

# **An Assessment of the Genetic Status of the Feral Horse Population of Assateague Island National Seashore**

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## **Summary**

This report is divided into two sections. The first section provides the results of the molecular genetic analysis of 175 Assateague horses that were alive during 2004/2005 as well as one horse that had died previously. While 37 samples were obtained using biopsy darts, most horses (N=137) were represented by fecal samples. Mitochondrial DNA (mtDNA) haplotypes and nuclear genotypes at 8 polymorphic loci were obtained and used to assess the level and distribution of genetic diversity within the herd. Previously described maternal pedigrees were verified and amended, and the affinities of most of the members of the X lineages were inferred. Once maternal pedigrees were confirmed and established, the computer program PARENTE was used to infer paternities for as many of the horses as possible. Finally, the breed affinity of the herd was examined using both mitochondrial and nuclear markers. As in previous studies, mtDNA results do not seem to be closely associated with breed, but the preliminary microsatellite analysis with a small number of loci suggest moderate levels of genetic differentiation from other established breeds that have been screened with the same microsatellite markers.

The second section analyzes the genetically-verified pedigree data from a demographic and population genetics perspective. The data are used to create a studbook for the population, to examine age structure for both males and females, and to estimate the rate of loss of genetic diversity. Examination of the relative contributions of the “founders” revealed that each was reasonably well represented in today’s herd, in agreement with the genetic results, which revealed a relatively high level of nuclear genetic diversity. Preliminary analyses in the computer program Population Management 2000 illustrate the potential effects of culling on the genetics and demography of the Assateague horse population and suggest that demographic considerations be seriously considered when selecting individuals for removal.

## **Introduction**

The feral horses of Assateague Island National Seashore (ASIS) are, perhaps, the park's most well known natural resource. Thousands of visitors are attracted to Assateague each year for the opportunity to view free-roaming horses in a natural barrier island setting. While considered an exotic species, the National Seashore's General Management Plan directs that the horses be managed as a "desirable feral species" within the context of the park's overall purposes. When the National Seashore acquired ownership of the horses in 1968, the herd numbered approximately 28. Since then, the population has expanded dramatically, reaching a maximum of 179 horses in 2002.

This dramatic increase in the horse population has created a series of negative effects on other natural resources in the area. Research has demonstrated that horses significantly influence the distribution and abundance of many native plant species, and can affect the dynamics of plant community composition. Over-grazing has also been shown to impact the physical integrity of sensitive dune features and marshlands through trampling and elimination of soil binding vegetation. Although the horses are an important part of the Assateague visitor experience, there is a pressing need to manage the population in ways that will both provide for the long-term health and viability of the herd as well as minimize adverse impacts to other park resources and values.

Achieving this balance will involve a wide range of management actions; some of which may entail increased risks to the horse population. For example, evidence suggests that the park can support between 110 and 125 horses with minimal resource impact. A proposed reduction of herd size would undoubtedly reduce grazing impacts, but may also increase risk to the horses from inbreeding. Neither is the current program of fecundity control using immuno-contraceptives without risk. The management of the ASIS horse population prior to 1968 has been poorly documented. It is believed that the island population has been subject to repeated introductions of new stock and removals of existing horses over time; a practice that is continuing today with the Chincoteague herd. However, the ASIS population after 1968 has expanded from 28 founders in the past four decades and any action that ultimately reduces genetic diversity has the potential to increase susceptibility to small population effects. Unfortunately, until now little scientific information was available to assess the genetic status of the horse population or assess the potential for future problems under alternative management scenarios.

The purpose of this project was to develop information describing the genetic status and condition of the Assateague feral horses. The results will be used to support appropriate long-term management of the horses, including potential actions to reduce the size of the population, manage reproductive rates, and enhance the long-term health and viability of the herd.

## **Part 1. Molecular Genetic Analysis of the Assateague Horse Population**

A total of 176 individuals were sampled by National Park Service personnel and volunteers. An initial set of 30 skin biopsies were collected for optimization and verification of the genetic markers that were chosen for the study. Fecal samples were then collected from the remaining living horses. As in many studies that use feces as a DNA source, we found that some fecal samples did not yield amplifiable DNA. Seven of those samples were re-collected as skin biopsies, and new fecal samples were collected for the others. When DNA sequencing revealed that one of the maternal lineages (T5) appeared to include multiple mtDNA haplotypes, a bone from a dead horse that belonged to the original T5 lineage was located and used to obtain her DNA to establish which haplotype characterized the original T5 lineage.

Total genomic DNA was extracted from all skin biopsy samples using the tissue protocol of the QIAGEN DNeasy™ DNA extraction kit (Qiagen Inc., Valencia, CA). Fecal samples were extracted using the Guanadine Thiocyanate/Silica method of Eggert *et al.* (2005). All extractions were performed in a laboratory devoted only to DNA extractions, and fecal samples were carefully extracted using established protocols described for non-invasively collected samples (Eggert *et al.* 2005).

### **Genetic Markers**

In mammals, the mitochondrion is passed from mother to all of her offspring in the egg. This organelle has its own genome (mtDNA), which can be used to investigate maternal lineages. We used the primers of Vilà *et al.* (2001) to amplify 355 base pairs of the left domain of the highly variable mtDNA control region. Sequences of the amplification products were obtained using the ABI BigDye ready reaction kit (Applied Biosystems), and detected in a capillary DNA sequencer (3100 Model; Applied Biosystems, Foster City, CA.).

To investigate the level of nuclear genetic variability and infer parentage, we chose to use simple tandem repeat, or microsatellite, loci. These loci are highly variable and are characterized by a series of repeats (eg. CACACACACACACACACA) flanked by conserved sequences that can be used to design amplification primers. We initially used the StockMarks® for Horses Equine Genotyping Kit (Applied Biosystems, Valencia, CA.) to screen 25 matched tissue and dung samples at 13 microsatellite loci that are commonly used in horse pedigree analysis. We found that four loci were both reliable and informative (VHL20, HTG4, HTG6, and HTG7). Five were found to be only marginally informative and unreliable on fecal samples. The final four were informative in biopsy samples, but had high rates of allelic dropout in fecal samples. Previous studies in our lab have shown that fragment size of the microsatellite locus has a direct effect on its amplification rates, particularly when trying to amplify DNA extracted from feces (i.e. the larger the locus the lower the amplification rates and reliability). Therefore, we downloaded from GenBank the published sequences of the clones used to design the original published primers of these four loci and redesigned new primers using a shorter flanking region that would amplify the same microsatellite tandem repeat region. Our new redesigned primers amplified fragment

sizes that are 39-68 base pairs shorter than the originally published fragment size of those four microsatellite loci (Table 1).

**Table 1.** Redesigned microsatellite loci used in this study.

| <b>Original Locus</b> | <b>Original Size (bp)</b> | <b>Redesigned Locus</b> | <b>New Size (bp)</b> | <b>Primer sequences</b>                                  |
|-----------------------|---------------------------|-------------------------|----------------------|----------------------------------------------------------|
| ASB2                  | 180                       | ASB2R                   | 112                  | F: ACTCTTTGGGATCTCCTTCC<br>R: GACTCTTAGCTAATGTTCCCAATG   |
| HMS3                  | 169                       | HMS3R                   | 130                  | F: TGTGATTCATAAAGGGGATGG<br>R: TTGTCACATAACAAGAAAACAGCTT |
| HMS6                  | 169                       | HMS6R                   | 118                  | F: TTCTTCAAATCAGAAACCCATATAGA<br>R: CCCAGCTCCATCTTGTGAAG |
| HMS7                  | 182                       | HMS7R                   | 116                  | F: CCCAGACGTGACAAAATTGC<br>R: CCATCCACCAATCTTATTTGG      |

All microsatellite analyses were performed on two extractions per sample, and results were scored after comparison with the sizes of the alleles in the ISAG approved control sample from the StockMarks® kit. This control sample is used to standardize allele sizes for paternity analyses in horses worldwide. Two Assateague horse biopsy samples were calibrated against that control sample, and thereafter those samples were used as size controls in all microsatellite reactions, along with the appropriate negative controls to detect contamination. Allele sizes were determined in an automated sequencer (ABI 3100, Applied Biosystems, Foster City, CA.) using fluorescently labeled primers and GeneScan technology (Applied Biosystems, Foster City, CA.).

### Data analyses

Sequences of the mtDNA control region were aligned and analyzed using Sequencher™ 4.5 for Macintosh (GeneCodes Corp., Ann Arbor, MI.). The mtDNA haplotypes found in this study were compared to the haplotypes included in the horse phylogenetic study of Vilà *et al.* (2001). Phylogenetic analysis of control region sequences was performed using the neighbor joining algorithm in PAUP\* 4.90b1.2 (Sinauer Assoc. Inc., Sunderland, MA.). For the neighbor joining analysis, we selected the same model of substitution determined by Vila *et al.* 2001 that best fit the data (Tamura and Nei model, assuming a proportion of invariable sites of 0.52 and a gamma rate heterogeneity of 0.76).

Nuclear microsatellite results were analyzed in GenePop using GenePop on the Web (<http://wbiomed.curtin.edu.au/genepop/>). That program allowed us to test for deviations from expectations under Hardy-Weinberg equilibrium and linkage disequilibrium between loci. It was also used to determine the number of alleles per locus as well as observed and expected heterozygosity values.

One way to quantify the number of loci needed to differentiate between individuals, in this case different potential sires, is a measure known as the probability of identity. We used Prob-id5, a program designed and provided by Lisette Waits of the University of Idaho (Waits *et al.* 2001). This program computes  $P(ID)_{\text{random}}$ , the power to differentiate between

randomly chosen individuals, as well as a more conservative measure,  $P(ID)_{sib}$ , the power to differentiate between siblings. The results of the more conservative test were used to determine the power of the loci used in this study (Waits *et al.* 2001). Paternity analyses were performed using PARENTE (Cercueil *et al.* 2002).

Phylogenetic analysis of the nuclear microsatellite data was performed using the computer program POPULATIONS 1.2.28, available on the web (<http://www.pge.cnrs-gif.fr/bioinfo/populations/>) using the shared allele distance, DAS, described by Jin and Chakraborty (1993). For this analysis, data from 20 Assateague horses genotyped from biopsy samples were combined with that of Vilà *et al.* (2001).

### Mitochondrial DNA sequence analysis

Within the Assateague population, we found three mitochondrial DNA lineages, or haplotypes, defined by twelve polymorphic bases in the sequence (Figure 1).

|           | Base # |    |    |     |     |     |     |     |     |     |     |     |
|-----------|--------|----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Haplotype | 47     | 51 | 78 | 159 | 207 | 223 | 277 | 294 | 327 | 364 | 367 | 383 |
| A         | C      | T  | G  | C   | A   | G   | G   | T   | C   | C   | A   | A   |
| B         | T      | C  | G  | C   | G   | A   | A   | T   | C   | T   | A   | G   |
| C         | C      | C  | A  | T   | A   | G   | A   | C   | T   | C   | G   | A   |

**Figure 1.** Polymorphic bases and their positions in 355 bp. of the left domain of the mtDNA control region in Assateague horses.

These haplotypes were mapped onto the presumed maternal pedigrees for all horses in the study. We would expect that, within a maternal lineage, all horses would have the same haplotype, but we found a few instances in which horses from one lineage had haplotypes that matched those of other lineages. This result could be explained in several ways:

- 1) In the field, a fecal sample from one horse could have been mistaken for that of another horse within the same band.
- 2) The presumed pedigree contained errors.

Samples were collected a second time for all horses involved in these discrepancies and this helped us determine that we had encountered both field misidentification and pedigree errors. The few errors in sample collection were resolved, and the proposed revised pedigrees are depicted in Appendix 3.

The three mtDNA haplotypes are very different: Haplotype A differs from Haplotype B at 7 positions, A differs from C at 7 positions, and B differs from C at 10 positions (Figure 1). The most common is Haplotype A, which was found in 124, or 71% of the horses we examined. Haplotypes B and C were much less common, and were found in 35 (20%) and 16 (9%) of the horses, respectively (Table 5). The Vilà *et al.* study showed that mitochondrial DNA diversity within breeds is high. They documented that, on average, 7.4 haplotypes occurred per breed (range = 3 - 9), and that the nucleotide diversity per breed averaged 0.022 (range = 0.012 to 0.027), comparable to that found in large wild ungulate populations. Because their study was based on approximately 20 horses per breed, we randomly selected 20 Assateague horses for comparison. In that sample, we found all three

mtDNA haplotypes and a nucleotide diversity value of  $0.011 \pm 0.006$ . This suggests that the levels of mitochondrial DNA diversity observed in the Assateague herd are low in comparison to those found in other established breeds.

Phylogenetic analysis of the three haplotypes compared with the haplotypes of breeds found worldwide from Vilà *et al.* (2001) showed that they do not cluster together, but appear to be related to haplotypes found in several established breeds (Figure 2). This is not surprising, given that Vilà *et al.* (2001) found that mtDNA haplotype was not closely associated with breed. These results suggest that the three Assateague haplotypes did not arise on the island within the past few hundred years, but were brought in from elsewhere by the founders of this population. Our results support the hypothesis that domestic horse populations were founded by a diversity of matrilineal lines that, as suggested by the archaeological record, was augmented by trade

Although the initial founding of the Assateague horse population may have involved incorporation of multiple (at least three) matrilineal lines, because of the lack of documentation it is difficult to establish if some of these matrilineal lines were the result of introduction events that occurred later in their history or if there were more original matrilineal lines that have been lost.

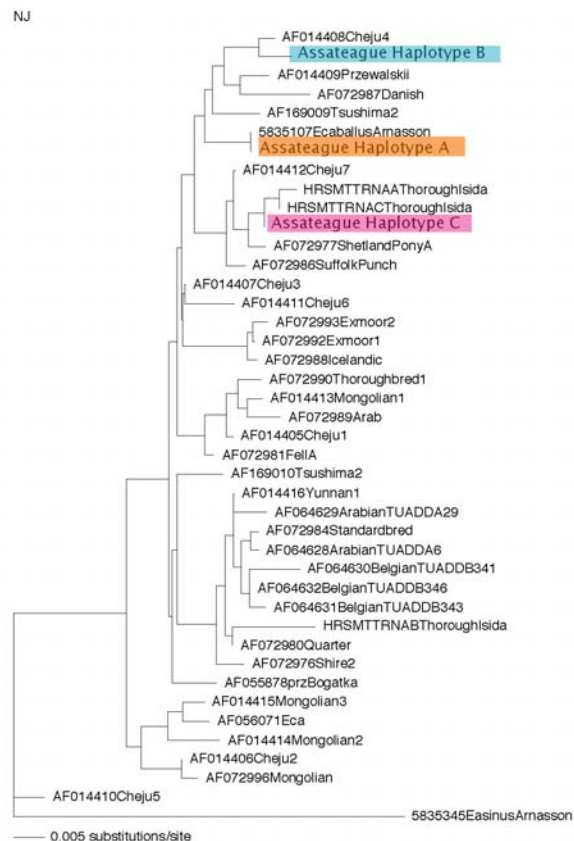


**Figure 2.** Phylogenetic analysis of the mtDNA haplotypes of the Assateague horses with respect to the 10 established horse breeds and horses from the Orient analyzed in Vilà *et al.* (2001).

### Nuclear microsatellite analysis

Of the 175 horses, 115 were genotyped successfully at all eight loci, 24 were genotyped at 7 loci, 20 were genotyped at 6 loci, and 12 were genotyped at 5 loci. In total, 98% of the samples were successfully genotyped and only four samples could not be genotyped successfully at enough loci to be included in this analysis.

With one exception (locus HTG6), all of the loci screened in this study were in Hardy-Weinberg equilibrium (Table 2). Locus HTG6 had an excess of heterozygotes, which was surprising since most studies using fecal DNA suffer from problems of allelic drop-out (alleles that do not amplify due to degraded and low copy number nuclear DNA). We have re-checked the scoring of this locus and confirmed its reliability. Since the slight excess of heterozygotes will not affect our ability to assess the genetic variability of the population and will enhance our ability to assess parentage, we have retained locus HTG6 in the study.

Despite the small number of mtDNA haplotypes, the Assateague horse population appears to have retained significant nuclear DNA diversity. The levels of allelic diversity and heterozygosity are high with a mean of 7.4 alleles per locus, and an average  $H_o = 0.704$ . This suggests that the nuclear DNA diversity for the population as a whole is relatively high (Table 2).

**Table 2.** Population level genetic diversity measures.

| Locus | No. Alleles | H observed | H expected |
|-------|-------------|------------|------------|
| ASB2R | 10          | 0.759      | 0.825      |
| HTG4  | 5           | 0.682      | 0.741      |
| HTG6* | 5           | 0.861      | 0.653      |
| HTG7  | 8           | 0.514      | 0.541      |
| HMS3R | 9           | 0.762      | 0.713      |
| HMS6R | 7           | 0.673      | 0.730      |
| HMS7R | 8           | 0.645      | 0.625      |
| VHL20 | 7           | 0.738      | 0.732      |
| AVG   | 7.4         | 0.704      | 0.695      |
| SD    | 1.8         | 0.102      | 0.086      |

The levels of allelic diversity in our study are higher than the diversity of worldwide breeds found in Vilà *et al.* (2001) (7.4 vs. 4.5). However, their study did not include populations in which all of the individuals were sampled and thus, not all of the rare alleles were detected. A better comparison is between the sample of 20 individuals from our study and the breeds included in the Vilà *et al.* (2001) study. When the results of 20 biopsy samples are examined, we find that the average of 4.9 alleles per locus is comparable to the 4.5 alleles found in the Vilà *et al.* (2001) study.

In addition, when examined separately, each maternal lineage contains comparable allelic diversity with a range from 3 to 6 alleles and an average of 3.8 alleles (Table 3). As we

might expect, allelic diversity appears to be correlated with the sample size of each lineage, i.e., X16 has the lowest values (2.3) and also the smallest sample size ( $n=3$ ) and N9 has the largest values (6.0) and the largest sample size ( $n=35$ ). Heterozygosity values are also comparable in most lineages with the exception of X16, again due to small sample size (Table 3).

**Table 3.** Allelic diversity and heterozygosity within maternal lineages.

| Lineage               | N  | Avg No Alleles | H observed | H expected |
|-----------------------|----|----------------|------------|------------|
| M2                    | 10 | 4.1            | 0.83       | 0.66       |
| M6                    | 8  | 3.0            | 0.60       | 0.55       |
| M17                   | 5  | 3.0            | 0.62       | 0.58       |
| N2                    | 12 | 3.4            | 0.74       | 0.64       |
| N6                    | 29 | 5.0            | 0.71       | 0.69       |
| N9                    | 35 | 6.0            | 0.72       | 0.72       |
| N10                   | 9  | 4.0            | 0.68       | 0.65       |
| T3                    | 14 | 4.0            | 0.65       | 0.63       |
| T5                    | 27 | 5.1            | 0.70       | 0.69       |
| T6                    | 14 | 4.0            | 0.68       | 0.63       |
| X16                   | 4  | 2.9            | 0.59       | 0.53       |
| Single horse lineages | 8  | 3.8            | 0.75       | 0.68       |

The distribution of rare microsatellite alleles, however, is not random (Table 4). This may be an important factor be considered in management planning of the population.

Microsatellite loci, especially dinucleotide loci such as those used in this study, are generally found in non-coding regions of the genome and are unlikely to affect the fitness of the individual. Here we view them as proxies for the genome as a whole, making the assumption that lineages that contain significant numbers of rare microsatellite alleles may also harbor significant numbers of rare alleles at loci that are likely to affect fitness. These results demonstrate that, while mtDNA haplotype C is the rarest, the horses of that lineage (all in the proposed T6 lineage) may not carry the rarest nuclear alleles. As we might expect, some of the lineages with the most horses (N6, N9, T5) also appear to contain individuals that harbor rare microsatellite alleles, a factor that may need to be considered in management planning.

**Table 4.** Distribution of rare microsatellite alleles. Individuals are coded by mtDNA haplotype (orange=A, blue=B, pink=C), and for each lineage the number of individuals with each of the rare alleles are shown. See appendix 4 for a list of the individuals included in this table.

| LINEAGE |        |      | M2 | M6 | M17 | N2 | N6 | N9 | N10 | T3 | T5 | T6 | X16 |
|---------|--------|------|----|----|-----|----|----|----|-----|----|----|----|-----|
| N       |        |      | 10 | 8  | 5   | 12 | 29 | 35 | 9   | 14 | 27 | 14 | 3   |
| LOCUS   | Allele | Freq |    |    |     |    |    |    |     |    |    |    |     |
| VHL20   | 98     | 0.01 |    |    |     |    |    | 1  | 1   |    | 1  |    |     |
| HMS6R   | 106    | 0.04 |    |    |     |    | 6  | 5  | 2   |    | 1  |    |     |
| HTG7    | 122    | 0.01 | 1  |    |     |    |    |    |     |    |    |    |     |
| HTG7    | 124    | 0.03 |    |    |     |    |    | 1  | 1   |    | 1  |    |     |
| HMS7R   | 106    | 0.03 |    | 1  |     |    | 3  | 6  |     |    | 3  | 2  |     |
| HMS7R   | 110    | 0.01 |    | 1  |     |    |    | 2  |     | 1  | 2  |    |     |
| HMS7R   | 114    | 0.01 |    |    | 1   |    |    | 1  |     |    |    |    |     |
| HMS3R   | 110    | 0.01 |    |    |     |    | 1  | 4  |     |    |    |    |     |

Results of paternity analyses were derived from several sources and listed in Table 5. Sires listed in the column labeled “genetically determined” were inferred using genetic data and confirmed by NPS personnel as being consistent with historic ranges and coat color inheritance. In some cases, the most likely sire is deceased and thus not available for genetic analyses. For those horses, the sire was inferred using NPS records along with consideration of coat color inheritance. The results of the probability of identity analysis indicated that the  $P(ID)_{\text{random}}$  for these loci is  $<0.00005$ , and the  $P(ID)_{\text{siblings}}$  is  $<0.002$ . This confirms that the loci we have selected for this study provide sufficient power to perform the paternity analyses with confidence.

**Table 5.** Results of paternity analyses.

| Sample # | Horse ID | Sex | mtDNA | Dam     | Sire                   | Sire(deceased)      | Notes        |
|----------|----------|-----|-------|---------|------------------------|---------------------|--------------|
|          |          |     |       |         | Genetically determined | inferred-other data |              |
| 1        | M12AG    | M   | A     | Unk     |                        |                     | Sire Unknown |
| 2        | M17G     | F   | A     | M17     |                        |                     | Sire Unknown |
| 3        | M17GJ    | F   | A     | 2       |                        |                     | Sire Unknown |
| 4        | M17GM    | F   | A     | 2       | 1(M12AG)               |                     |              |
| 5        | M17GMV   | F   | A     | 4       | 46(N4GM)               |                     |              |
| 6        | M17JP    | F   | A     | M17J    | 15(M2EK)               |                     |              |
| 7        | M2E      | F   | A     | M2      |                        |                     | Sire Unknown |
| 8        | M2EI     | F   | A     | M2(unk) |                        |                     | Sire Unknown |
| 9        | M2EIN    | F   | A     | 8       |                        | M7D                 |              |
| 10       | M2EINR   | F   | A     | 9       | 123(T5BHL)             |                     |              |
| 11       | M2EINR-B | F   | A     | 10      | 133(X11)               |                     |              |
| 12       | M2EINRY  | M   | A     | 10      | 133(X11)               |                     |              |
| 13       | M2EINS   | F   | A     | 9       |                        | M12AF               |              |
| 14       | M2EIQ    | F   | A     | 8       | 114(T5AEK)             |                     |              |
| 15       | M2EK     | M   | A     | 7       |                        | M7D                 |              |
| 16       | M2M      | M   | A     | M2      |                        | M7D                 |              |
| 17       | M4J      | F   | A     | M2      |                        | M1                  |              |
| 18       | M6I      | F   | B     | T5B     |                        | K1                  |              |
| 19       | M6IM     | F   | B     | 18      | 167(X6)                |                     |              |
| 20       | M6IMQ    | F   | B     | 19      | 1(M12AG)               |                     |              |
| 21       | M6IMQ-A  | M   | B     | 20      | 15(M2EK)               |                     |              |
| 22       | M6IMQV   | M   | B     | 20      | 15(M2EK)               |                     |              |

|    |        |   |   |     |           |            |                                          |
|----|--------|---|---|-----|-----------|------------|------------------------------------------|
| 23 | M6IMQX | M | B | 20  | 15(M2EK)  |            |                                          |
| 24 | M6IX   | F | B | 18  | 146(N4GM) |            |                                          |
| 25 | M6J    | M | B | M6  |           | K1         |                                          |
| 26 | M6K    | M | B | M6  |           | K1         |                                          |
| 27 | M6L    | F | B | M6  |           | K1 OR N6BF |                                          |
| 28 | M6LP   | M | B | 27  | 111(T3M)  |            |                                          |
| 29 | M6M    | F | B | M6  |           | N6BF       |                                          |
| 30 | M6MQ   | M | B | 29  |           | M6CG       |                                          |
| 31 | M6MS   | F | B | 29  |           | M6CG       |                                          |
| 32 | M6MSY  | M | B | 31  | 111(T3M)  |            |                                          |
| 33 | N10    | F | A | Unk |           |            | Sire Unknown                             |
| 34 | N10E   | F | A | 33  |           | N1         |                                          |
| 35 | N10ET  | F | A | 34  |           | X10        | X10 is not deceased (he was transferred) |
| 36 | N10O   | F | A | 33  |           | N6D        |                                          |
| 37 | N10OS  | M | A | 36  |           | N9CH       |                                          |
| 38 | N10OY  | M | A | 36  | 61(N6EHP) |            |                                          |
| 39 | N10R   | F | A | 33  |           | N6D        |                                          |
| 40 | N10T   | F | A | 33  |           | N6D        |                                          |
| 41 | N2B    | F | A | Unk |           |            | Sire Unknown                             |
| 42 | N2BM   | F | A | 41  |           | N2E        |                                          |
| 43 | N2BMR  | M | C | 149 | 25(M6J)   |            |                                          |
| 44 | N2BQ   | M | A | 41  |           | K1         |                                          |
| 45 | N2BR   | F | A | 41  | 105(T3F)  |            |                                          |
| 46 | N4GM   | M | A | Unk |           |            | Sire Unknown                             |
| 47 | N6BI   | F | B | T5B |           |            | Sire Unknown                             |
| 48 | N6BIQ  | F | B | 47  |           | N10D       |                                          |
| 49 | N6BIR  | F | B | 47  |           | N10D       |                                          |

|    |         |   |   |       |            |              |
|----|---------|---|---|-------|------------|--------------|
| 50 | N6BIR-B | M | B | 49    | 103(T3DMT) |              |
| 51 | N6BIRU  | F | B | 49    |            | N10D         |
| 52 | N6BIRUY | F | B | 51    | 136(X13P)  |              |
| 53 | N6BIRY  | F | B | 49    | 111(T3M)   |              |
| 54 | N6BK    | F | A | N6B   |            | M8           |
| 55 | N6BKO   | F | A | 54    |            | M8           |
| 56 | N6BKOS  | F | A | 55    |            | X10          |
| 57 | N6BP    | F | A | N6B   |            | M8           |
| 58 | N6BS    | M | A | N6B   |            | M2I          |
| 59 | N6E     | F | A | N6    |            | N1           |
| 60 | N6EH    | F | C | 113   |            | M8           |
| 61 | N6EHP   | M | C | 60    |            | N4E          |
| 62 | N6EL    | F | A | 59    |            | K1           |
| 63 | N6ELP   | M | A | 62    |            | K1           |
| 64 | N6ELS   | F | A | 62    | 135(X13N)  |              |
| 65 | N6EM    | F | A | 59    |            | K1           |
| 66 | N6EMS   | F | A | 65    | 135(X13N)  |              |
| 67 | N6EMSZ  | F | A | 66    | 136(X13P)  |              |
| 68 | N6G     | F | A | N6    |            | Sire Unknown |
| 69 | N6GP    | M | B | 156   |            | N4E          |
| 70 | N6GR    | M | A | 68    |            | N4E          |
| 71 | N6GS    | M | A | 68    |            | N4E          |
| 72 | N6H     | F | A | N6    |            | N1           |
| 73 | N6KR    | M | A | N6K   | 25(M6J)    |              |
| 74 | N9BF    | F | A | N9B   |            | N1           |
| 75 | N9BFNS  | F | A | N9BFN |            | N4E          |
| 76 | N9BFNSZ | M | A | 75    | 136(X13P)  |              |

|     |        |   |   |       |           |      |              |
|-----|--------|---|---|-------|-----------|------|--------------|
| 77  | N9BFNY | M | A | N9BFN |           | N2BO |              |
| 78  | N9BFOY | M | A | N9BFO | 126(T5Q)  |      |              |
| 79  | N9BFP  | M | A | 74    |           | N6D  |              |
| 80  | N9BFQ  | F | A | 74    |           | N6D  |              |
| 81  | N9BFQX | F | A | 80    |           | N4E  |              |
| 82  | N9BFT  | F | A | 74    |           | N6D  |              |
| 83  | N9BFTZ | F | A | 82    |           | N4E  |              |
| 84  | N9BFV  | F | A | 74    | 157(X22P) |      |              |
| 85  | N9BJN  | F | A | N9BJ  |           | N4E  |              |
| 86  | N9BLQ  | M | A | N9BL  | 25(M6J)   |      |              |
| 87  | N9BM   | F | A | N9B   |           | N4E  |              |
| 88  | N9BMR  | M | A | 87    | 168(X7)   |      |              |
| 89  | N9BN   | F | A | N9B   |           | N4E  |              |
| 90  | N9BNT  | M | A | 89    |           | N9CH |              |
| 91  | N9BNZ  | M | A | 89    | 69(N6GP)  |      |              |
| 92  | N9BO   | F | A | N9B   |           | N4E  |              |
| 93  | N9BO-A | F | A | 92    | 61(N6EHP) |      |              |
| 94  | N9BOX  | M | A | 92    |           | N4E  |              |
| 95  | N9BQ   | M | A | N9B   |           | N4E  |              |
| 96  | N9E    | F | A | N9    |           |      | Sire Unknown |
| 97  | N9EL   | M | A | 96    |           |      | Sire Unknown |
| 98  | N9H    | M | A | N9    |           | K1   |              |
| 99  | T2BE   | F | C | Unk   |           | M9   |              |
| 100 | T3DH   | F | A | T3D   |           | K1   |              |
| 101 | T3DHT  | F | A | 100   |           | N10G |              |
| 102 | T3DM   | F | A | T3D   |           | K1   |              |
| 103 | T3DMT  | M | A | 102   | 125(T5L)  |      |              |

|     |       |   |   |      |          |                                               |              |
|-----|-------|---|---|------|----------|-----------------------------------------------|--------------|
| 104 | T3DMY | M | A | 102  | 26(M6K)  |                                               |              |
| 105 | T3F   | M | A | T3   |          | K1                                            |              |
| 106 | T3K   | F | A | T3   |          | K1                                            |              |
| 107 | T3KR  | F | A | 106  | 168(X7)  |                                               |              |
| 108 | T3KV  | M | A | 106  | 168(X7)  |                                               |              |
| 109 | T3L   | F | A | T3   |          |                                               | Sire Unknown |
| 110 | T3LV  | F | A | 109  | 167(X6)  |                                               |              |
| 111 | T3M   | M | A | T3   |          | M6CG                                          |              |
| 112 | T3Q   | F | A | T3   |          | M6CG                                          |              |
| 113 | T5A   | F | C | T6   |          |                                               | Sire Unknown |
| 114 | T5AEK | M | B | T5BH |          | M9                                            |              |
| 115 | T5AF  | F | A | N9   |          |                                               | Sire Unknown |
| 116 | T5AFP | F | A | 115  |          | M8                                            |              |
| 117 | T5AFS | M | A | 115  |          | X10                                           |              |
| 118 | T5AG  | F | A | N9   |          |                                               | Sire Unknown |
| 119 | T5AGS | M | A | 118  |          | N10G                                          |              |
| 120 | T5AP  | F | C | 113  |          | M8                                            |              |
| 121 | T5APS | M | C | 120  |          | M2I or X10<br>X10 or stray refuge<br>stallion |              |
| 122 | T5AR  | F | C | 113  |          |                                               |              |
| 123 | T5BHL | M | B | T5BH |          | M9                                            |              |
| 124 | T5BQ  | M | B | T5B  |          | M6CG or N10D                                  |              |
| 125 | T5L   | M | B | T5   |          | K1                                            |              |
| 126 | T5Q   | M | B | T5   | 105(T3F) |                                               |              |
| 127 | T6K   | F | C | T6   |          | K1                                            |              |
| 128 | T6K-A | F | C | 127  | 133(X11) |                                               |              |
| 129 | T6KQ  | M | C | 127  |          | M7D                                           |              |
| 130 | T6M   | F | C | T6   | 105(T3F) |                                               |              |

|     |        |   |   |      |             |              |                                    |
|-----|--------|---|---|------|-------------|--------------|------------------------------------|
| 131 | T6MS   | M | C | 130  | 125(T5L)    |              |                                    |
| 132 | X1     | F | A | Unk  |             |              | Sire Unknown                       |
| 133 | X11    | M | A | Unk  |             | M7D          |                                    |
| 134 | X13    | F | A | 41   |             | K1           |                                    |
| 135 | X13N   | M | A | 134  |             |              | Sire Unknown                       |
| 136 | X13P   | M | A | 134  |             | N6BG         |                                    |
| 137 | X13S   | F | A | 134  |             | N6BG or M17E |                                    |
| 138 | X13S-A | F | A | 137  | 114 (T5AEK) |              |                                    |
| 139 | X13S-B | F | A | 137  | 114 (T5AEK) |              |                                    |
| 140 | X13SX  | M | A | 137  | 167(X6)     |              |                                    |
| 141 | X15    | F | B | T5   |             |              | Sire Unknown                       |
| 142 | X15N   | F | B | 141  | 26(M6K)     |              |                                    |
| 143 | X15NY  | M | B | 142  | 97(N9EL)    |              |                                    |
| 144 | X15P   | F | B | 141  | 25(M6J)     |              |                                    |
| 145 | X15W   | M | B | 141  | 114 (T5AEK) |              |                                    |
| 146 | X16    | F | A | Unk  |             |              | Sire Unknown                       |
| 147 | X16-A  | M | A | 146  | 94(N9BOX)   |              |                                    |
| 148 | X16P   | M | A | 146  |             | N9CH         |                                    |
| 149 | X17    | F | C | ?    |             | N1           |                                    |
| 150 | X17U   | F | C | 149  | 61(N6EHP)   |              |                                    |
| 151 | X21    | F | A | N9BI |             | N1           |                                    |
| 152 | X21P   | M | A | 151  |             | N4E          |                                    |
| 153 | X21Q   | M | A | 151  |             | N4E          |                                    |
| 154 | X21T   | F | A | 151  |             | N4E          |                                    |
| 155 | X21T-A | F | A | 154  | 148(X16P)   |              |                                    |
| 156 | X22    | F | B | 47   |             |              | Poor genotype-unable to infer Sire |
| 157 | X22P   | M | A | 68   |             | N4E          |                                    |

|     |         |   |   |     |                                            |                 |
|-----|---------|---|---|-----|--------------------------------------------|-----------------|
| 158 | X22R    | M | B | 156 | N4E                                        |                 |
| 159 | X24     | F | A | N6B | M8                                         |                 |
| 160 | X24M    | F | A | 159 |                                            | Sire Unknown    |
| 161 | X24MR   | M | A | 160 | N9BK                                       |                 |
| 162 | X24R    | M | A | 159 | N10G                                       |                 |
| 163 | X25     | F | A | N6B | M8                                         |                 |
| 164 | X25S    | M | A | 163 | N9CH                                       |                 |
| 165 | X25T    | F | A | 163 | N9CH                                       |                 |
| 166 | X25TW   | F | A | 165 | 152(X21P)                                  |                 |
| 167 | X6      | M | B | Unk | K1                                         |                 |
| 168 | X7      | M | A | Unk | K1                                         |                 |
|     |         |   |   |     | 90(N9BNT) more<br>likely than<br>37(N10OS) |                 |
| 169 | N10T-B  | F | A | 40  |                                            |                 |
| 170 | T3LV-C  | F | A | 110 | 114(T5AEK)                                 |                 |
| 171 | N6ELS-C | F | A | 64  | 135(X13N)                                  |                 |
| 172 | X13S-C  | F | A | 137 | 114(T5AEK)                                 |                 |
| 173 | X25T-C  | M | A | 165 | 152(X21P)                                  |                 |
| 174 | X16-C   | M | A | 146 | 61(N6EHP)                                  | Foal found dead |
| 175 | X25TW-D | M | A | 166 | 32(M6MSY)                                  |                 |

### **Phylogenetic analysis of microsatellite results**

Previous studies by Vilà *et al.* (2001) have shown that microsatellite genotype appears to be more closely associated with breed affiliation than mtDNA haplotype. The results of our phylogenetic analysis using a subset of four microsatellite loci used in that study show similar results (Figure 3). Because all other breeds in the analysis were represented by up to 20 individuals, we selected 20 Assateague horses from various lineages that were represented by reliable and complete genotypes derived from biopsy samples. The combined analysis resulted in 19 of the 20 Assateague horses clustering together. However, the next closest cluster of horses is of mixed breeds (at least six breeds are represented) and the relationship of Assateague horses to other breeds is therefore not clearly established with these few markers. The fact that most of the Assateague horses form a cluster using only a few loci in the analysis supports the findings that microsatellite divergence between breeds was more marked than that observed with mtDNA sequences. The development of phenotypically distinct breeds may in fact be a different process characterized by a limited founding stock and restricted breeding. A sex bias occurs in breeding practices in which select breeding males are used as stud for up to twenty or more females. Similarly, Assateague wild horses form harems where one stallion breeds with many females biasing the distribution of male mediated gene flow.

Before we accept these results, they will need to be confirmed. There are two potential pitfalls: first, we have found that the study of Vilà *et al.* (2001) did not calibrate their microsatellite scoring using the standard positive control contained in the ISAG approved paternity kit. While their results remain valid (this does not in any way affect the results of their study), it means that the two studies cannot be directly compared. If we are able to obtain a small number of samples from their study, we can calibrate our results with theirs. If there are no available samples from their study, we will either gather data from other directly comparable studies (there have been several less complete studies) or obtain and genotype a few samples from Spanish pony populations and established breeds in the United States. Second, our results are likely to be strongly influenced by the fact that they are based on only four loci. Expanding our analysis to the full 15 loci with size controls based on the ISAG standard will allow us to have the maximum confidence in these results.

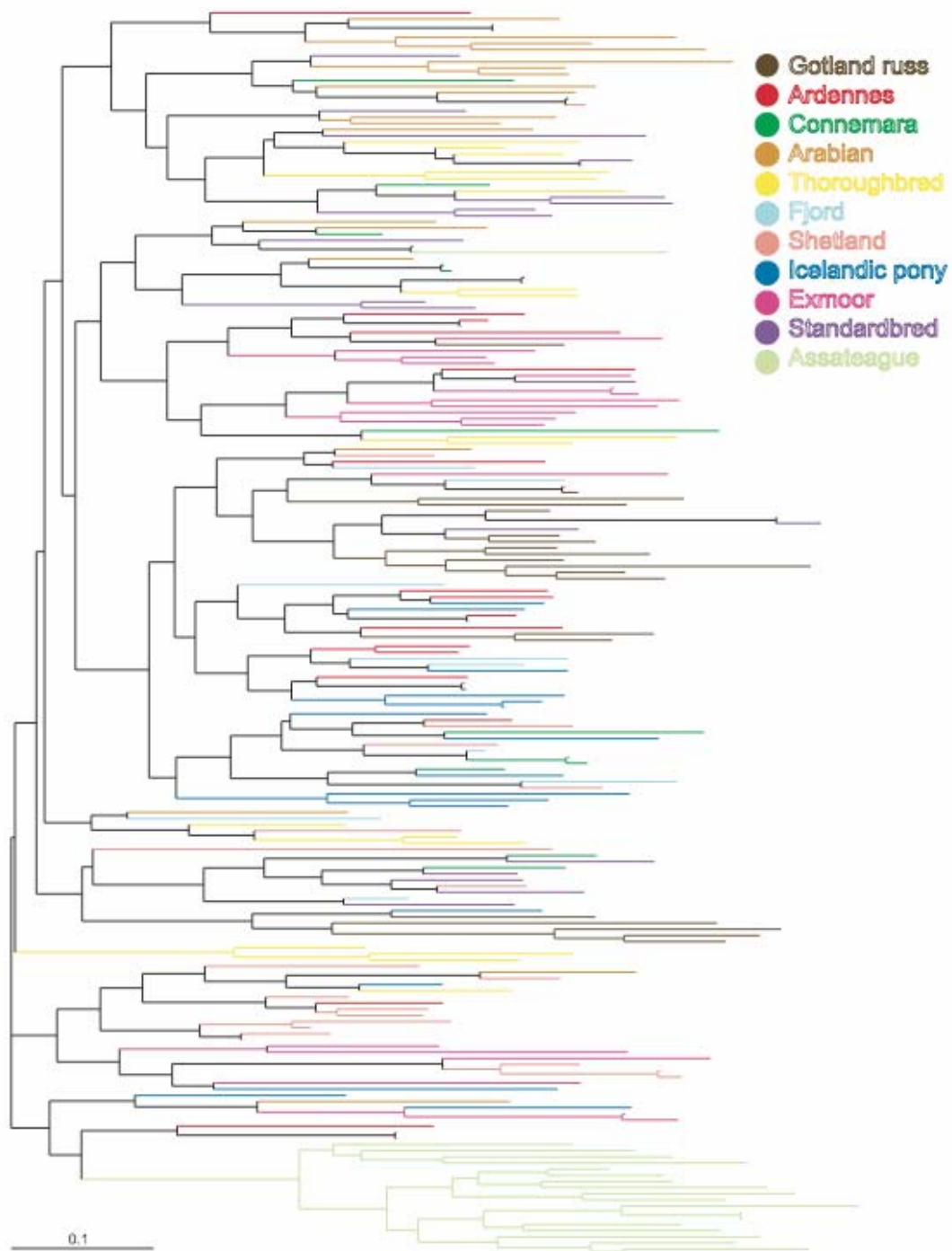


**Figure 3.** Phylogenetic analysis of microsatellite data for 20 Assateague horses representing all extant maternal groups. The Assateague horses were analyzed in conjunction with the data from established breeds worldwide (from Vilà *et al.* 2001).

### **Conclusions from the molecular genetics study**

The results presented in this report characterize the current level and distribution of genetic diversity within the Assateague horse population. We analyzed both mitochondrial and nuclear genetic markers and found that there is less mitochondrial DNA diversity than has been found in established breeds, but the level of nuclear diversity is comparable to that found in established breeds. Using the genotype data, we confirmed and amended the maternal pedigrees, and inferred the relationships between the horses that were born when records were incomplete (the “X” horses) and others in the herd. We successfully assigned all but one of these horses to an existing matriline.

Once the maternal pedigrees were confirmed, we performed paternity analysis on the dataset. The results presented infer paternity for horses with living sires, and other data from NPS records was used to infer paternity for horses whose sires are deceased and could not be included in the genetic study.

Preliminary results of a phylogenetic analysis using microsatellite data suggests that the Assateague horses may be somewhat differentiated genetically from other breeds worldwide. However, we need to work out methods of calibrating our results with those of Vilà *et al.* (2002) or other studies of established breeds, or have genotyped samples from Spanish ponies and established breeds in the U. S., we will be more confident in this result. Furthermore, we propose that a more focused study with approximately 20 samples and at least 11 additional microsatellite loci may be needed to more firmly establish whether the Assateague horses represent a genetically differentiated breed.

## **Part 2. Demographic and genetic analysis of the Assateague horses using the pedigree.**

Many captive populations of conservation interest in zoos are managed using a set of software tools developed by geneticists and population biologists. This first tool is S.P.A.R.K.S. (Single Population Animal Records Keeping System, v. 1.52, International Species Information System, 2005). SPARKS is used to generate a “studbook” of the population. Studbooks include all known living and deceased individuals that are considered to be a part of the managed population. A SPARKS database can include a wealth of information about individuals (age, sex, parentage, health variables, contraception data, movements between facilities, etc.). While the SPARKS software is capable of some analyses of population genetics and demographics, the best practice today is to export the SPARKS database to Population Management 2000 (PM2000 v. 1.211; Pollak *et al.*, 2005). This software program enables managers to conduct more sophisticated analyses of populations and includes many options not available in SPARKS. SPARKS is available by purchase from the International Species Information System and PM2000 can be downloaded from the internet at <http://www.vortex9.org/pm2000.html> for free and includes a software manual.

The genetically-verified pedigree data were entered into SPARKS in order to create the studbook of the Assateague horse population. Pedigree data beyond the scope of the genetic analyses were also included when NPS staff felt confident that parentage determinations were correct. These additions were a very small fraction of the studbook. In cases where only the year of birth or death were known for an individual, July 1 was arbitrarily chosen as the date. Three putatively living individuals in the studbook (#181, 184, 220) were transferred out of the Maryland population in the early to mid-1990s. These individuals were not included in demographic or culling analyses, but their data were used in genetic calculations if they left descendants on the island. In any studbook there are animals with unknown parentage; however, at some point in the past those animals descended from wild “founders”. Wild caught founders have to be identified in any studbook in order to analyze how the population has retained or lost that genetic diversity that was initially captured. We do not have the complete studbook data that traces the lineages of the current population back to the founders that were first placed on the island. However, we can trace many of the lineages back to individuals that were part of the original population as described by Keiper (e.g., M1, M2, K1), or at least to first-generation descendants from them (e.g., T2B). In the absence of any more data on the history of these individuals, we assigned their unknown parents “Wild” status (note: when using these software tools, designating parentage as “Wild” indicates to the software that these individuals are the “founders” of the population and that this is as far back as the pedigrees can be traced. Founders are assumed to be unrelated. All genetic analyses - level of inbreeding, retention of genetic diversity, etc., are then relative to this set of “founders”). These changes are reflected in the studbook. Admittedly, treating each of these founder individuals as unrelated to any other “wild” founder represents the most optimistic scenario and is likely erroneous. We know for example that some of the earliest records on the herd indicated that there were 28 individuals in the herd when the NPS took over management of the horses. However, we have no additional data available on how these individuals were

related to one another. Regardless of what the true founder situation was, the task today is to manage the remaining genetic diversity for the long term. A common goal in genetic management is to maintain 90% of the genetic diversity present for at least 100 years, regardless of the true amount of allelic diversity present. Genetic studies of the captive black-footed ferret population, which had only 14 founders, have found that managing animals from a mean kinship perspective (described below) has been effective at retaining much of the genetic diversity present in that small number of founders (Wisely et al., 2003).

We first used PM2000 to look at the current demographic status of the horse population. PM2000 takes the demographic information from the SPARKS database and generates a life table for the population. Variables in the life table can then be manipulated to determine their effects on demographics over time (i.e. if we delay the first age of reproduction, what effect does that have?). We did not conduct any of these sorts of analyses as they were beyond the scope of this project; however, it must also be remembered that the life table we generated is based on a contracepted population for which we only have a small number of generations of biased data (maybe 2 to 3 generations at most, and biased in the sense that individual reproductive rates have been artificially controlled). In the population management sense, “generation” means more than just having offspring, grand-offspring, etc. It refers to the age, on average, at which females are reproducing. In PM2000, generation time is calculated by taking the average of the ages at which animals give birth (or sire offspring). Estimates of generation time for males and female are given in Table 6. We know that contraception has altered the reproductive characteristics of the population and has extended longevity in females, so any projections would be subject to those caveats.

**Table 6.** Generation time estimates for Assateague horses.

| Sex     | Generation Time (years) |
|---------|-------------------------|
| Males   | 11.44                   |
| Females | 9.99                    |

We also generated an age pyramid of the population and visually inspected that to determine if there were any concerns for the long-term health of the population (Figure 4 below). The age pyramid is somewhat “top heavy” with a higher proportion of older individuals than younger individuals, particularly and more importantly for females. We suspect that this is a by-product of the contraception program. Having a smaller population of young animals, particularly females, now means that the population will have a limited opportunity to grow in the future. This is probably desirable for the management of this population, but a decreased number of breeding females puts the population at greater genetic and demographic risk, particularly if some unknown event led to an increase in mortality of females.

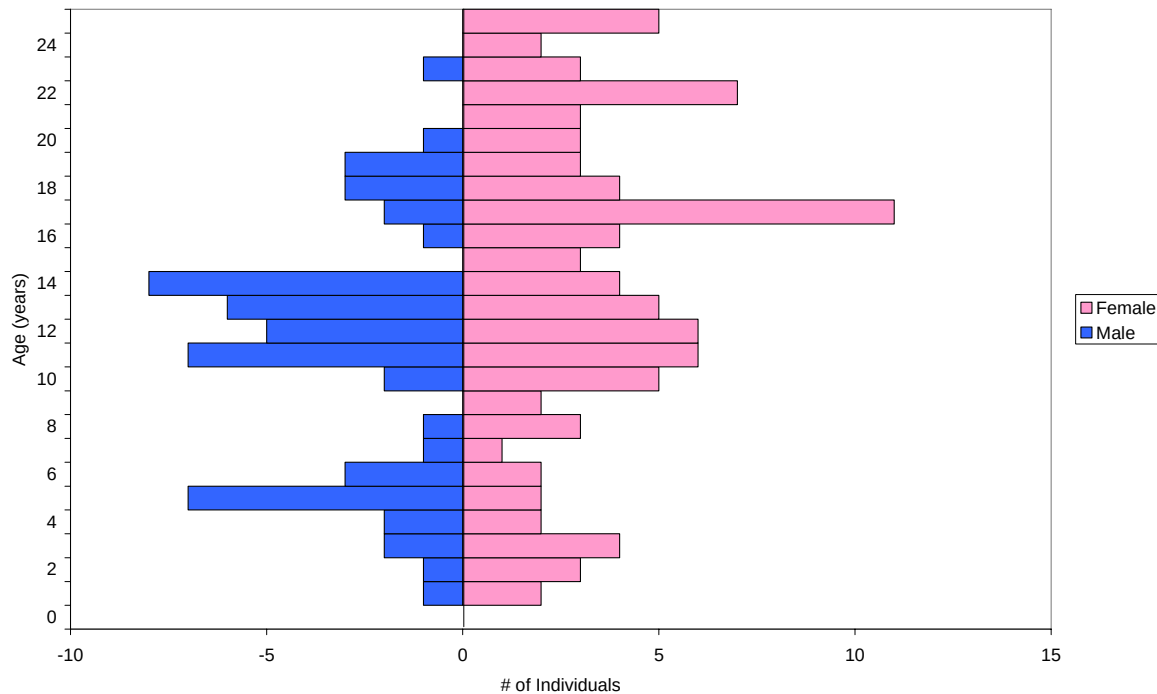


**Figure 4.** Age pyramid for the current Assateague horse population.

Our pedigree for the Assateague horse population was 86.4% known. This is pretty complete, and better than many captive populations, but we recommend any efforts that would decrease the number of individuals with unknown parentage when possible. For example, there may be cases where an unknown stallion might have sired multiple offspring. If we could link these offspring to the same unknown sire, we could create a hypothetical individual in the studbook. Alternatively where a group of stallions is suspected of siring an offspring, their reproductive history could be examined to determine who might be the most likely sire.

The first step in our genetic analysis was to look at founder representation in the current, living population. Based on our studbook, there were 39 founders in the population. One measure of how well the genes of those founders have been maintained in the population is “founder genome equivalents”. FGE is a measure of how many wild caught individuals would have the same genetic diversity as the current captive population. The FGE on Assateague currently is 14.58, but our analyses indicate that this could be increased to as much as 27.11 if breeding efficiency increased.

A genetic analysis of the part of the pedigree that is known indicates that the current population has retained 96.6% of the genetic diversity of the population from which it was derived (i.e., the population extant at the time of Keiper’s studies 2-3 horse generations ago). This loss of 3.4% of the diversity over this time equates to an average genetically effective population of 30-45, which is about 20-30% of the total number of individuals. Estimating the effective size of a population allows one to project loss of genetic diversity and increase in average kinship in the population into the future. The loss of genetic diversity can be estimated using:

$$GD_t = GD_{(t-1)} * [1 - 1/2N_e]$$

where  $GD_t$  is the proportional retention of genetic diversity at generation  $t$  and  $N_e$  is the effective population size (Kimura & Crow 1963).

For example, if the population retained an effective population size of 25, the % of retained genetic diversity would drop to 87%, and the average level of kinship among individuals in the population would increase to 13%. As half-sibs share kinships of 12.5%, with this effective population size, horses in the population five generations from now would be more closely related than half-sibs (Table 7). Of course, this is ignoring any accumulation of inbreeding that might already have occurred prior to the “founders” of this studbook population.

**Table 7.** Predicted losses of genetic diversity over future generations.

| Generations<br>from now | % Retained<br>Genetic Diversity | Average level of<br>kinship in the<br>population |
|-------------------------|---------------------------------|--------------------------------------------------|
| 0                       | 97%                             | 3%                                               |
| 1                       | 95%                             | 5%                                               |
| 2                       | 93%                             | 7%                                               |
| 3                       | 91%                             | 9%                                               |
| 4                       | 89%                             | 11%                                              |
| 5                       | 87%                             | 13%                                              |

It is also useful to examine the relative contribution of founders to the current living population. In Figure 5, representation refers to the proportion of the current gene pool that the individual has contributed. You will note that some of the horses on the x-axis are identified with labels such as “P015”. These are wild caught animals that were not in the studbook but which mated with a known individual in the studbook. So P015 is the wild caught individual that bred with studbook #15. Though the number of founders may be artificially high since we assumed that many of the animals in the “original” population were unrelated, it is clear that these founders have all contributed relatively equally to the current gene pool. This is one of the factors we try to consider when managing captive populations. We attempt to have all founders as equally represented as possible (Ballou & Foose 1996). The outstanding exception is Studbook #178, stallion K1, which has fathered a number of offspring in the population. The fact that we have good founder representation generally agrees with the finding that we have a good amount of nuclear genetic diversity in the population.

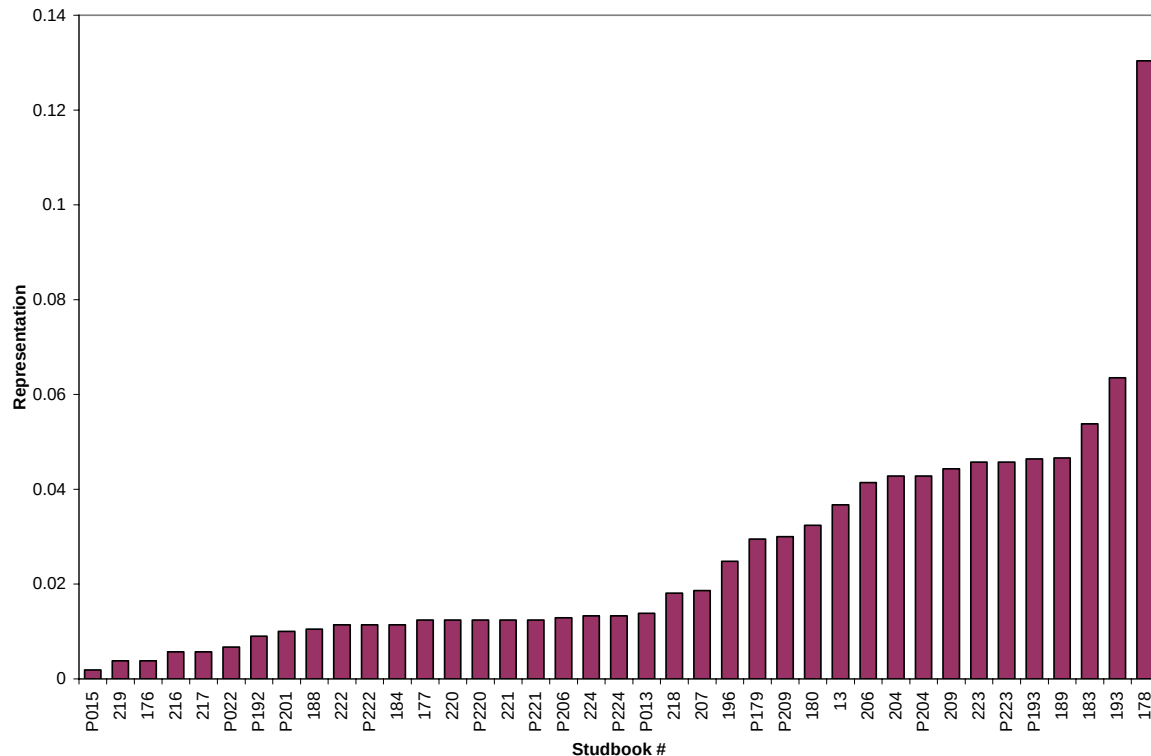


**Figure 5.** Founder representation in the current Assateague horse population.

Current practice in managed North American zoos is to pair animals based on Mean Kinship (MK; (Ballou & Lacy 1995)). MK is a measure of how related that individual is to the population. Thus, individuals with low MK are usually considered genetically valuable and are paired with other low MK individuals. Pairing animals based on MK has the benefit of reducing inbreeding and equalizing founder representation in the population, and it has been shown to effectively retain genetic diversity in the population. The MK of an individual changes whenever it reproduces or when other animals reproduce or die. Therefore MK values are reviewed annually in many programs after birth/death information has been obtained from holding facilities. A list of horses included in the PM2000 analyses and their MK values is included in Appendix 1 of this report. Four individuals in this table have an MK value of 0.5 because their parentage is completely unknown so we only know that they are related to themselves, hence the 0.5 MK; these values were not included in any further calculations.

### **Potential Effects of Culling on Genetics and Demography**

PM2000 has a culling feature that allows managers to look at the genetic and demographic impacts of culling selected individuals from the population. We recommend that NPS personnel obtain the PM2000 software so that they can examine this feature. NZP personnel can provide assistance with training. The studbook population that was taken to PM2000 for analysis included 153 individuals. Using the culling feature, we culled the 25 and 50 individuals in the population with the highest MK values (Note: the four individuals

with unusually high MK values described above were also culled). The results are presented below.

**Table 8.** Predicted results of culling on the genetics of the Assateague horse population.

| Parameter            | Original Population | 25 culled | 50 culled |
|----------------------|---------------------|-----------|-----------|
| Gene Diversity       | 0.966               | 0.9694    | 0.9710    |
| Average Mean Kinship | 0.034               | 0.0306    | 0.029     |
| Gene Value           | 0.9657              | 0.9630    | 0.9641    |
| FGE                  | 14.58               | 16.35     | 17.24     |

As expected, culling procedures have the potential to immediately improve the genetic status of the population (Table 8). Gene diversity is an estimate of the proportion of genetic diversity in the current population relative to the genetic diversity of the population from which the “founders” came – in this case the population studied by Keiper 2-3 horse generations ago (Lacy 1995). Gene value goes one step further by incorporating age-specific reproductive rates into this estimate (Ballou & Lacy 1995); however, keep in mind that rates of reproduction on Assateague are artificially controlled. The overall population mean kinship decreases with culling whereas the number of founder genome equivalents increases.

While it may appear the culling will improve the genetic status of the population, we recommend that culling decisions be made based upon the molecular analyses as well. Some questions to be asked about the potential effects of culling include: 1) does culling based on MK lead to greater risk of losing one of the two more rare maternal haplotypes? and 2) do culled individuals carry any rare alleles in the population? It is also very likely that the selection of individuals for removal will depend on other factors such as age. Another consideration in culling is what effect it will have on the demographic status of the population. Figure 6 is the age pyramid generated after culling 25 individuals from the population based solely on MK values.

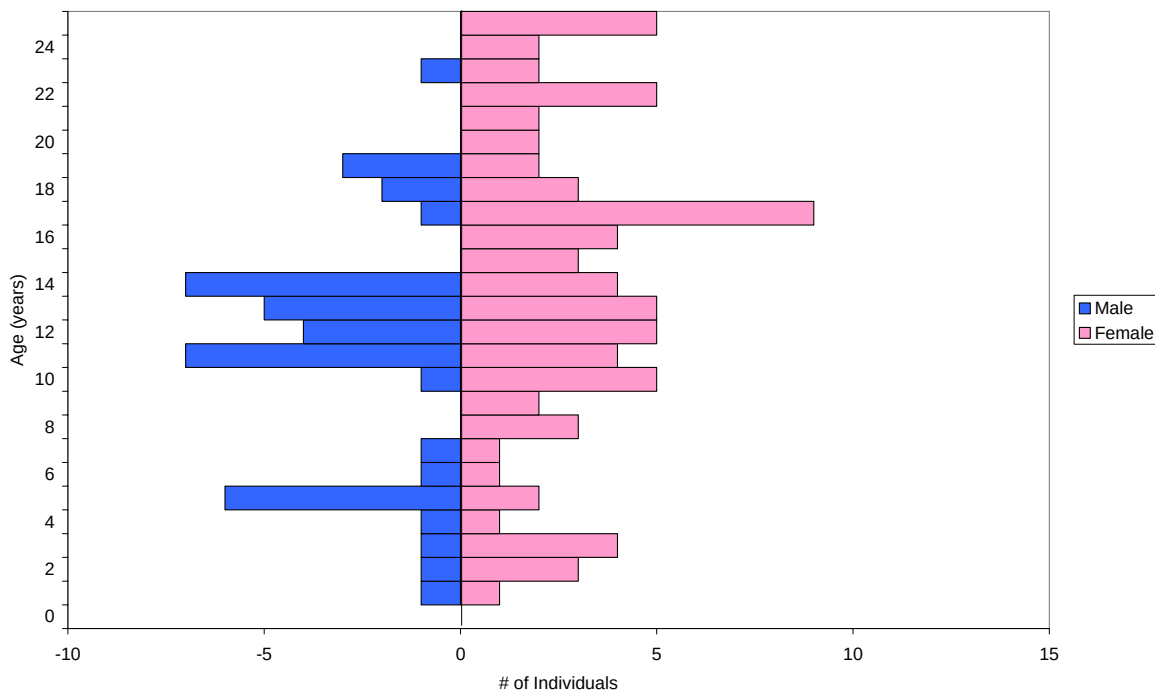


**Figure 6.** Age pyramid after culling 25 individuals from the population based only on Mean Kinship values.

It appears that mainly males would be affected by culling the 25 highest MK horses in the population, leaving only 12 males in the younger age groups (ages 0 to 7 years). Only 13 young females would remain after this cull.

Figure 7 is the age pyramid generated after culling the 50 highest MK horses from the population. An additional two young males and four young females would be removed from the population. These culling simulations suggest demographic factors be given serious consideration when making decisions for removal. Data sets used to generate these age pyramids are included at the end of this report in Appendix 2.

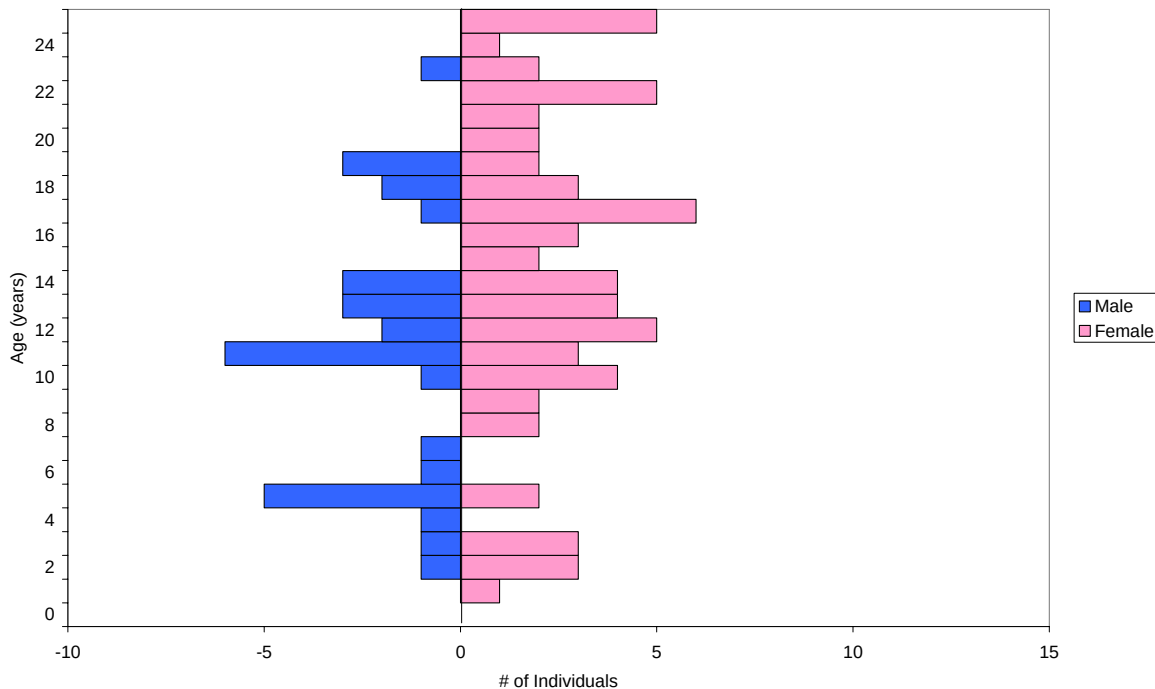


**Figure 7.** Age pyramid after culling 50 individuals from the population based only on Mean Kinship values.

### Conclusions

This project was designed to develop information to ascertain the genetic status and condition of the Assateague feral horse population. We make recommendations that we hope will be useful in the appropriate long-term management of the population, including potential actions to reduce the size, manage reproductive rates, and enhance their long-term health and viability.

PM2000 provides the tools that could be used to evaluate both the genetic and demographic effects of various culling strategies. There are clear trade-offs in genetic and demographic effects that should not be ignored. For example, while culling genetically “over-represented” animals will immediately increase the genetic diversity, the resulting reduction in population size will reduce the population’s genetically effective size. The immediate benefits of selective genetic culling may then be offset by a more rapid rate of loss of genetic diversity due to smaller effective size. Various strategies should be examined and tested.

We suggest that the next step in development of a long-term management plan for the Assateague horses is a population and habitat viability analysis (PHVA). These analyses enable the modeling of population persistence over long periods of time given the ecological, behavioral, and genetic background of the species. Anthropogenic and management factors can also be included in these models. PHVAs are currently a best practice in the management of populations of varying sizes and degree of fragmentation or isolation. Smithsonian Institution staff can be of assistance with the development of a PHVA workshop in the future.

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## **Appendix**

- 1.** Mean Kinship Values Before Culling Simulation
- 2.** Age Pyramid Data: male values are negative only for the purposes of graphing.
- 3.** Proposed pedigrees.
- 4.** List of individual horses that carry rare microsatellite alleles.

**Appendix 1: Mean Kinship Values Before Culling Simulation**

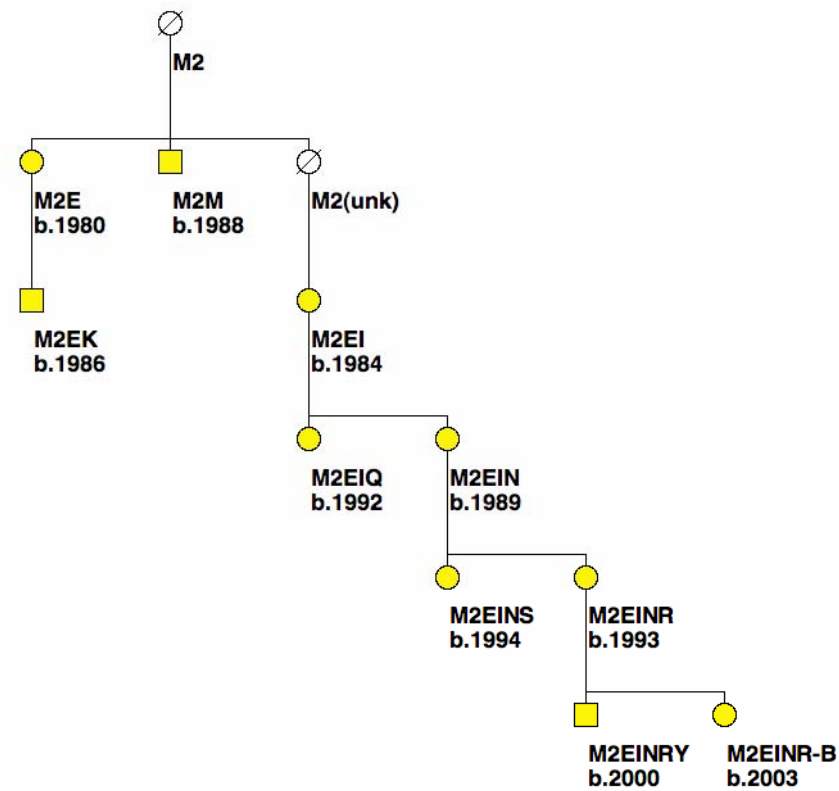
| Studbook# | Sex | Mean Kinship | Studbook# | Sex | Mean Kinship | Studbook# | Sex | Mean Kinship |
|-----------|-----|--------------|-----------|-----|--------------|-----------|-----|--------------|
| 36        | F   | 0.006        | 98        | F   | 0.029        | 73        | M   | 0.041        |
| 220       | F   | 0.006        | 101       | F   | 0.029        | 102       | M   | 0.041        |
| 20        | F   | 0.009        | 34        | F   | 0.03         | 117       | M   | 0.041        |
| 19        | F   | 0.01         | 84        | F   | 0.03         | 122       | M   | 0.041        |
| 25        | F   | 0.01         | 126       | F   | 0.03         | 168       | M   | 0.041        |
| 35        | F   | 0.01         | 142       | M   | 0.03         | 83        | M   | 0.042        |
| 24        | M   | 0.011        | 160       | M   | 0.03         | 91        | M   | 0.042        |
| 110       | F   | 0.012        | 208       | F   | 0.03         | 157       | M   | 0.042        |
| 106       | F   | 0.013        | 29        | F   | 0.031        | 58        | F   | 0.043        |
| 51        | F   | 0.014        | 60        | F   | 0.031        | 79        | M   | 0.043        |
| 55        | F   | 0.014        | 67        | F   | 0.031        | 137       | F   | 0.043        |
| 70        | F   | 0.014        | 68        | F   | 0.031        | 31        | F   | 0.044        |
| 135       | F   | 0.015        | 129       | F   | 0.031        | 44        | F   | 0.044        |
| 48        | F   | 0.016        | 134       | F   | 0.031        | 107       | M   | 0.044        |
| 81        | M   | 0.016        | 72        | F   | 0.032        | 37        | M   | 0.045        |
| 38        | M   | 0.017        | 128       | M   | 0.032        | 39        | M   | 0.045        |
| 62        | F   | 0.017        | 153       | M   | 0.032        | 59        | F   | 0.045        |
| 119       | M   | 0.017        | 4         | F   | 0.033        | 63        | F   | 0.045        |
| 125       | F   | 0.017        | 26        | F   | 0.033        | 69        | F   | 0.045        |
| 7         | F   | 0.018        | 40        | F   | 0.033        | 75        | M   | 0.045        |
| 92        | F   | 0.018        | 165       | F   | 0.033        | 144       | F   | 0.045        |
| 86        | M   | 0.019        | 167       | F   | 0.033        | 158       | F   | 0.045        |
| 147       | M   | 0.019        | 3         | F   | 0.034        | 161       | F   | 0.045        |
| 152       | M   | 0.019        | 71        | M   | 0.034        | 159       | M   | 0.046        |
| 166       | F   | 0.019        | 5         | F   | 0.035        | 41        | F   | 0.047        |
| 111       | F   | 0.02         | 64        | F   | 0.035        | 154       | M   | 0.047        |
| 113       | F   | 0.02         | 90        | M   | 0.035        | 47        | M   | 0.048        |
| 120       | M   | 0.02         | 141       | F   | 0.035        | 145       | M   | 0.048        |
| 50        | M   | 0.021        | 155       | M   | 0.035        | 163       | M   | 0.049        |
| 121       | M   | 0.022        | 213       | M   | 0.035        | 104       | M   | 0.05         |
| 148       | M   | 0.023        | 17        | F   | 0.036        | 130       | M   | 0.05         |
| 30        | F   | 0.024        | 61        | F   | 0.036        | 156       | F   | 0.05         |
| 54        | F   | 0.024        | 66        | F   | 0.036        | 87        | M   | 0.051        |
| 112       | M   | 0.024        | 80        | F   | 0.036        | 33        | F   | 0.052        |
| 16        | F   | 0.025        | 139       | F   | 0.036        | 105       | F   | 0.052        |
| 18        | F   | 0.025        | 150       | F   | 0.036        | 138       | M   | 0.052        |
| 23        | F   | 0.025        | 85        | F   | 0.037        | 57        | F   | 0.053        |
| 49        | M   | 0.025        | 93        | M   | 0.037        | 143       | F   | 0.054        |
| 78        | F   | 0.026        | 118       | F   | 0.038        | 45        | F   | 0.055        |
| 88        | F   | 0.026        | 149       | M   | 0.038        | 53        | F   | 0.056        |
| 97        | F   | 0.026        | 22        | F   | 0.039        | 146       | M   | 0.056        |
| 124       | M   | 0.026        | 76        | M   | 0.039        | 12        | F   | 0.059        |
| 133       | F   | 0.026        | 96        | M   | 0.039        | 115       | F   | 0.059        |
| 140       | M   | 0.027        | 100       | F   | 0.039        | 65        | M   | 0.061        |
| 151       | F   | 0.027        | 103       | M   | 0.039        | 171       | F   | 0.061        |
| 162       | F   | 0.027        | 164       | F   | 0.039        | 74        | M   | 0.062        |
| 42        | M   | 0.028        | 169       | F   | 0.039        | 123       | F   | 0.062        |
| 46        | M   | 0.028        | 172       | F   | 0.039        | 8         | F   | 0.5          |
| 109       | M   | 0.028        | 43        | F   | 0.04         | 10        | F   | 0.5          |
| 132       | F   | 0.028        | 89        | F   | 0.04         | 32        | F   | 0.5          |
| 6         | F   | 0.029        | 127       | F   | 0.04         | 56        | M   | 0.5          |

**Appendix 2:** Age Pyramid Data: male values are negative only for the purposes of graphing.

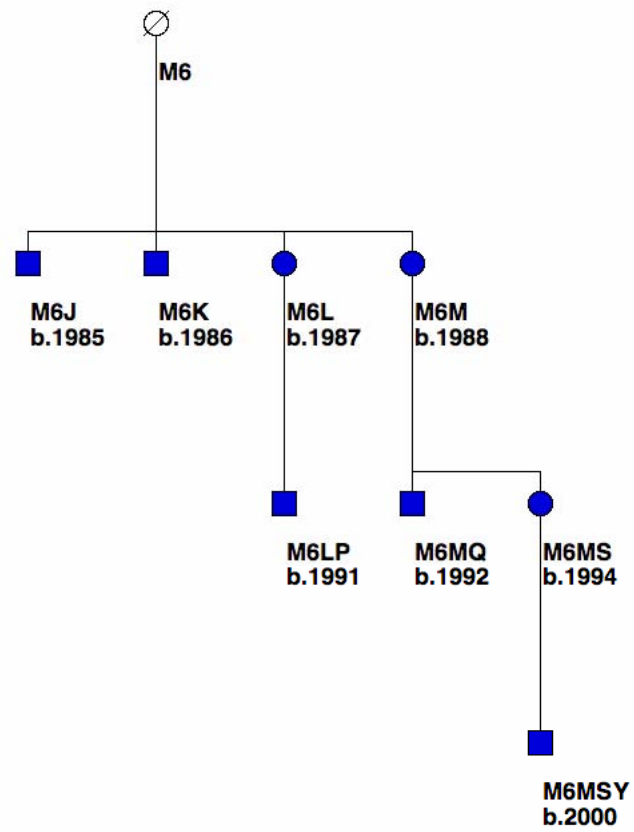
| Original Population |      |        | After culling 25 individuals |      |        | After culling 50 individuals |      |        |
|---------------------|------|--------|------------------------------|------|--------|------------------------------|------|--------|
| Age                 | Male | Female | Age                          | Male | Female | Age                          | Male | Female |
| 0                   | 0    | 0      | 0                            | 0    | 0      | 0                            | 0    | 0      |
| 1                   | -1   | 2      | 1                            | -1   | 1      | 1                            | 0    | 1      |
| 2                   | -1   | 3      | 2                            | -1   | 3      | 2                            | -1   | 3      |
| 3                   | -2   | 4      | 3                            | -1   | 4      | 3                            | -1   | 3      |
| 4                   | -2   | 2      | 4                            | -1   | 1      | 4                            | -1   | 0      |
| 5                   | -7   | 2      | 5                            | -6   | 2      | 5                            | -5   | 2      |
| 6                   | -3   | 2      | 6                            | -1   | 1      | 6                            | -1   | 0      |
| 7                   | -1   | 1      | 7                            | -1   | 1      | 7                            | -1   | 0      |
| 8                   | -1   | 3      | 8                            | 0    | 3      | 8                            | 0    | 2      |
| 9                   | 0    | 2      | 9                            | 0    | 2      | 9                            | 0    | 2      |
| 10                  | -2   | 5      | 10                           | -1   | 5      | 10                           | -1   | 4      |
| 11                  | -7   | 6      | 11                           | -7   | 4      | 11                           | -6   | 3      |
| 12                  | -5   | 6      | 12                           | -4   | 5      | 12                           | -2   | 5      |
| 13                  | -6   | 5      | 13                           | -5   | 5      | 13                           | -3   | 4      |
| 14                  | -8   | 4      | 14                           | -7   | 4      | 14                           | -3   | 4      |
| 15                  | 0    | 3      | 15                           | 0    | 3      | 15                           | 0    | 2      |
| 16                  | -1   | 4      | 16                           | 0    | 4      | 16                           | 0    | 3      |
| 17                  | -2   | 11     | 17                           | -1   | 9      | 17                           | -1   | 6      |
| 18                  | -3   | 4      | 18                           | -2   | 3      | 18                           | -2   | 3      |
| 19                  | -3   | 3      | 19                           | -3   | 2      | 19                           | -3   | 2      |
| 20                  | -1   | 3      | 20                           | 0    | 2      | 20                           | 0    | 2      |
| 21                  | 0    | 3      | 21                           | 0    | 2      | 21                           | 0    | 2      |
| 22                  | 0    | 7      | 22                           | 0    | 5      | 22                           | 0    | 5      |
| 23                  | -1   | 3      | 23                           | 0    | 2      | 23                           | -1   | 2      |
| 24                  | 0    | 2      | 24                           | 0    | 2      | 24                           | 0    | 1      |
| 25                  | 0    | 5      | 25                           | 0    | 5      | 25                           | 0    | 5      |
| 26                  | 0    | 0      | 26                           | 0    | 0      | 26                           | 0    | 0      |
| 27                  | 0    | 0      | 27                           | 0    | 0      | 27                           | 0    | 0      |
| 28                  | 0    | 0      | 28                           | 0    | 0      | 28                           | 0    | 0      |
| 29                  | 0    | 1      | 29                           | 0    | 1      | 29                           | 0    | 1      |
| 30                  | 0    | 0      | 30                           | 0    | 0      | 30                           | 0    | 0      |

## Appendix 3: Proposed pedigrees

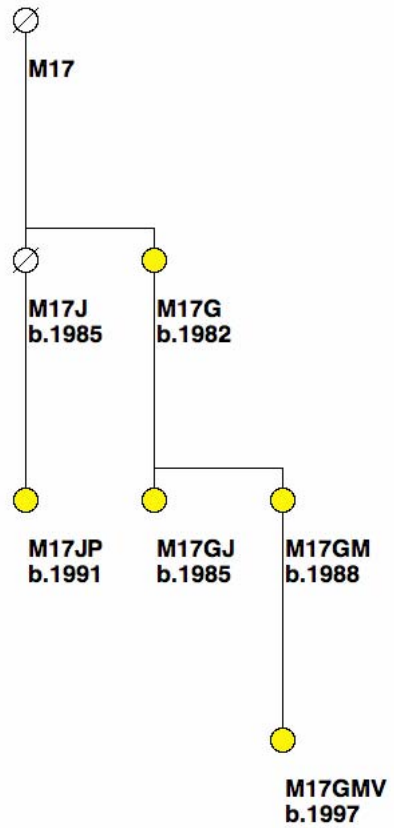
## M2 Lineage



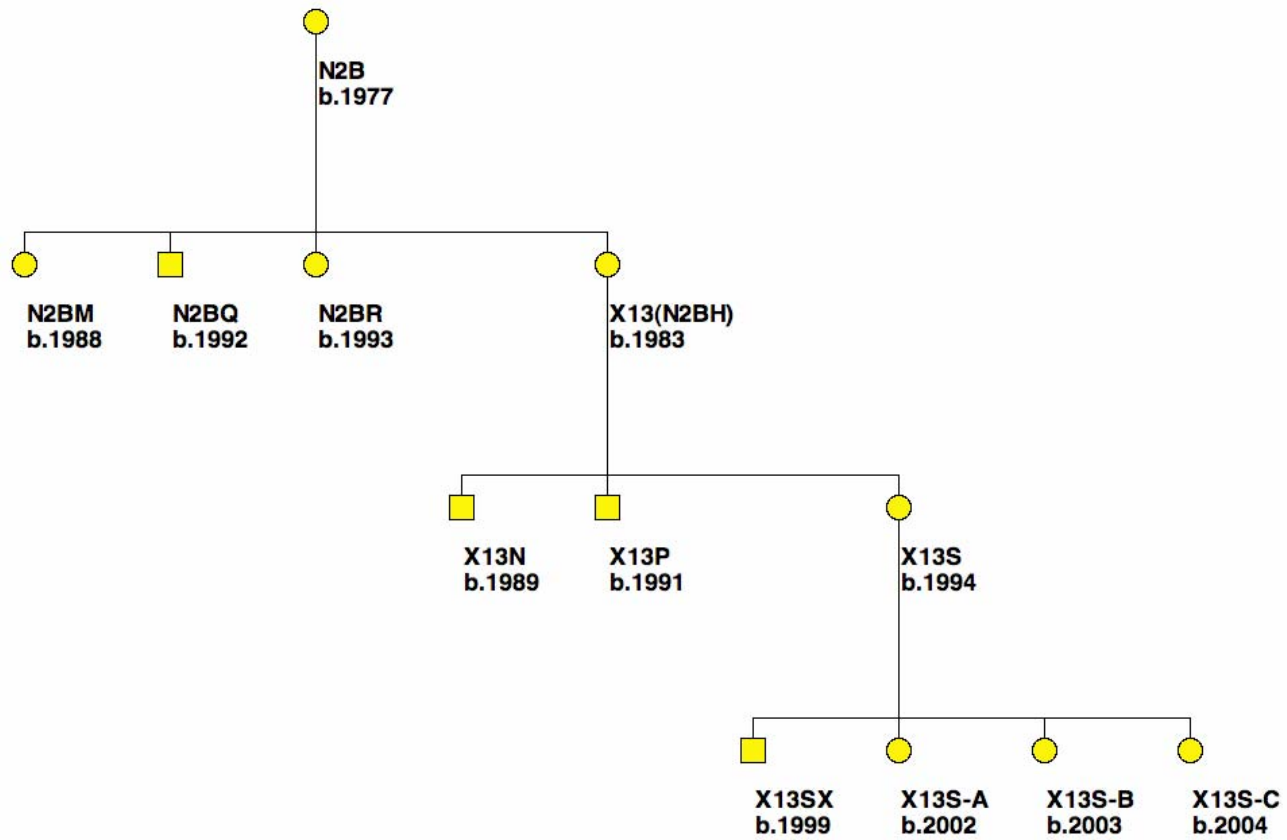
## M6 Lineage



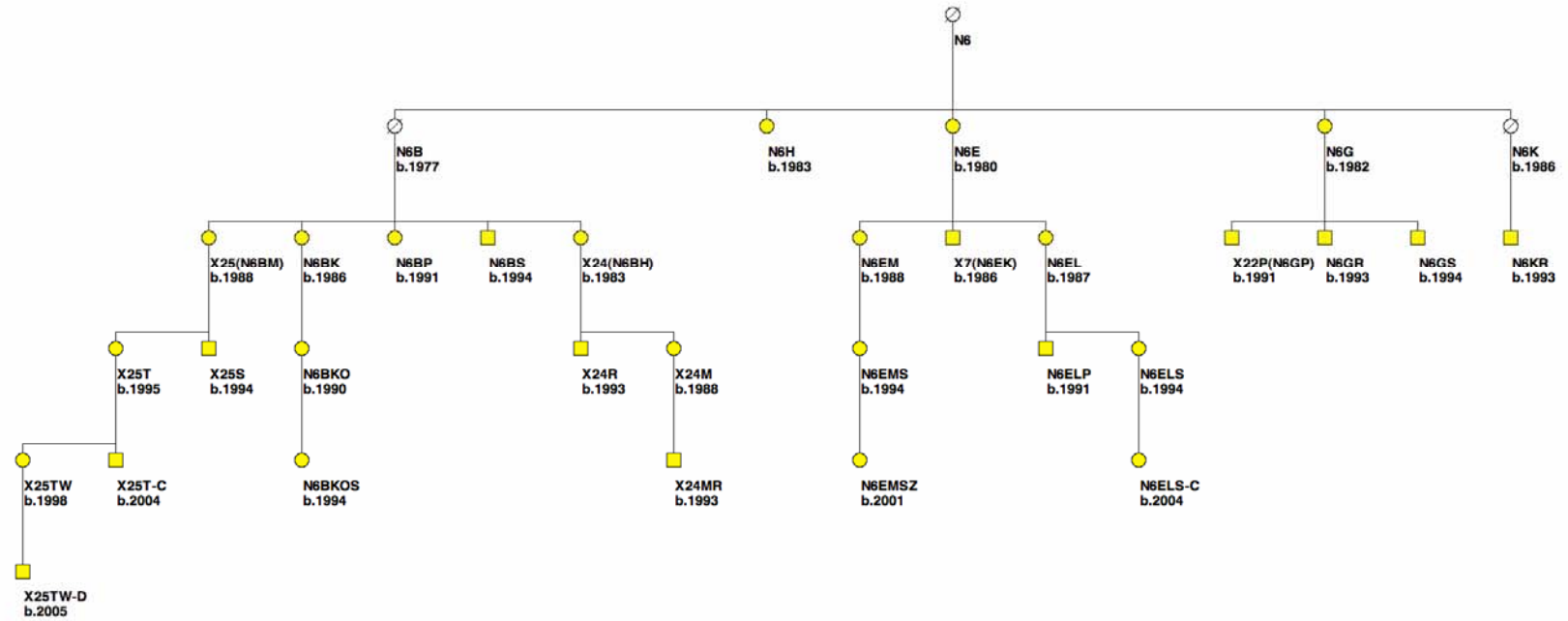
## M17 Lineage



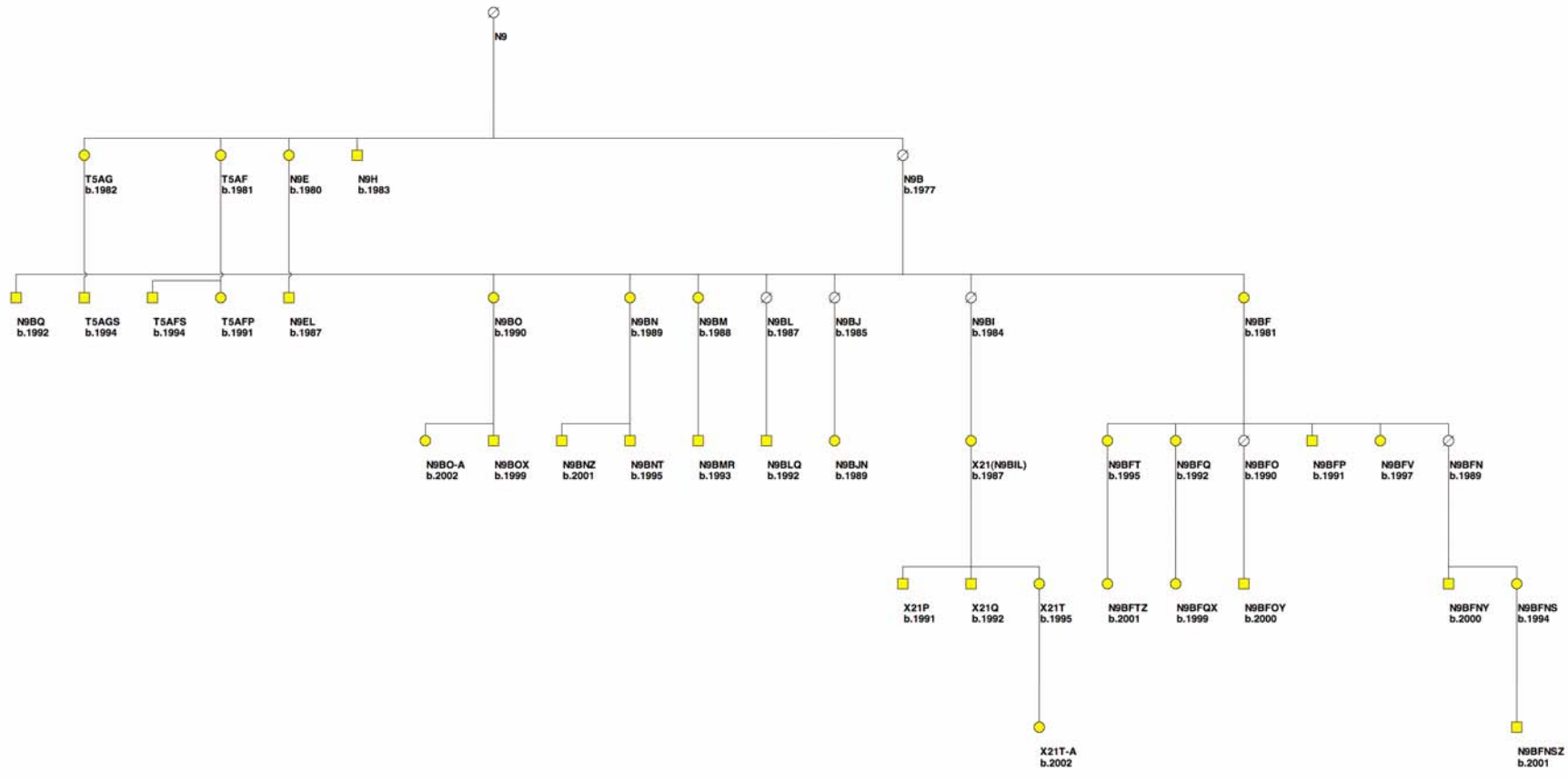
## N2 Lineage



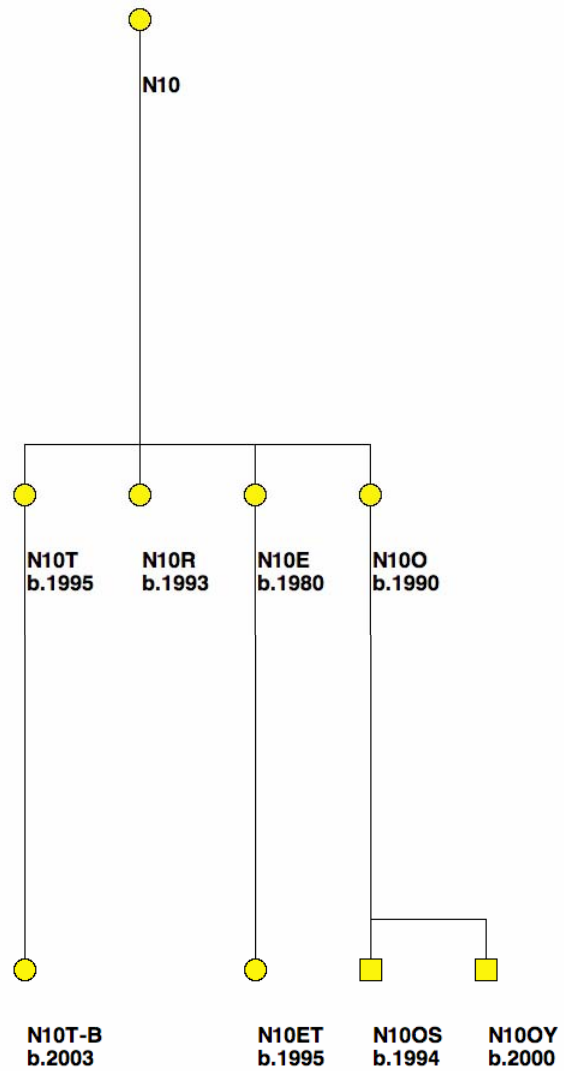
## N6 Lineage



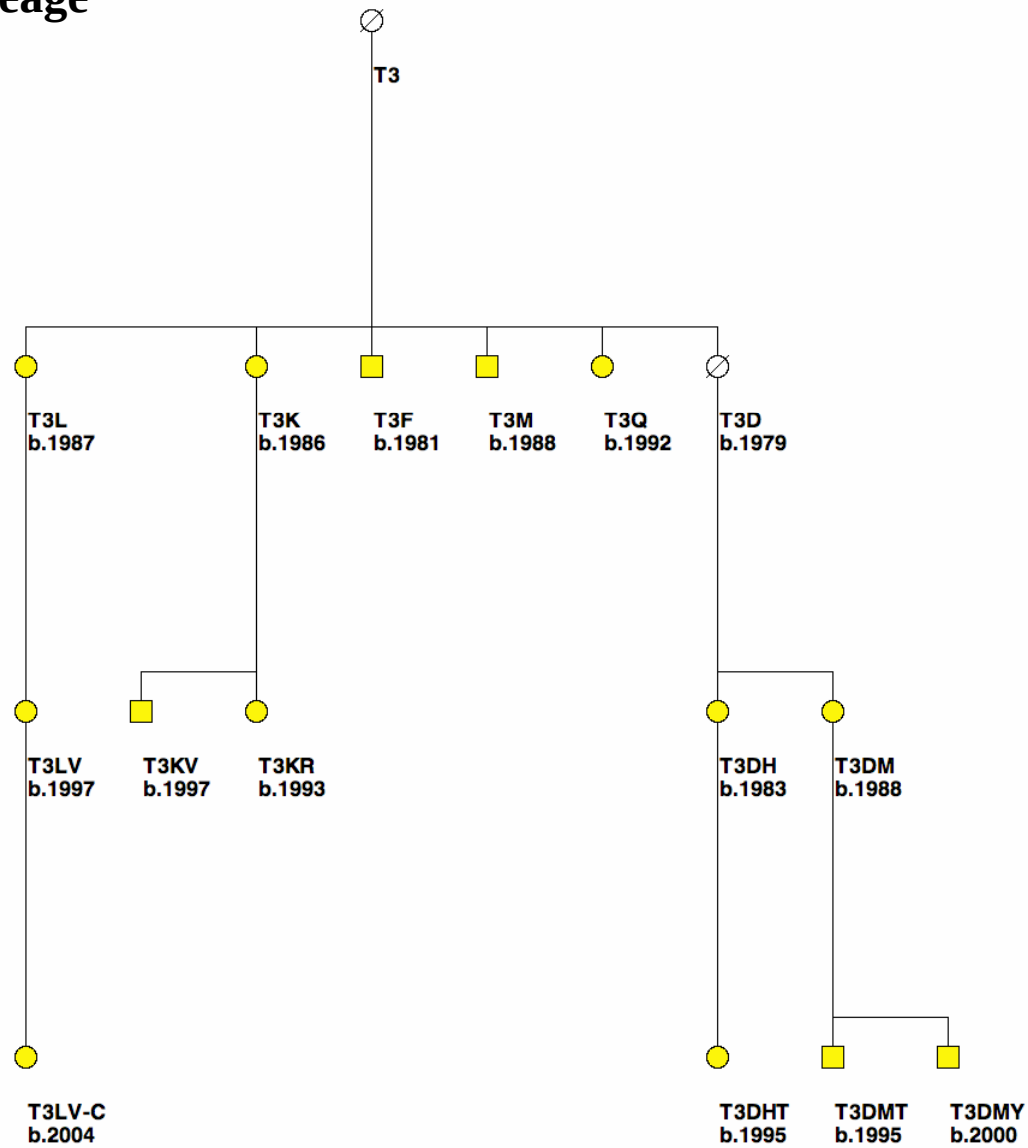
# N9 Lineage



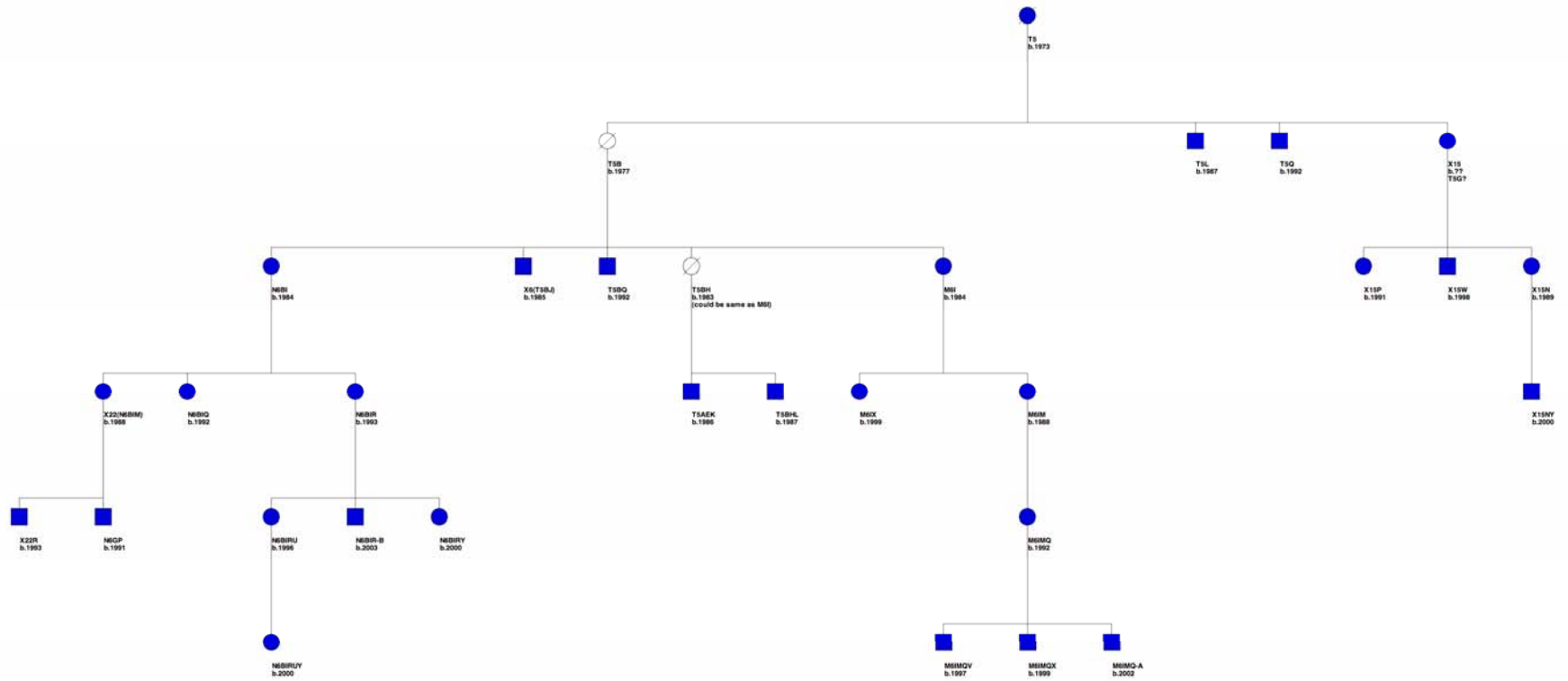
## N10 LINEAGE



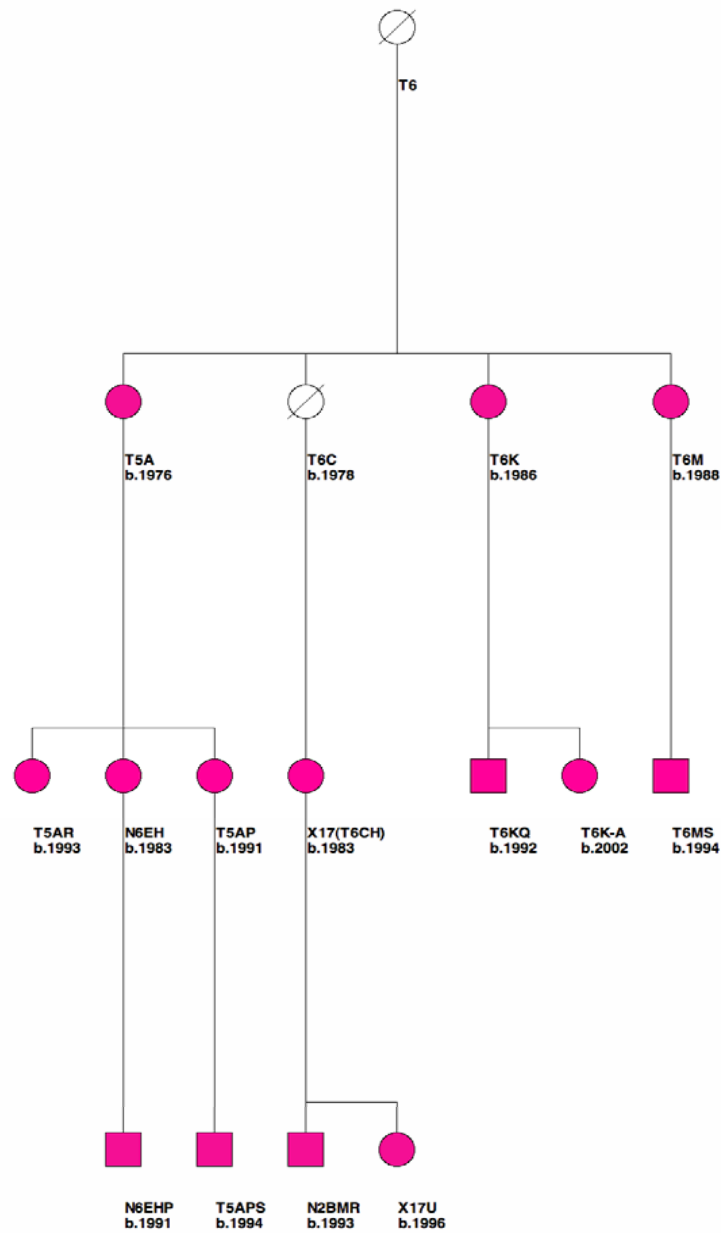
## T3 Lineage



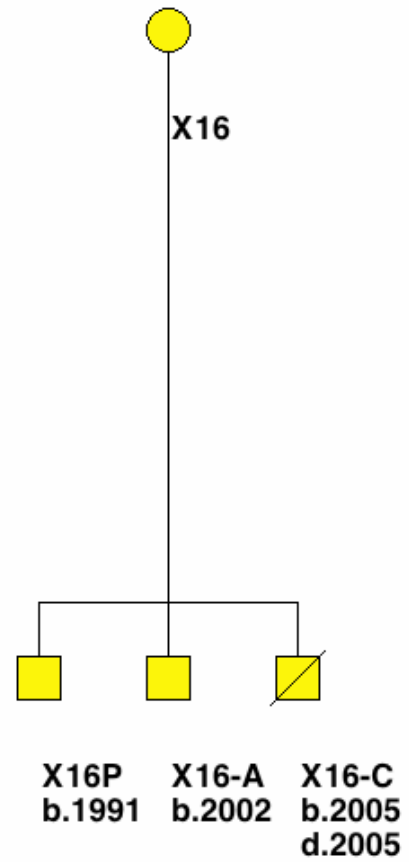
## T5 Lineage



## T6 Lineage



## X16 Lineage



**Appendix 4:** List of individual horses that carry rare microsatellite alleles. Individuals are coded by mtDNA haplotype (orange=A, blue=B, pink=C).

| LINEAGE |        |      | M2   | M6   | M17   | N2 | N6      | N9     | N10   | T3  | T5      | T6   | X16 |
|---------|--------|------|------|------|-------|----|---------|--------|-------|-----|---------|------|-----|
| N       |        |      | 10   | 8    | 5     | 12 | 29      | 35     | 9     | 14  | 27      | 14   | 3   |
| LOCUS   | Allele | Freq |      |      |       |    |         |        |       |     |         |      |     |
| VHL20   | 98     | 0.01 |      |      |       |    |         | N9EL   | N10   |     | X15NY   |      |     |
| HMS6R   | 106    | 0.04 |      |      |       |    | N6E     | X21    | N10   |     | N6BIQ   |      |     |
|         |        |      |      |      |       |    | N6EL    | X21P   | N10OY |     |         |      |     |
|         |        |      |      |      |       |    | N6ELS   | X21Q   |       |     |         |      |     |
|         |        |      |      |      |       |    | N6ELS-C | X21T   |       |     |         |      |     |
|         |        |      |      |      |       |    | N6EM    | X21T-A |       |     |         |      |     |
|         |        |      |      |      |       |    | N6EMSZ  |        |       |     |         |      |     |
| HTG7    | 122    | 0.01 | M2EI |      |       |    |         |        |       |     |         |      |     |
| HTG7    | 124    | 0.03 |      |      |       |    |         | N9H    | N10OS |     | M6IMQX  |      |     |
| HMS7R   | 106    | 0.03 |      | M6M  |       |    | X25     | N9BFQ  |       |     | N6BIRUY | T5AP |     |
|         |        |      |      |      |       |    | X25T    | N9BFT  |       |     | M6IMQ-A | T6KQ |     |
|         |        |      |      |      |       |    | X25TW   | N9BFTZ |       |     | M6IMQX  |      |     |
|         |        |      |      |      |       |    |         | T5AFS  |       |     |         |      |     |
|         |        |      |      |      |       |    |         | X21    |       |     |         |      |     |
|         |        |      |      |      |       |    |         | X21P   |       |     |         |      |     |
| HMS7R   | 110    | 0.01 |      | M6MQ |       |    |         | N9BMR  |       | T3M | N6BIRY  |      |     |
|         |        |      |      |      |       |    |         | X21T-A |       |     | N6BIR-B |      |     |
| HMS7R   | 114    | 0.01 |      |      | M17GM |    |         | N9BLQ  |       |     |         |      |     |
| HMS3R   | 110    | 0.01 |      |      |       |    | N6E     | X21    |       |     |         |      |     |
|         |        |      |      |      |       |    |         | X21P   |       |     |         |      |     |
|         |        |      |      |      |       |    |         | X21T   |       |     |         |      |     |
|         |        |      |      |      |       |    |         | X21T-A |       |     |         |      |     |