0.0580
	181	0.1449
	183	0.2246
	185	0.2826
	187	0.2681
	189	0.0145
G10D	169	0.0652
	171	0.3261
	173	0.2101
	175	0.0453
	177	0.0870
	179	0.1087
	181	0.1449
	183	0.0145

Table 2. Estimated allele frequencies for the Maine black bear population (cont'd).

Locus	Allele	Frequency
G10H	255	0.0448
	259	0.1567
	261	0.0821
	263	0.2388
	266	0.0522
	268	0.0075
	270	0.0746
	272	0.1269
	274	0.1716
	278	0.0149
	280	0.0149
	285	0.0075
	286	0.0075

Table 3. Individual genotypes for population of black bear in Maine study.

Bear	Locus B		Locus L		Locus A		Locus D		Locus H	
B1	149	153	154	158	183	183	169	181	259	272
B2	147	155	129	160	179	187	173	173	259	266
B3	149	155	150	160	183	187	173	181	261	272
B4	149	155	150	158	181	183	169	179	263	266
B5	153	153	129	144	181	187	173	173	259	263
B6	149	157	144	150	168	183	171	183	259	263
B7	149	155	131	160	181	187	181	181	259	266
B8	153	153	133	144	185	187	169	171	272	272
B9	153	155	148	152	183	185	171	179	263	270
B10	149	155	133	137	179	183	171	173	259	280
B11	147	149	131	150	185	187	171	173	261	263
B12	149	149	129	154	183	185	169	171	272	274
B13	157	157	150	152	181	185	171	173	255	259
B14	149	155	133	154	179	187	171	181	263	266
B15	148	151	123	131	181	183	173	181	263	263
B16	149	151	129	144	181	183	171	177	263	268
B17	149	151	131	133	179	181	171	173	259	259
B18	151	151	133	152	181	187	171	179	261	274
B19	149	159	129	143	183	187	173	179	261	261
B20	149	159	133	143	183	183	171	179	259	272
B21	149	153	133	150	181	183	171	175	270	270
B22	153	153	148	148	183	185	171	181	263	270
B23	153	157	133	148	183	189	173	183	259	263
B24	149	157	129	130	187	187	171	179	259	259
B25	149	155	131	133	181	187	171	173	263	274
B26	149	157	131	144	183	185	171	177	274	278
B27	149	151	129	137	179	185	171	177	266	270
B28	149	149	129	131	179	187	171	173	259	266
B29	149	149	148	152	183	187	173	173	261	280
B30	149	153	152	158	185	187	171	171	274	274
B31	149	149	150	150	181	187	171	177	263	270
B32	149	151	129	133	183	185	173	181	272	274
B33	147	149	133	143	183	183	171	171	263	274
B34	149	153	141	152	181	185	173	175	263	274
B35	149	153	129	133	181	183	169	171	270	285
B36	149	151	131	152	181	185	171	175	263	270
B37	149	157	148	150	181	187	171	173	272	274
B38	153	157	129	131	181	181	171	181	263	263
B39	149	151	133	148	185	185	177	179	266	274
B40	151	151	131	148	179	185	171	173	261	274
B41	153	157	148	150	179	187	173	173	263	274
B42	151	151	148	148	183	185	173	173	263	274

Table 3. Individual genotypes for population of black bear in Maine study (cont'd).

Bear	Locus									
	B		L		A		D		H	
B43	147	149	129	133	185	185	179	181	272	274
B44	149	149	129	152	181	185	171	175	259	261
B45	149	153	133	148	185	187	171	173	272	274
B46	149	149	129	144	185	189	179	181	272	274
B47	149	157	131	152	183	187	175	177	259	263
B48	147	151	129	148	187	187	171	173	259	286
B49	149	149	129	129	187	187	173	179	261	261
B50	149	157	129	154	183	185	171	181		
B51	149	151	133	150	183	187	171	173	255	263
B52	147	149	129	129	185	187	171	177	259	278
B53	149	151	131	143	183	183	169	179	263	272
B54	149	151	133	133	181	185	175	181	274	274
B55	153	157	131	133	185	187	171	179	261	272
B56	149	149	129	150	181	185	177	181	274	274
B57	149	149	150	152	185	185	171	177	255	263
B58	149	149	150	152	185	185	177	181	263	263
B59	149	157	150	152	185	185	177	181	255	259
B60	149	157	150	150	185	185	177	181	259	263
B61	153	153	133	144	185	187	169	171	272	274
B62	153	153	133	144	185	185	171	171	255	255
B63	153	153	133	144	185	187	169	171	272	274
B64	151	153	129	152	187	187	171	181	263	263
B65	147	151	152	152	187	187	171	181	263	272
B66	151	153	129	152	187	187	173	179	263	263
B67	149	157	129	129	185	187	171	179		
B68	147	151	129	152	183	183	169	179	270	272
B69	149	157	129	133	183	187	171	181	259	270
B70	149	155	131	160	183	183	169	181	259	272

Table 4. Observed number of heterozygotes (H_O) and expected number of heterozygotes (H_E) at each locus.

	H_O	H_E
G10B	51	52.710
G10L	61	55.994
G10A	51	54.109
G10D	61	56.195
G10H	55	60.895

Table 5. Observed heterozygosity (H_O) and estimated heterozygosity (H_E) at each locus.

	H_O	H_E
G10B	0.729	0.753
G10L	0.871	0.800
G10A	0.729	0.773
G10D	0.871	0.803
G10H	0.786	0.870

DISCUSSION

This study succesfully genotyped seventy bears at five microsatellite loci. Microsatellite analysis can provide information about the genetic diversity of the Maine black bear population. Statistical analysis of the data can help determine the relative frequencies of alleles in the population for specific locus and allow for the creation of an allelic frequency database. This information can be used for forensic applications.

Hardy-Weinberg equilibrium

When using the Hardy-Weinberg Equilibrium (HWE), it is always assumed that the population is composed of a large number of diploid, randomly mating individuals that are free from mutation and migration (Hartl, 1988). It is also a common assumption that the markers being analyzed are inherited in a Mendelian fashion, and are selectively neutral.

In the data collected through this study, a significant ($p < 0.05$) deviation from HWE was found for the H locus. However, the deviation here was marginally significant ($p=0.0458$). If rounded the deviation is no longer significant. Because this deviation is marginally significant its deviation is attributed to chance alone.

There are several different explanations why there were deviations from the HWE, most of which would be attributable to technical problems. Possible reasons for the deviation could have been the result of sampling, allelic dropout (an instance where a laboratory artifact masks the visualization of a larger allele), or even linkage (Inman et al., 1997). Null alleles, or nonamplifying alleles, are another source of deviations from

HWE where point mutations at a primer site can prevent primers from annealing and thus amplifying a product in the PCR reaction (Waits et al. 2000). Null alleles can cause incorrect scoring of alleles and an excess of homozygotes. However, estimates of frequencies for null alleles were exceptionally small; it is unlikely that they were present at the tested loci. Deviation from HWE could also have been caused by inequality of sampling a true range for the population, meaning the sample size did not truly represent the diversity within the population. However, with 70 animals captured over a wide area (Figure 1), this was unlikely to be a concern.

The black bear population in Maine is considered to be healthy and stable with a population size of approximately 23,000 animals. It is improbable then to assume that genetic drift is responsible for any deviation with the HWE. Based on the levels of heterozygosity below, it can be reasonably assumed that random mating is occurring in the population with respect to these genetic markers.

Heterozygosity

The level of heterozygosity within a population can indicate the occurrence of inbreeding. In this study low levels of heterozygosity were not observed within the population consistent with the conclusion that inbreeding within the population is minimal.

DNA wildlife forensics significance

The genetic information presented here in the allelic frequency database will be very useful in the processing of wildlife forensic cases for Maine authorities. In forensic

cases, often, two or more samples of unknown origin are presented. Genetic analysis is done on the samples to determine if they are different, or if they share the same genotype.

If two unknown samples differ from each other by one or more alleles, it can be asserted that they do not come from the same individual; this is known as exclusion. For example, bear blood is found atop a person's boot and poached bear meat is found in a freezer. The two samples are genotyped and produce the following results:

Genotype of bear blood:

G10B 153/157, **G10L** 148/150, **G10A** 181/185, **G10D** 171/173, **G10H** 259, 286

Genotype of bear meat:

G10B 153/157, **G10L** 148/150, **G10A** 185/187, **G10D** 171/173, **G10H** 259, 286

Since these two genotypes differ at locus G10A with one different allele they do not match and cannot be from the same animal. Exclusion shows that the person whose boots were examined did not have the poached bear's blood on them, meaning the person cannot be linked to the poaching incident through the bloody boot. Determining exclusion does not rely upon knowledge of population genetic structure but rather on the accurate processing and analysis of genetic information from the samples.

If the samples share the same alleles at each locus tested, then the samples may or may not have come from the same individual. However, it cannot be asserted with 100% certainty that two samples with identical genotypes are from the same individual. The existence of an allelic frequency database permits determination of the significance of a match occurring by chance alone. The probability that the same multi-locus genotype

occurring in two separate individuals picked at random from the population i.e., inclusion, can be estimated if the frequencies at which the alleles occur are known for the population in general.

Inclusion

If the probability of a random match of several alleles is low and the alleles of the different samples are found infrequently in the population, then it would be unusual to find two bears in the population that share the same genotype at the loci studied. If, on the other hand, the probability for a match is high then it is can be concluded that many individuals within the population may share the same genotype. If two samples share the same alleles at all the loci examined, then depending upon allele frequencies, it can be said with a reasonable amount of certainty that the two samples are possibly from the same individual.

The Third Law of Probability states that the probabilities associated with independent events may be multiplied together to determine the overall probability of the events occurring simultaneously. If the alleles observed were inherited independently, then the probability of finding an individual in the population with a certain genotype would equal the product of the frequencies of the genotypes in the population in accord with the Hardy-Weinberg Law. The probability of observing two different alleles in an individual at the same locus is given by 2pq (where p and q are the frequencies of the two alleles in the population), when the individual is a heterozygote at a given locus. A homozygote is an individual who expresses two identical alleles at a given locus; the probability of observing them in the population is p^2.

For example, say a poached bear is found in the Maine woods and bear meat is found in a suspect's freezer; samples from both are sent to the University of Maine Molecular Forensics Laboratory. The two samples are genotyped at the 5 loci that have been characterized. If the two samples are found to have different genotypes then it can be concluded that the two samples are not from the same individual. If the genotypes are identical, then the significance of this match can be calculated.

The significance of a match can be calculated as in the following example. Assume that the two bear samples have been found to have the same multilocus genotype:

G10B 153/157, **G10L** 148/150, **G10A** 181/185, **G10D** 171/173, **G10H** 259/286

The frequency of each allele occurring in the Maine black bear reference population will be taken from the available database. Using Table 2, the frequencies of alleles within the population are:

G10B 153= 0.1812, 157= 0.1159

G10L 148= 0.0942, 150= 0.1232

G10A 181= 0.1449, 185= 0.2826

G10D 171= 0.3261, 173= 0.2101

G10H 259= 0.1567, 286= 0.0075

The probabilities each genotype occurring in the population are then multiplied together to provide a probability that an individual bear in Maine could have all five genotypes. In this example, the individual is a heterozygote for all five loci, so the probability calculation is: $2pq*2pq*2pq*2pq*2pq$, or $2(0.1812)(0.1159)*2(0.0942)(0.1232)*2(0.1449)(0.2826)*2(0.3261)(0.2101)*2(0.1567)(0.0075)$. The result, a

probability of 2.57 x 10^{-8}, is the probability of observing two individuals in the population with that genotype. Thus, it is expected that approximately 1/2.57 x 10^{-8}, or 1 out of 38,910,506 Maine black bear will have this genotype. Because this probability is very small and greatly exceeds the population size, it can be asserted that the two samples are likely from the same individual.

Multiple paternity

Known mother-offspring were genotyped in this study. With the information gathered about specific families, multiple paternity was investigated in families with offspring of two or more. No cases of polyandry or multiple paternity were observed in this study. One possible reason multiple paternity was not observed was because the number of family genotypes obtained was small. If more genotypes had been produced, the chances of observing multiple paternity in the population would have been greater.

CONCLUSION

The production of an allele frequency database for the Maine black bear population can aid in wildlife forensic cases across the State. The database will allow for the determination of the probability of a random match occurring between two samples in wildlife poaching cases. The existence of this database and the information provided in it will hopefully be a deterrent for future bear poaching cases in the State of Maine.

APPENDIX

Figure 3.
Multilocus genotypes for individuals in family 13 with pedigree.

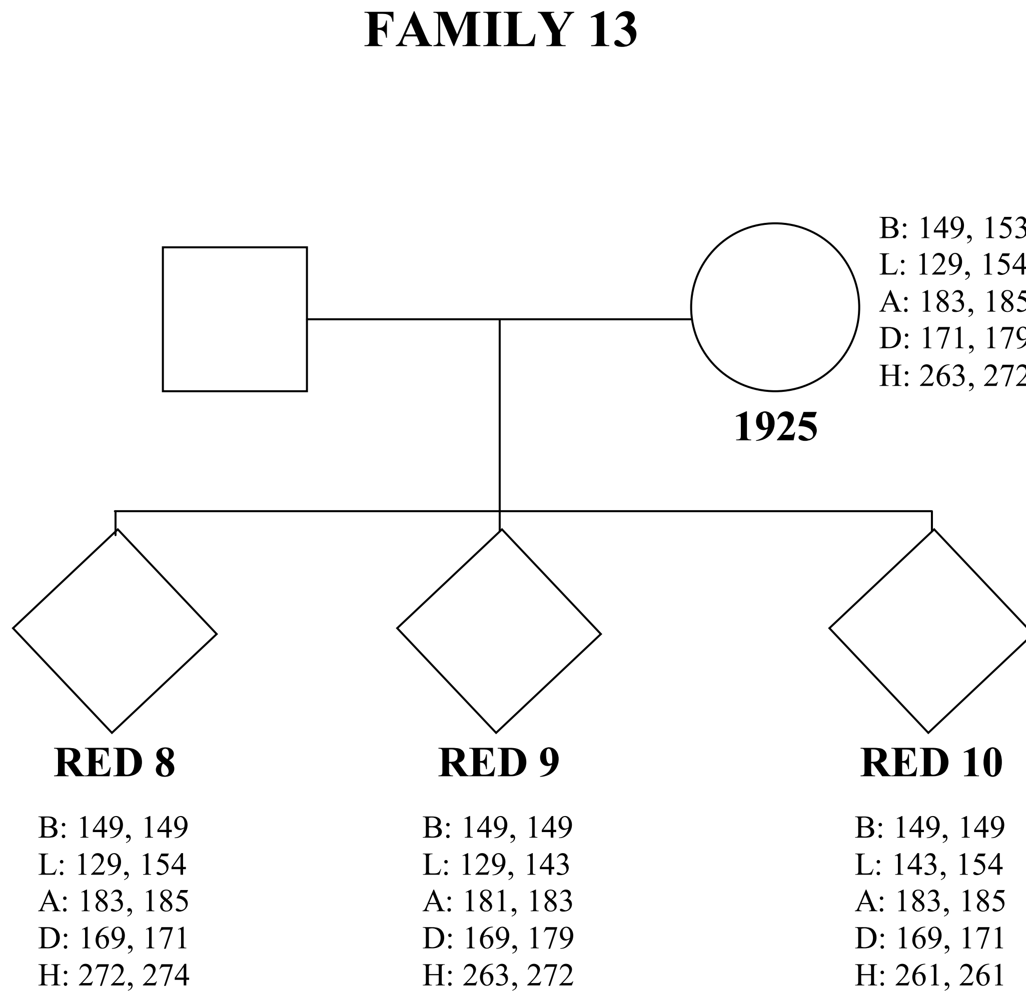
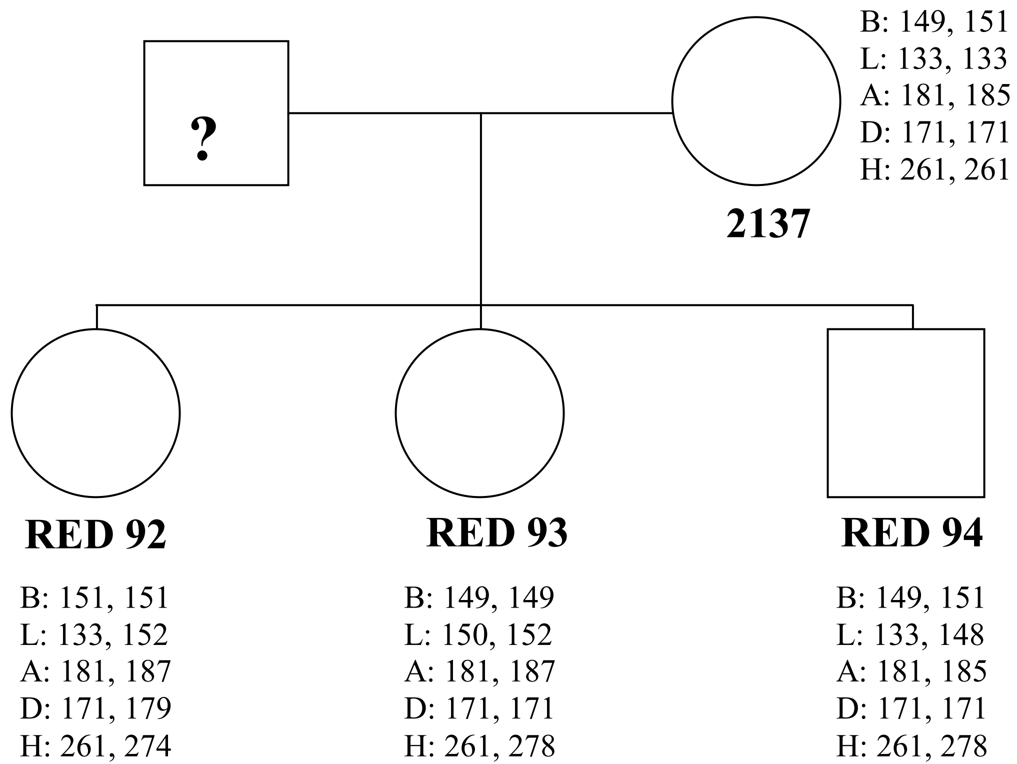
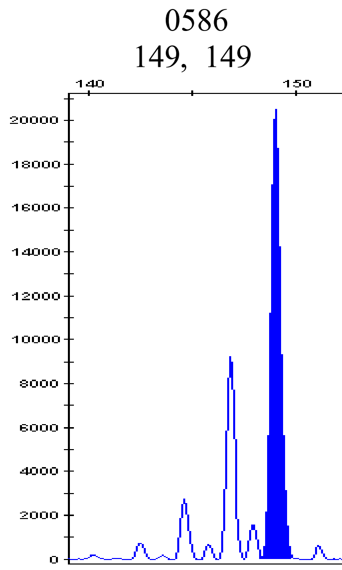


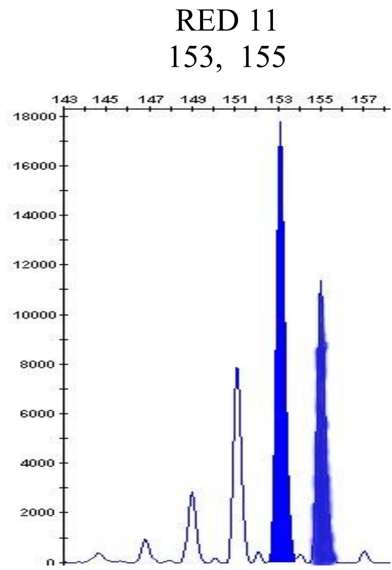
Figure 4.
Multilocus genotypes for individuals in family 1 with pedigree.

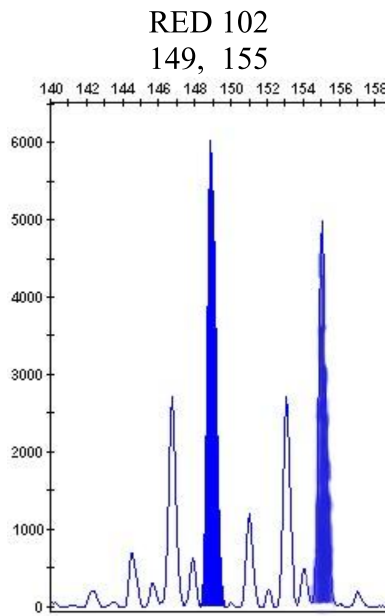


LOCUS B

Figure 5.
Chromatographs of typical allelic patterns for locus B.

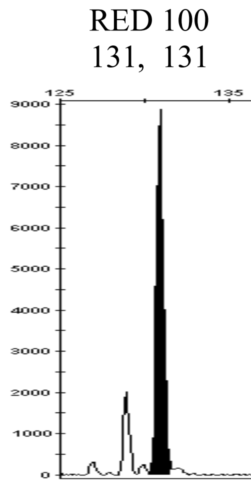


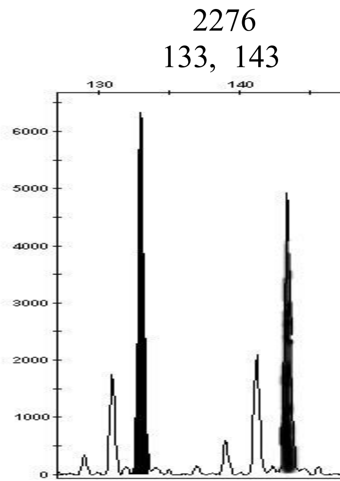


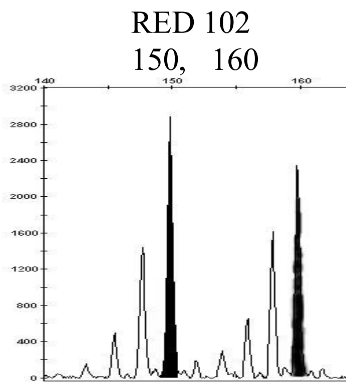


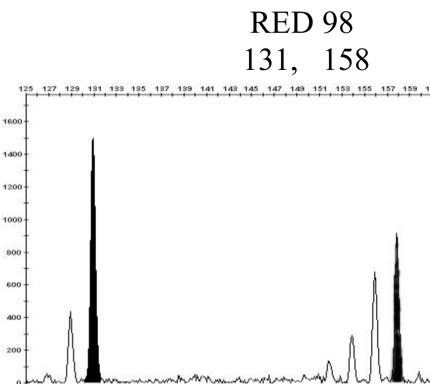
LOCUS L

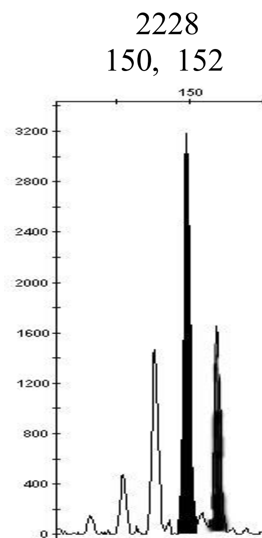
Figure 6.
Chromatographs of typical allelic patterns for locus L.





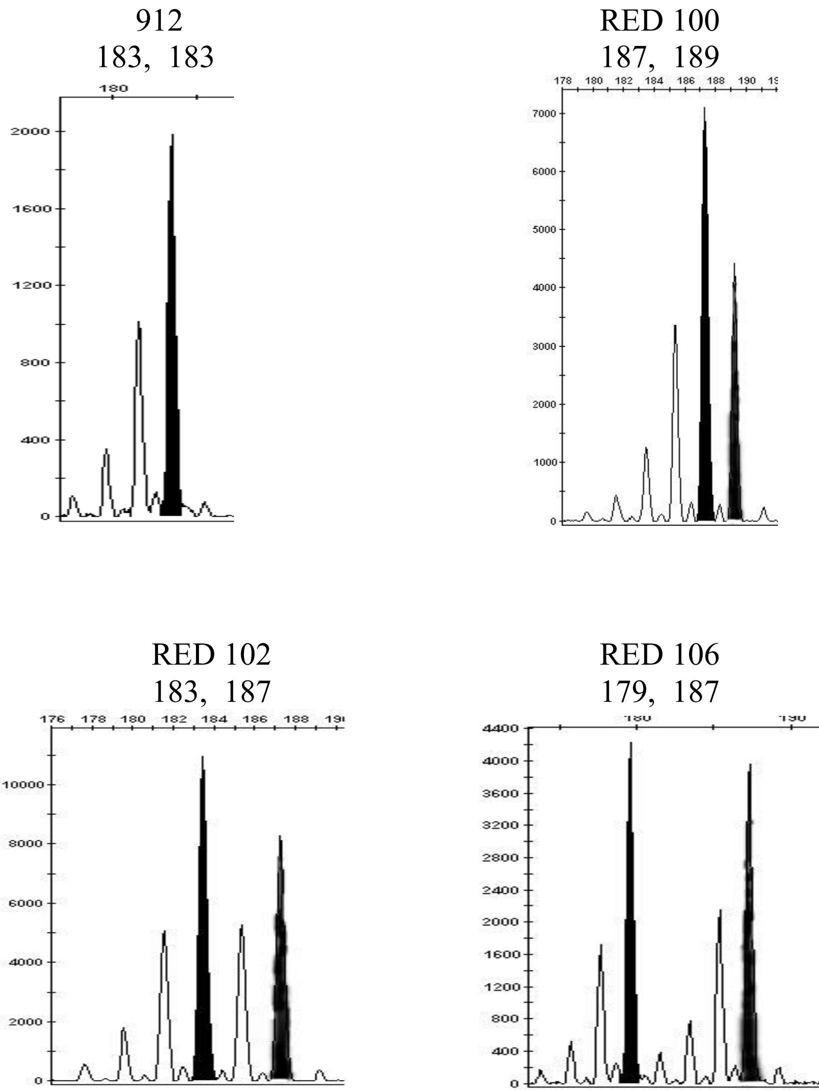






LOCUS A

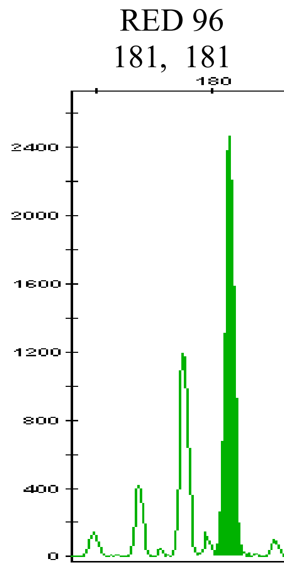
Figure 7.
Chromatographs of typical allelic patterns for locus A.



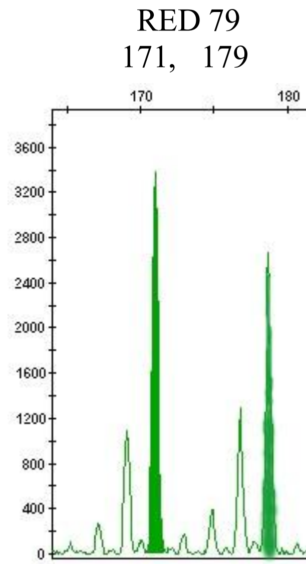
LOCUS D

Figure 8.
Chromatographs of typical allelic patterns for locus D.

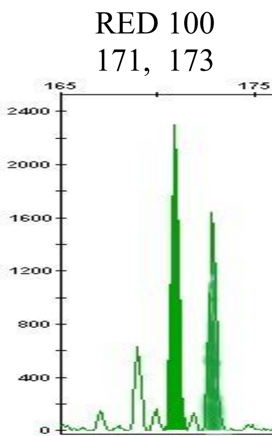
RED 96
181, 181



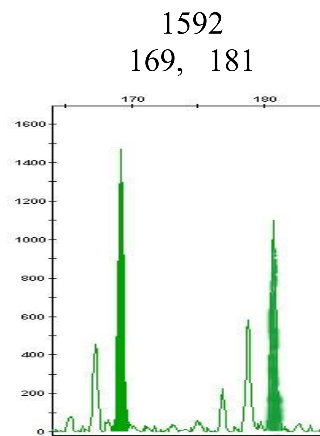
RED 79
171, 179



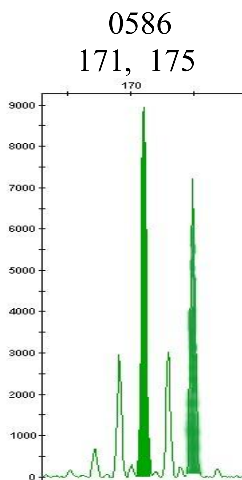
RED 100
171, 173



1592
169, 181

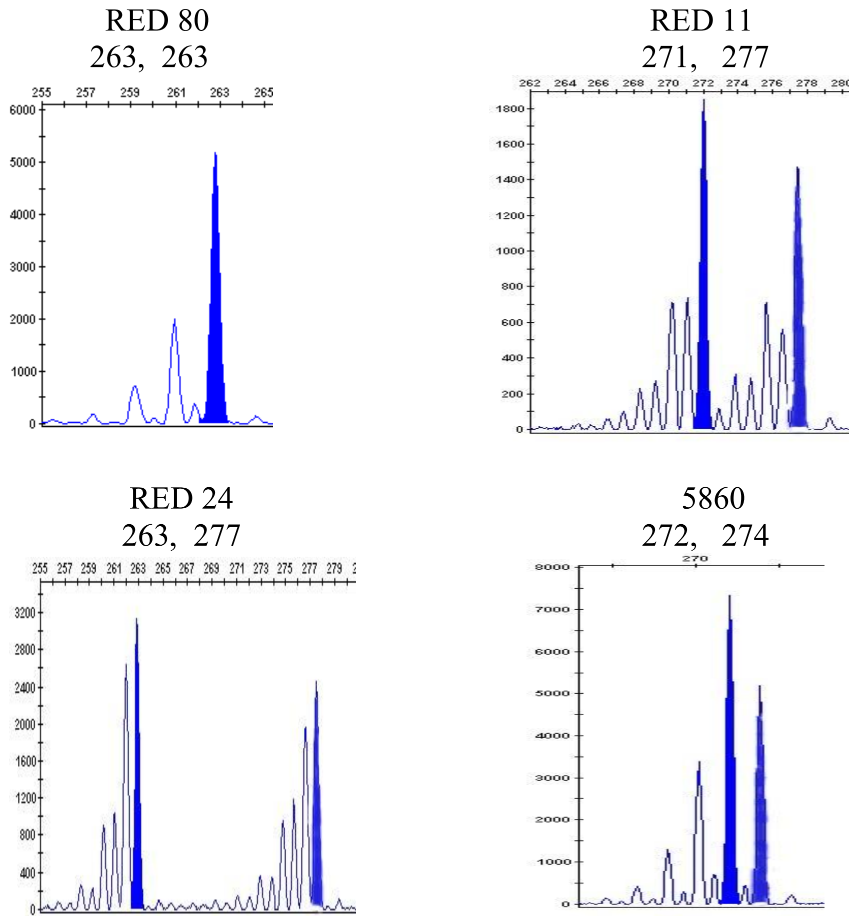


0586
171, 175



LOCUS H

Figure 9.
Chromatographs of typical allelic patterns for locus H.



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Amanda K. Thibodeau was born in Bangor, Maine on September 26, 1984. She was raised in Marshfield, Maine and graduated from Machias Memorial High School in 2003. Majoring in biology, Amanda has a minor in chemistry. She is a member of Alpha Zeta honors fraternity.

Upon graduation Amanda plans to spend a few years traveling the world before she heads to medical school to pursue a career in emergency medicine.