MORPHOLOGICAL AND MOLECULAR APPROACHES TO SPECIES IDENTIFICATION IN EQUID CHEEKTEETH FROM GODIN: TERMINOLOGY, TAXONOMY, AND FURTHER IMPLICATIONS

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MA thesis – Summer 2008

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ABSTRACT

The study discussed herein concerns modern Eurasian equids; for the moment, the horse, the donkey, and the onager. These equids are represented in a specific archaeological context (Godin, Iran) by their lower cheekteeth, or premolars and molars. Using morphological techniques developed by Eisenmann (1986), eligible cheekteeth were assigned to one of the three relevant species (horse, donkey, or onager) based on occlusal morphology (specifically, the shape of the linguaflexid and the depth of the ectoflexid). Teeth assigned to species using this morphological technique were subjected to molecular analysis. A region of the mitochondrial D-loop variable amongst the three species was targeted and said sequence was compared to species-known controls for each tooth with a tentative species identification. The initial objective of this study was to compare morphological and molecular techniques for determining species from an archaeological assemblage. However, this study also involved a significant struggle with both terminology and taxonomy, leading to epistemological questions about the relationship between the biological reality of the natural world and human cultural understanding of that world.
INTRODUCTION

Archaeologists are frequently faced with the task of creating or “discovering” typology when presented with material culture. Specific items are separated or grouped together based on diagnostic attributes. This organization of archaeological data is a necessary part of understanding that data. When confronted with biological materials, the process is more complex. The biological materials most commonly found in archaeological contexts are faunal remains: animal bones and teeth. Faunal remains constitute a major source of potential information regarding the lives of ancient people (Klein and Cruz-Uribe, 1984), including subsistence practices (Domínguez-Rodrigo and Pickering, 2003), ritual activities (Alekshin, 1983), and environmental reconstructions (Henry et al., 1985). However, even obtaining basic information about faunal remains from a specific archaeological context can lead to major questions: what type of justification (diagnostic attributes) should be used to organize biological data into a typology or taxonomy (based on species- or genus-level identification, etc.) that agrees with a coherent terminology? Why is this organization (read: categorization) done, and is it necessary? These larger theoretical concerns arise even when undertaking a relatively constrained project.

Three successive issues raise their collective heads, both at the outset of this paper and during the course of this project: terminology, taxonomy, and justification. The first issue concerns terminology, or naming the animals correctly. The issue of terminology leads to a more substantive concern: taxonomy, or correct categorization of the animal in
question. The issue of taxonomy leads to a much larger concern: justification, or how and why we know what this animal is. This issue of justification is best summed up in the following question: what is the relationship between the biological reality of the natural world and our human (cultural) understanding of it?

_Equus: terminology and taxonomy_

This project concerns modern Eurasian equids; for the moment, the horse (_Equus caballus_), the donkey (_Equus asinus_), and the onager (_Equus hemionus_). These equids are represented in a specific archaeological context (Godin, Iran) by their lower cheekteeth, or premolars and molars. Using morphological techniques developed by Eisenmann (1986) and used by subsequent researchers (Davis, 1976, 1980; Forstén, 1986, 1998; Bennett and Hoffmann, 1999; Eisenmann and Mashkour, 1999; van der Made, 1999), eligible cheekteeth were assigned to one of the three relevant species (horse, donkey, or onager) based on occlusal morphology (specifically, the shape of the linguaflexid and the depth of the ectoflexid). Teeth assigned to species using this morphological technique were subjected to molecular analysis. In this molecular analysis, a region of the mitochondrial D-loop variable amongst the three species was targeted and sequenced. A sequence from each morphologically-identified tooth was then compared to the sequences from species-known controls (a horse and a donkey; an onager sequence was not available either from a fresh tissue sample or from a database such as GenBank). The first objective of this study was to compare morphological and molecular techniques for determining species from an archaeological assemblage.
However, terminology (the scientifically “correct” names of these equids) and, therefore, taxonomy (which is interdependent with scientific terminology) rose as major obstacles at the outset of the study. How does one assign species (place into a category) if one cannot properly name that species (category)?

It is important to note that, in the context of this study, it is almost impossible to discuss equid terminology without simultaneously discussing equid taxonomy. The interrelatedness of terminology and taxonomy can border on tautological, although underlying taxonomic structure tends to have a strong impact on resultant terminology. A perusal of the current literature relevant to this study (i.e., concerning living and fossil horses, donkeys, onagers, and other close relatives) leads the credulous reader to believe that equids are protean in nature.

The horse is the common name almost universally associated with the Linnaean or scientific term *Equus caballus*. However, there is debate about whether *E. caballus* constitutes a monolithic species or an umbrella species that includes a number of living and extinct subspecies (Forstén, 1998; Bennett and Hoffmann, 1999). This is perhaps best illustrated by asking whether Przewalskii’s wild horse or the takhi (equivalent common names) is a horse. The terms *Equus przewalskii* and *Equus caballus przewalskii* are both used for this equid. The first term is consistent with the monolithic species status of *E. caballus* (*E. przewalskii* being a separate species by the rules of nomenclature); the second term is consistent with the umbrella species status of *E. caballus* (Przewalskii’s wild horse is a subspecies of *E. caballus* and, therefore, a horse). Some researchers use *E. przewalskii* and *E. caballus przewalskii* simultaneously (Bennett and Hoffmann, 1999), while still others employ terms inconsistent with the rules of
nomenclature (*E. przewalskii* as a subspecies of *E. caballus*; Forstén, 1998), further muddying the waters. This lack of consensus on equids within the scientific community, in this case with regards to terminology, will be a recurring theme in this project. The donkey is the common name almost universally associated with the Linnaean or scientific term *Equus asinus*. However, a number of researchers (Grigson, 1993) characterize both *Equus africanus* and *Equus asinus* as donkeys, differentiated solely by their domesticated status (feral and domesticated, respectively). Others categorize *Equus africanus* as the “African wild ass” (Oakenfull et al., 2000) or the “Nubian ass” (Ivankovic et al. 2002). There is also widespread use of the term “ass” to apply to a variety of common names – donkey, onager, khulan – and Linnaean terms – *E. hemionus onager* (Saltz and Rubenstien, 1995; Reading et al., 2001). Yet others distinguish between the ass and the hemione (Davis, 1980), although the same Linnaean terms (*asinus* and *hemionus*, respectively) are invoked.

The Linnaean term *Equus hemionus* is most commonly associated with the common name onager (Grigson, 1993; Vilà et al., 2006). This is by no means universal. Other common names include: “Asiatic/Asian wild ass” (Meadow, 1989; Saltz and Rubenstien, 1995; Reading et al., 2001), “hemione Asiatic ass” (Oakenfull et al., 2000), “wild half-ass” (Vilà et al., 2006), and “khulan or dziggetai” (van Dierendonck and de Vries, 1996; Reading et al., 2001). *Equus hemionus* is further broken down into subspecies: *E. h. onager*, *E. h. khur*, *E. h. luteus*, and *E. h. kulan* by some authors (Oakenfull et al., 2000; Reading et al., 2001). Notice that the same researchers use different common names or Linnaean terms for the same animal and the wide variety of common names when compared to *E. caballus* and *E. asinus*. 
To enter into a discussion of the myriad species of fossil equids (let alone genuses of fossil perissodactyls) of Eurasia before the Holocene is to go down the rabbit hole (Loomis, 1926; Lindsay et al., 1989; MacFadden, 1992; Alberdi et al., 1998; van der Made, 1999) and will not be attempted here. Even including the extant African equids clouds an already murky picture of the subjects at hand.

Terminologies are invented and implemented differentially by molecular biologists, paleontologists, morphologists, ecologists, conservationists, and zooarchaeologists based on different lines of evidence and for myriad purposes. While confusion over names might be excused at a colloquial (cultural?) level, it is surprising that biological or scientific terminology does not present a unified position on these equids.

For both morphologists and molecular biologists, the taxonomy of the equids is still up for debate. There is, in fact, what might be described as a “high level of uncertainty” (Oakenfull et al., 2000:343) regarding extant Equus taxonomy. This uncertainty about taxonomy translates more precisely into confusion regarding the nature—the biological relationships between and identities of—members of the genus Equus. According to Ishida et al., “the phylogenetic relationship within the species of this genus (Equus) is, however, not well understood” (1995:180). This lack of consensus amongst both morphologists and molecular biologists has very immediate repercussions for zooarchaeologists as they attempt to reliably and consistently identify equid remains (Gilbert et al., 1990). However, in spite of this level of uncertainty, researchers (including zooarchaeologists) continue to conduct morphological and molecular studies of both fossil and extant equids (Rossel et al., 2008).
The third issue, that of justification, will be addressed in later stages of this paper (see Discussion: epistemological considerations). For the moment, it should be noted that this project will take two common approaches to identification and assignment of species: morphological analysis using nonmetric traits and molecular analysis using mitochondrial DNA. Both methodological approaches – morphological and molecular – are widely used in species identification and assignment of equids (Ishida et al., 1995; Bennett and Hoffmann, 1999; Eisenmann and Mashkour, 1999; van der Made, 1999; Orlando et al., 2006; Rossel et al., 2008). However, a widely held assumption is that results based on molecular analysis are more objective or reliable than those based on morphological analysis (Orlando et al., 2006). This pervasive notion deserves some discussion.

However, it is also important to recognize that this project deals with a sample from an archaeological context, and that that context dictates much of the merit of this study.

**Context: archaeological setting and considerations**

This study is grounded to a particular archaeological context that provides another layer of issues and questions. The multi-period archaeological site of Godin is located in central western Iran and includes occupation levels dating from the late Neolithic (Young and Levine, 1974) through the later Bronze Age with a period of interruption until the 8th-6th centuries BC (Young, 1969; Gilbert et al., 1990), with evidence up until the 18th century (Gilbert, 1979). However, the archaeological layers containing faunal materials from which this study’s sample were drawn come from four periods: VI (mid-fourth millennium BC) through II (750 BCE; M. Rothman, pers. comm. 2008). The resultant
faunal assemblage from these four layers (VI, IV, III, and II), which is both large and diverse, contains a number of equid remains. Of these equid remains, 42 cheekteeth (premolars and molars) were isolated for species identification. Two separate paths of species identification were possible: morphological and molecular analyses.

Godin represents “a major town on the principal E-W trade route through the central Zagros Mts (that) was almost certainly involved in the movement of goods between lowland Mesopotamia and the Persian plateau from at least as early as the mid-4th century BC” (Gilbert et al, 1990:40). During the five periods relevant to this study, Godin underwent a series of demographic transitions: expansion from the mound/tepe into the nearby valley and doubling of the number of village sites (period VI: 4,000-3,000 BCE); influx of the Yanik or Early Trans-Caucasian II culture (period IV: 3,000-2,600 BC); and decentralization with commensurate intensification of local commerce and possible return or rise of pastoral nomadism (period III: 2,600-1,600 BC; M. Rothman, pers. comm. 2008). In particular, the presence of equid remains at a site spanning this time period engages some highly relevant archaeological issues: nomadic pastoralism, migration, and equid domestication.

Equid remains have appeared in association with human (or Homo, to be more precise) archaeological sites in Eurasia since at least the early Middle Pleistocene (van der Made, 1999), long before the domestication of either the horse (Equus caballus) or donkey (E. asinus; see Levine, 1999; Olsen, 2006; Vilà et al., 2006; Rossel et al., 2008). The process and timing of horse (Olsen, 2006) and donkey (Vilà et al., 2006) domestication is up for debate but domesticated equids could be represented at Godin as early as period VI (Chalcolithic), although this is much more likely to be true for the later
periods (Bronze Age and later; but see Grigson, 1993). Equid remains at Godin constitute a relatively small percentage of the total faunal assemblage throughout these four levels (Gilbert, 1979). This relatively small percentage remains fairly constant at between 2.7% and 3.6% throughout the first three levels (VI through III), but more than doubles (7.5%) in period II (P. Crabtree, pers. comm. 2008). In preliminary work at Godin, Gilbert noted “an increase in the number of equids…during period IV” (1979:131), which he attributed this to the presence of the Yanik culture’s pastoralism. It is unclear whether these equids represented consumed or worked (ridden, in traction) animals in a nomadic pastoral society. More recent evidence has pointed to the possibility that this trend is more reflective of herd management practices than nomadism (P. Crabtree, pers. comm. 2008) in the Yanik culture.

As stated above, period IV is thought to mark the migration of the Yanik culture to Godin (Young, 1969). The Yanik culture group is so-named due to “ceramic and architectural similarities” with “the Early Bronze strata at the site of Yanik Tepe in Iranian Azerbaidjan” (Gilbert, 1983:95). This phenomenon is, according to Gilbert, representative of a larger mass movement or migration of peoples from northern regions. Widespread, rapid migration events are caused by many factors, but such events can be aided by advances in transportation technology: the domestication of equids for traction or riding.

Gilbert (1979) cautiously employed the terms “equid” and “equine” in recognition of the possibility that more than one species of Equus was present at the site and that distinguishing between them was difficult; however, he argued that “both hemione and caballine forms were present at the site” (1979:7). While Gilbert expresses confidence in
the presence of both *E. caballus* and *E. hemionus* at Godin, he does not mention *E. asinus* or expound on whether either species of equid present were domesticated and used for purposes other than food production (a major issue for *E. hemionus* in particular, see Vilà et al., 2006).

In addition to morphological analyses, previous researchers working with the equid remains from periods IV and III:4 (3,000-2,600 BCE and 2,600-1,600 BC, respectively) at Godin attempted to identify the materials to species using protein radioimmunoassay (RIA; Gilbert et al., 1990), although no successful results were obtained. This study, then, represents the next step in a long history of attempts to identify and assign species to the equids of Godin: both morphological and molecular approaches not yet attempted, to the best of the author’s knowledge, on the faunal assemblage.

**MATERIALS AND METHODS**

**Morphological analysis**

Forty-two equid mandibular cheekteeth (premolars and molars) were isolated from a larger sample of *Equus* sp. remains from the archaeological site of Godin. These teeth were photographed and then scored for two nonmetric traits described by Eisenmann (1986) that aide in species identification. Of the original 42, it was only possible to tentatively assign species to 14 based on the study’s criteria.
Description of morphological traits. Two nonmetric traits of equid mandibular cheekteeth for species identification outlined by Eisenmann (1986) were used in the morphological analysis of the Godin sample. These two traits were both features of the complex enamel patterning of the occlusal surface of equid dentition (Hillson, 1986): the depth of the ectoflexid (see Fig. 1) and the shape of the linguaflexid or “double knot” (see Fig. 2). According to Eisenmann (1986), a shallow ectoflexid is most commonly seen in *E. asinus*, an ectoflexid of middling length in *E. hemionus*, and a deep ectoflexid in *E. caballus*. “Double knot” refers to the enamel morphology on the anterolingual aspect of the occlusal surface of equid mandibular cheekteeth (Fig. 2). Unlike ectoflexid depth, which differs in depth, the “double knot” (linguaflexid) is theoretically manifested in three different shapes amongst the three equid species in question. This “double knot” shape, therefore, has “three basic morphologies, which can be called ‘caballine’, ‘hemionine’, and ‘asinine’” (Eisenmann, 1986, p76). Eisenmann (1986) describes the “caballine” (for *E. caballus*) morphology as U-shaped, but it is perhaps more accurately described as a half-square when viewing her figures (see her Fig. 25). “Asinine” (for *E. asinus*) morphology is basically described as V-shaped: “deep and pointed” (Eisenmann, 1986, p76). “Hemione” (for *E. hemionus*) is simply described as being uniformly shallow on the anterolingual aspect.
Figure 1. Ectoflexid morphology in equid mandibular cheekteeth. A) An example of a “shallow” ectoflexid, most commonly associated with *Equus asinus*. B) An example of a “deep” ectoflexid, most commonly associated with *E. caballus*. Notice how the ectoflexid almost makes contact with the linguaflexid (aspect of the “double knot”) in the “deep” conformation, although no flattening occurs in this case. The blue highlights the idealized conformation of the ectoflexid. The scale (small bars indicate mm) is placed on the lingual aspect of both teeth.

A tooth was assigned to a specific species only if scored as the same species for both nonmetric traits. For example, tooth id 11 (see Table 1) has both a V-shaped
linguaflexid and a shallow ectoflexid; it was therefore scored as *E. asinus*. On the contrary, tooth id 5F has a V-shaped “double knot” (linguaflexid) but also has a deep ectoflexid; therefore, it could not be assigned to species using the nonmetric criteria of this study. This restrictive process was designed to maximize the efficacy of morphological analysis by only assigning species to teeth that displayed definitive traits. Only teeth meeting these criteria would be subjected to molecular analysis to a balanced test of morphological methods.

**Tooth selection.** Forty-two equid mandibular cheekteeth from the multi-period archaeological site of Godin were photographed in occlusal aspect with a Nikkon Digital Camera for two purposes: to preserve a record of the key traits in anticipation of the possible damage due to drilling (see Molecular analysis), and to provide the author with the opportunity to examine the morphological features in two-dimensional perspective (presuming higher compatibility with the 2-D line drawings of previous methods; see Eisenmann, 1986). Each tooth was positioned in two orientations for different images: “occlusal” and “flat”. “Occlusal” orientation best recreated the tooth in its original position in the mandible. The “occlusal” orientation is only a reasonable extrapolation of *in situ* position for the purposes of retaining the real-life topography of the occlusal surface, not a morphologically accurate positioning. The “flat” orientation was created by placing a circular level on a small piece of flat plastic (ruler) on the occlusal surface and leveling it. This orientation was used to minimize the impact of natural topography on the image of the occlusal surface.
Figure 2. The three types of “double knot” morphology seen in equid mandibular cheekteeth (occlusal aspect). A) Caballine U-shape, most frequently associated with *Equus caballus*. B) Asinine V-shape, most frequently associated with *E. asinus*. C) Hemione shallow and flat configuration, most frequently associated with *E. hemionus*. The red highlights the idealized conformation of the “double knot” for each type. The scale (small bars indicate mm) is placed on the lingual aspect of both teeth.

After images were captured, the teeth were sorted for suitability to the morphological techniques for differentiation of mandibular permanent cheekteeth outlined by Eisenmann (1986). Forty-two teeth were selected for morphological analysis because they were: 1) part of the permanent dentition (no subadult teeth), 2) $P_3$-$M_2$ (Eisenman recommends using only these teeth for her techniques), and 3) well-preserved...
[teeth were rejected if key traits were potentially obscured or distorted (see Table 1)].

Therefore, species assignment based on the two nonmetric traits was attempted with 42 teeth. Of the 14 equid mandibular cheekteeth assigned to species via morphological analysis, only ten of these teeth were then subjected to molecular analysis.

**Table 1**

Equid mandibular cheekteeth submitted to morphological analysis for species assignment based on two nonmetric traits, following Eisenmann (1986). All teeth listed are part of the permanent dentition (no subadult teeth) and in good condition (well-preserved, no more than moderately worn). Though it is difficult to assign specific position to most ex situ equid cheekteeth, none of these teeth represent P₂, which would be grounds for elimination from analysis.

<table>
<thead>
<tr>
<th>Tooth ID</th>
<th>Ectoflexid</th>
<th>Linguaflexid</th>
<th>Species</th>
<th>Context (period at Godin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1I*</td>
<td>Shallow</td>
<td>V-shaped (double knot)</td>
<td>E. asinus</td>
<td></td>
</tr>
<tr>
<td>1T*</td>
<td>Shallow/moderate</td>
<td>Flat, shallow</td>
<td>E. hemionus</td>
<td>III</td>
</tr>
<tr>
<td>1Z*</td>
<td>Moderate</td>
<td>Flat, shallow</td>
<td>E. hemionus</td>
<td>Mixed IV through IV</td>
</tr>
<tr>
<td>1.Y.A*</td>
<td>Shallow</td>
<td>V-shaped</td>
<td>E. asinus</td>
<td>Between II and III</td>
</tr>
<tr>
<td>2Y*</td>
<td>Shallow</td>
<td>V-shaped</td>
<td>E. asinus</td>
<td>Between II and III</td>
</tr>
<tr>
<td>3Z</td>
<td>Shallow/moderate</td>
<td>Flat, shallow</td>
<td>E. hemionus</td>
<td></td>
</tr>
<tr>
<td>5Z</td>
<td>Shallow/moderate</td>
<td>Flat, shallow</td>
<td>E. hemionus</td>
<td></td>
</tr>
<tr>
<td>G1B*</td>
<td>Shallow</td>
<td>V-shaped</td>
<td>E. asinus</td>
<td>Mixed II and III</td>
</tr>
<tr>
<td>G1D*</td>
<td>Deep</td>
<td>Flat, shallow</td>
<td>E. hemionus</td>
<td>Mixed II and III</td>
</tr>
<tr>
<td>G1F</td>
<td>Deep</td>
<td>Flat, shallow</td>
<td>E. hemionus</td>
<td></td>
</tr>
<tr>
<td>G1G*</td>
<td>Shallow</td>
<td>Flat, shallow</td>
<td>E. hemionus</td>
<td>Mixed II and III</td>
</tr>
<tr>
<td>G2E</td>
<td>Shallow</td>
<td>Flat, moderately shallow</td>
<td>E. hemionus</td>
<td></td>
</tr>
</tbody>
</table>
M1B* Very deep U-shaped *E. caballus
M1C* Very deep U-shaped *E. caballus

*Subjected to molecular analysis (see Table 2).

**Molecular analysis**

Of the 14 equid mandibular cheekteeth assigned to species via morphological analysis, only ten of these teeth were then subjected to molecular analysis. Each tooth was drilled to obtain powdered tooth material. Mitochondrial DNA was extracted from the powdered tooth material, amplified using equid-specific primers designed by the author, and sequenced. These sequenced regions of mitochondrial DNA were then compared to mitochondrial DNA of known equid species (from fresh tissue samples obtained by the author) using statistical analyses to assign the unknown teeth to species.

*Primer design.* Six primers (three forward, three reverse) were designed by the author to target an amplicon in the 12s region of the equid mitochondrial genome to the exclusion of other members of the Order Perissodactyla [i.e., rhinoceros (Rhinocerotidae: Gray, 1821), tapir (Tapiridae: Gray, 1821)]. The target segment (∼500 bp amplicon) of the 12s region was selected for its variability between all three species to maximize molecular differentiation. The six primers were designed to flank this target region in both *E. asinus* and *E. caballus*, as well as for their physiochemical qualities: within an optimal distance from the target region, a relatively high GC content and melting point, and optimal primer length for proper binding to the target region (see Fig. 3).
Extraction. Two major phases of mitochondrial DNA extraction occurred: fresh tissue samples collected by living animals of known species and powdered dental material collected from the 10 unknown archaeological samples (see Table 2). Fresh tissue samples were prepared following the Qiagen DNeasy sample preparation protocol (Scherczinger et al., 1997; see Appendix A). These extractions were tested for the presence of mtDNA using 12s universal primers (designed to amplify a ~500 bp region in all vertebrates) before being used to test the equid-specific primers designed by the author (see below). The unknown samples were prepared as follows: teeth were cleaned to remove any residual geological matrix and treated with a 1:10 bleach solution to remove foreign DNA. Each tooth was drilled to obtain at least 300 mg or more (except one tooth, which only yielded 112 mg) of powdered material. Mitochondrial DNA was extracted from this powdered tooth material following a modified version of the GENECLEAN Ancient DNA extraction protocol (BIO 101, Inc., 1997; see Appendix A).

Table 2

Fresh tissue samples and unknown archaeological samples (Godin, Iran) subjected to molecular analyses. See text for further details

<table>
<thead>
<tr>
<th>Source</th>
<th>Sample type</th>
<th>DNA?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Godin</td>
<td>Tooth</td>
<td>Yes&lt;sup&gt;c,e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Living E. caballus (horse)</td>
<td>Blood</td>
<td>Yes&lt;sup&gt;a,b,c,d,e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Buccal swab</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Hair</td>
<td>No</td>
</tr>
<tr>
<td>Living E. asinus (donkey)</td>
<td>Blood</td>
<td>Yes&lt;sup&gt;a,h,d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Buccal swab</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Hair</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Scrapped skin (scab)</td>
<td>Yes&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
a Primers designed to target the ~500 bp region of the equid mitochondrial genome were tested on these samples.

b Successful PCR amplification with universal vertebrate primers.

c Successful PCR amplification with equid-specific primers.

d Successful sequencing of mtDNA.

e Successful sequencing of target mitochondrial region.

F1: 5’ – CGA TAG CTA AKC CCC AAA CTG G – 3’
E. caballus: - - - - - - - - A - - - - - - - - - - 
E. asinus: - - - - - - - - G - - - - - - - - - - 

F2: 5’ – ACC TCT GAC CAC ACG ATA G – 3’
E. caballus: - - - - - - - - - - - - - - - - - - - - - 
E. asinus: - - - - - - - - T - - - - - - - - - - - 

F3: 5’ – CAC GAC AGC TAA GGC CCA AAC TGG GA – 3’
E. caballus: - - - - T - - - - - - - - - - - - - - - - 
E. asinus: - - - - T - - - - - - - A - - - - - - - - - 

RJ1: 5’ – CGA CTT GTC TCC CTT CAT ATG GTT GG – 3’
E. caballus: - - - - - - - - - - - - - - - - - - - - - 
E. asinus: - - - - - - - - - - - - - - - - - - - - - 

RJ2: 5’ – CCT TGT TAC GAC TTG TCT CCT TTC – 3’
E. caballus: - - - - - - - - - - - - - - - - - - - - 
E. asinus: - - - - - - - - - - - - - - - - - - - - 

RJ3: 5’ – CTT CTA GGT GTA AGC TGG ATG CT – 3’
E. caballus: - - - - - - - - - - - - - - - - - - - - 
E. asinus: - - - - - - - - - - - - - - - - - - - - 

Figure 3. Primers designed to target ~500 bp in a region of the mitochondrial genome variable amongst the relevant extant equid species. Primers were designed to have a GC content approaching 50% or greater, minimize delta G value (representing risk of self-dimerization), and for optimal length (between 20 and 25 bp) and melting point (55-59 °C). GenBank sequences from E. hemionus are not available for this entire
amplicon; however, they are available for a good portion of the 12s mitochondrial region and allow for phylogenetic comparisons.

**Test of equid-specific primers.** The six primers discussed above were tested in all possible combinations on successful fresh tissue extractions in order to determine which forward and reverse primer combination amplified the target sequence most effectively. Mitochondrial DNA from *Equus asinus* and *E. caballus*, respectively, were collected from a variety of fresh tissue samples (see Table 2). A fresh tissue sample from *E. hemionus* was not available to the author at the time of this analysis. A polymerase-chain reaction (PCR) protocol was designed for these six equid-specific primers and the amplicon (target sequence) desired for this project (see Appendix A). After a number of trials, a forward-reverse primer combination of F1 and RJ3, respectively, was chosen to amplify the desired sequence from the 10 extraction samples. This forward-reverse PCR combination also yielded sequences from the fresh *E. caballus* sample to be used in later analyses (see Molecular modeling and analysis).

**Amplification and sequencing of unknown samples.** Using the F1-RJ3 forward-reverse primer combination above, the 10 unknown samples were subjected to the optimal PCR protocol for amplification in order to achieve the requisite amount of the target sequence (see Appendix A for PCR protocol followed). Successful PCR amplification was determined by running a current through an agarose gel (1.2% in 1X TBE buffer) containing the PCR products (not shown), demonstrating successful amplification of the target sequence from three teeth: G1B, M1B, and G1D (see Table 1). These three successful PCR products from the unknown samples were then sequenced
(sequencing protocol can be found in Appendix A). All sequencing was performed on a 3730 sequencer.

**Molecular modeling and analysis.** Target sequences successfully amplified via PCR and sequenced from both unknown Godin and known fresh tissue samples were analyzed using a variety of computer programs. Target sequences were first imported from the 3730 sequencer and visually examined in Sequencher (Nishimura, 2000). This program also allowed for the editing of said sequences, especially in the case of “obvious errors” or “messy tails”, where the primer binding site may have allowed for the pick-up of extraneous bases. After visual examination and editing, all the sequences were run through BLASTalign (available online at: [www.ncbi.com](http://www.ncbi.com)) that compared the sequences to sequence DNA on file at GenBank to give rough species/genus affiliation and type of DNA sequenced (mtDNA, nuclear, etc.). All three successfully sequenced unknown samples and a fresh tissue *E. caballus* sample amplified with the same primer set (positive control) was aligned with published complete mitochondrial genomes of *E. asinus*, *E. caballus*, and *E. hemionus* from GenBank using TCOFFEE (Notredame et al., 2000) and Muscle (Edgar, 2004). Two complete rhinoceros mitochondrial genomes (*Ceratotherium_simum* and *Rhinoceros unicornis*, also from GenBank) were included for comparative purposes and to serve as outgroups for later analyses. This alignment of the three target sequences and published GenBank mtDNA sequences was imported into MacClade (Maddison and Maddison, 2005) and visually inspected, much as in the initial step of analysis (see above), in that the sequences could be edited in order to give a “better” or “more accurate” alignment.
RESULTS

Morphological analysis

Using Eisenmann’s two nonmetric traits for equid mandibular cheekteeth, it was possible to assign a number of the Godin teeth to species. However, of the original 47 intact teeth, it was only possible to assign species to 14 of these teeth: four to *E. asinus*, two to *E. caballus*, and eight to *E. hemionus*. The results of morphological analysis can be seen in Table 1. A tooth was assigned to a specific species if both nonmetric traits conformed to the typology described for that species. However, based on Eisenmann’s (1986) protocol for morphological scoring, some variation in strict typology is expected, which compounds the difficulty of species assignment (see Discussion). All four teeth assigned to *E. asinus* displayed a V-shaped linguaflexid and a shallow ectoflexid, although one tooth with a moderate ectoflexid was still scored as *E. asinus*. Both teeth assigned to *E. caballus* displayed a U-shaped linguaflexid and a very deep ectoflexid. However, both of these teeth were found in situ in the same mandible; therefore, it is unsurprising that they were scored identically. All eight teeth assigned to *E. hemionus* displayed a shallow linguaflexid; however, as Eisenmann (1986) did not prescribe a strict conformation for the ectoflexid of *E. hemionus* (an intermediate conformation between *E. asinus* and *E. caballus* seems assumed), the *E. hemionus* group display shallow-moderate to deep ectoflexids (see Table 1).

Molecular analysis
Of the 14 equid teeth from the Godin archaeological sample assigned to species (see above), only 10 teeth were subjected to molecular analysis. Of these 10 teeth, working mitochondrial sequence of the target length using equid-specific primers was only obtained from three teeth. Cursory comparison of a global alignment with known equid mitochondrial sequences, the positive control, and the three unknown Godin sequences (see Table 2) yields interesting albeit tentative findings. Sequence from G1B (morphologically identified as *E. asinus*) most closely resembles known published *E. asinus* sequence. Sequence from G1D (morphologically identified as *E. hemionus*) most closely resembles known published *E. hemionus* sequence. Sequence from M1B (morphologically identified as *E. caballus*) most closely resembles known published *E. caballus* sequence as well as the known *E. caballus* positive control sequence.

The amplified and sequenced mitochondrial DNA from these teeth represents an exciting find; however, these sequences represent the initial stages of what would be a more extensive research project and cannot be treated as more than preliminary results. The “global” sequence alignment (see Methods), containing the one known *E. caballus*, *E. asinus* and *E. hemionus* from GenBank, and three unknown Godin target sequences, requires further analysis in order to come to a more firm conclusion about the species assignment of the three unknown Godin sequences.

These preliminary results are nonetheless encouraging for three reasons. First, the target sequences from teeth G1B, G1D, and M1B are all different from one another. Second, teeth G1B and G1D are also divergent from the positive control (known *E. caballus*, see Table 2). Tooth M1B is slightly more difficult to interpret, as it is very
similar to the known *E. caballus* sequences (both published and the positive control) yet not identical to either: it is not clear whether this target amplicon shows variation within *E. caballus* or not. However, both of these facts demonstrate that these preliminary findings are not the result of laboratory contamination. Third, this indicates that DNA recovery is possible and that further research and experimentation on the Godin equid dental assemblage is merited. This is particularly important considering past difficulties encountered when trying to recover biochemical information from the equid assemblage at Godin for the purposes of species identification (Gilbert et al., 1990). Furthermore, the above three reasons indicate that the target mitochondrial sequences from the three Godin teeth can be reasonably interpreted to agree with their species assignment based on the two morphological criteria. However, this is a tentative inference based on the available evidence rather than a definitive statement.

**DISCUSSION**

**Species assignment: morphological and molecular approaches**

Forty-two equid mandibular cheekteeth (premolars and molars) were isolated from a larger sample of *Equus* sp. remains from the archaeological site of Godin and assigned to species based on two nonmetric traits described by Eisenmann (1986): ectoflexid depth and linguaflexid shape. Of these 42, it was only possible to tentatively assign species to 14 based on these morphological criteria (see Table 1). Ten of these 14 teeth were then subjected to morphological analysis, of which only three yielded genetic
material suitable for species assignment. Although the results of the molecular analysis are preliminary, the comparison of morphological and molecular methods and results is justified.

Morphological techniques of species assignment identified the three posited species of equids at Godin: *E. asinus* (I1, Y.A., 2Y, and G1B), *E. caballus* (M1B and M1C), and *E. hemionus* (1T, 1Z, 3Z, 5Z, G1D, G1F, G1G, and G2E). Molecular analysis, although tentative, yielded divergent sequences from teeth G1B, M1B, and G1G. It is too early to state with surety that the molecular results agree with the morphological species assignment; however, their possible agreement is supported by the fact that the three target mtDNA sequences are different from one another and thus possibly represent three different species based on molecular criteria.

Based on morphological results alone, the three possible species of equid – *E. asinus*, *E. caballus*, and *E. hemionus* – are represented in the Godin archaeological assemblage. This is an important finding, given that previous researchers have only documented the presence of *Equus* sp. rather than identified particular species present in the Godin assemblage (Young, 1969; Young and Levine, 1974; Gilbert, 1979; Gilbert et al., 1990). However, we are interested in species assignment based on the molecular methods. The current molecular results are preliminary and require statistical modeling of their relationships to one another and known equid mitochondrial DNA sequences in order to provide strong support for or negation of morphological results. More also needs to be known about intraspecies variation within the target equid sequence; this issue is not unique to *Equus* nor this project, but it does underscores some of the difficulties in species assignment and identification with even molecular data.
Confirmation of the preliminary molecular results would lead to a consensus on species assignment arrived at independently via morphological and molecular methods. The implications of this hypothetical situation would be important for future work regarding equid species identification in other assemblages, most significantly in that the consensus between morphological and molecular species assignment could allow researchers to employ the less labor- and resource-intensive morphological methods with the same confidence as molecular methods. There are, however, greater issues regarding the premises of this project that deserve serious attention.

**Epistemological considerations**

The focus of this study is the identification of equid teeth discovered at the archaeological site of Godin in Iran. The study is relatively straightforward: the assignment of species to this sample of equid teeth. In this study, the assignment of individual teeth to species was a two-step procedure. Step one was morphological analysis; step two was molecular analysis. Morphological analysis for this project meant examining lower cheekteeth and comparing two nonmetric traits (visible features) of each tooth to those nonmetric traits from teeth typed/classified by a zooarchaeologist specializing in equid morphology (Eisenmann). A tooth was assigned to a type or species if it conformed to Eisenmann’s type to a reasonable degree; if it looked very much like the published version for the type or species. Molecular analysis for this project meant extracting mitochondrial DNA (mtDNA) from teeth assigned to species in the first step. A target region (amplicon) from the 12s region of the mitochondrial genome was
compared to said region/amplicon drawn from animals of known species. The somewhat unspoken assumption going into the project was that, one way or another, results from these two methods would yield straightforward results. While these methods did yield results, this project also led to questions about the role of the scientist as interpreter of pure scientific data.

Two major questions about whether the scientific inquiry in this project holds a mirror up to objective truth or reality, or whether it operates as a necessary lens that categorizes that information into comprehensible categories of thought deserve mention. First, is the notion of “species” a strictly scientific construct or is it also informed by the cultural context? Second, is molecular analysis actually a better way of arriving at objective reality than morphological analysis? Each of these two questions will be addressed in turn.

First, is the notion of “species” a strictly scientific construct or is it also informed by the cultural context? The sample examined in this project consisted of a number of teeth from an archaeological site. These teeth are equid teeth, meaning that they belong to one of the many members of the genus Equus. These equid teeth were sorted from other teeth at the site by a zooarchaeologist. This assessment was made before my work with the teeth began and its accuracy is not in dispute. The sample’s temporogeographic context was such that three possible species could be considered: Equus caballus, E. asinus, and E. hemionus. From a layman’s point of view, determining whether the teeth were horse (E. caballus) or donkey (E. asinus) was a fairly straightforward task. I know what a horse is; I know what a donkey is. Equus hemionus was a different matter entirely.
What about *Equus hemionus*? When we don’t ‘know’ what an animal is, our scientific understanding of it reflects this. If I ‘know’ what a horse is and I ‘know’ what a donkey is, I cannot honestly say that I do not ‘know’ what *Equus hemionus* is. First, I looked for a common name, like ‘donkey’, to go with *Equus hemionus*. The Linnaean name seemed most frequently associated with the common name ‘onager’ (notably a Roman siege weapon). However, kiang, kulan, and Asiatic wild ass also appeared in a variety of combinations. Cross-checking the term ‘onager’ brought up new Linnaean names (*E. hemionus onager*, *E. hemionus hemionus*, *E. luteus*) and variations on ‘onager’ (Persian onager). Why wasn’t there a straight and consistent association between *Equus hemionus* and onager?

‘Onager’ was unfamiliar to me because I had no cultural exposure to it. I could not conceptualize it. I did not ‘know’ what an onager was. I first reasoned that, as a relatively rare undomesticated species, it made sense that the onager did not get the same treatment as the horse and the donkey. This view was entirely untenable – many rare wild animals live in our phrases (“the elephant in the room”), parables (“can the leopard change its spots?”), verbs (to lionize someone), songs (“Eye of the tiger”, “Baby beluga”), and even commercials (the Coca-Cola polar bears). Like some of these wild animals, the onager purportedly had close associations with historical groups as deep in the past as ancient Mesopotamia (Zarins, 1978, Gilbert et al., 1990; but see Vilà et al., 2006) who had profound influences on the modern West. As noted above, the Romans named one of their siege weapons after the onager and its mighty kick. Yet, for whatever reason, the onager does not have a place in Western cultural context like the horse and donkey do. This is perhaps the root of the problem. I do not ‘know’ the onager in a
cultural sense. Therefore, it is difficult to define the onager and *Equus hemionus* in a scientific, species-concept sense. When we don’t ‘know’ what an animal is, our scientific understanding of it reflects this.

What we ‘know’ unscientifically about an animal may reflect what we know scientifically about it. The horse and the donkey both have a firm place in modern Western culture. We ‘know’ what they are. We can conceptualize both, having had significant cultural exposure to them. This cultural exposure is how we ‘know’ what the horse and donkey are. The horse is fast: (the Kentucky Derby), beautiful (Anne Sewell’s “Black Beauty), powerful (horsepower of a car), a friend of conquerors (Bucephalus) and children (“A horse and his boy”, “National Velvet”) alike, and associated with prestige and sexuality. The donkey is stubborn (or so goes its association with the Democratic National Committee), moody (A.A. Milne’s Eeyore), an object of amusement (pin-the-tail-on-the-donkey), an insult (“jackass”), and associated with comic relief (Sancho Panza on his donkey, Bottom in “A Midsummer’s Night Dream”) and moralistic punishment (Midas’ ears, the boy-donkey metamorphoses of Disney’s “Pinocchio”). These cultural contexts for the horse and the donkey impact what we ‘know’ about the animals.

Specifically, the horse and the donkey constitute different cultural entities and we accept that they are in fact different things.

We ‘know’ that the horse and the donkey are different from one another. They are, therefore, easily accepted as different species. We assume that the onager must also be different from both a horse and a donkey. But species is not a ‘negative’ definition in terms of either terminology (naming) or taxonomy (categorization): the donkey is not a “not-horse”. With species we create a category to encompass what we observe about an
animal that also differentiates it from animals we ‘know’ to be different. Categorization illustrates how we are able to think about and understand these animals. A “species” distinction is most understandable, and perhaps most useful, if it reflects a distinction we already accept.

If the equid teeth are to be sorted by species, it is important to spend a moment on species concept. The scientific concept of species is basically putting living things into categories based on certain similarities. A and B are different. They constitute different species. Is C like A or B? Is C enough like A to be called ‘A’? This categorization can be done in many ways, as there a number of competing definitions of ‘species’. Do the two things share a common ancestor (cladistics)? Do the two things look alike (morphology)? Do the two things produce offspring (interbreeding)? Species concept is a major subject of scientific debate and will not be fully addressed here. However, the traditional biological species concept is often invoked as a basic test of whether two animals belong to the same species.

The traditional biological definition of species concept is that two animals belong in different species if they cannot interbreed to produce viable offspring. However, the horse and the donkey can interbreed to produce mules (the offspring of a male donkey and a female horse) and hinnies (the offspring of a female donkey and a male horse). In fact, some female mules or hinnies (mollys) are fertile and able to bear offspring with either a horse or a donkey in rare cases (Xu et al., 1996). This does not change the scientific view that the horse and the donkey are two different species. *Equus caballus*, the horse, and *Equus asinus*, the donkey, are seen as genetically and morphologically distinct. They are, therefore, different species.
‘Horse’, ‘donkey’, and ‘onager’ are all separate cultural and scientific categories. Overall, species concept represents scientific organization by categorization. Species are categories designed to make the living world understandable. Species, therefore, represent both the objective reality of the living world and our ability to organize and understand it through categorization. The scientific concept of species is most useful when it confirms our cultural understanding of an animal (arrived at ‘unscientifically’). The horse-donkey-onager example illustrates this aptly. We accept the scientific species distinction between the horse and the donkey fairly easily because it conforms to what we already ‘know’ culturally about them. We have more trouble with *Equus hemionus*/onager because we have no cultural exposure to it. Archaeologists, conservation biologists, morphologists, and molecular biologists frequently use local or indigenous (read: nonscientific) names for *Equus hemionus*/onager in scientific literature: khulan, dziggetai, kiang, etc. This lack of cultural understanding makes it more difficult for us to understand the scientific species or category.

Now to the second question: is molecular analysis actually a better way of arriving at objective reality than morphological analysis? This question arose during the implementation of both methods. Therefore, a closer look at the two types of methods is merited.

In this project, the morphological methodology focused on nonmetric traits, which is quantitatively and qualitatively different than metric approaches to morphology. This difference is critical. Particularly concerning nonmetric methodologies, morphology in the broadest sense deals with how things look. The morphological method for this project compared patterns on the lower cheekteeth from an archaeological sample to
drawings of equid teeth from known species by Eisenmann, a zooarchaeologist specializing in morphology. Teeth are categorized based on whether they look like those of one of the known species. Does the tooth in your hand look more like the morphologist’s drawing of a horse tooth or of a donkey tooth? As implied above, morphological analysis can be conducted in many ways, but this is a technique developed for these categories (horse-donkey-onager) and for this type of sample (teeth). The technique’s author (Eisenmann, 1986) makes it fairly evident that it is open to ‘human error’. This is a nonmetric methodological approach: there is no quantification or statistical test to mediate between the data and the scientist/observer’s interpretation of the data. The scientist/observer makes a straightforward assignment based on what she sees.

Molecular evidence and analysis are widely considered to be more objective than their morphological counterparts (Orlando et al., 2006). A relatively short sequence (approximately 500 base-pairs) of the 12s region of the equid mitochondrial genome that varied between what is on record for the three species is supposed to provide ‘better’ insight on the animal in question and which category or species it belongs in. In fact, molecular results were assumed to have the power to reject or confirm morphological results. A statistician specializing in biological research made the argument that molecular data is more objective than morphological data: a position that, to his credit, he quickly qualified (D. Tranchina, pers. comm.). However, the assumption that molecular results should trump morphological results remained and is a widespread albeit rarely stated belief. What is the basis for this view, and is it supported by a comparison of the two methods?
There are two basic reasons to suppose that molecular results trump morphological results: quantity of data and the way those data are interpreted. Molecular data comes in a much larger quantity and is also more easily quantifiable. The morphological analysis of this study consisted of scoring two nonmetric traits on equid teeth. The molecular analysis of this study consisted of comparing approximately 500 bp of the 12s mitochondrial genome. Quantity alone allows for more possible information to be gleaned from the data. More information, in theory, should allow for a better species assignment due to a greater number of points of comparison. However, data of great quantity (and quality) still need to be interpreted. This is where a comparison of the interpretation of morphological vs. molecular data is useful.

Unlike morphological data, molecular data cannot be interpreted without the aid of advanced technology and sophisticated statistical analyses. With morphological analysis, the scientist directly observes the traits on the tooth and make an assessment at to which category their pattern belongs to. The only tools used are eyes and judgment derived from training and experience. With molecular analysis, the scientist must first extract the ‘unobservable’ data (mitochondrial DNA, which cannot be viewed by the naked eye), amplify it, sequence it, then use a variety of statistical analyses (maximum parsimony, maximum likelihood, and Bayesian analysis) to decide which of the three types (horse, donkey, or onager) the sequence belongs to. In this molecular analysis, a wide variety of practical and theoretical tools were used. However, the scientist’s judgment also came into play.

In this molecular analysis, a number of decisions about how to carry out the analysis were made based on experience and ‘knowing what I was looking for’. In any
molecular analysis, the scientist has to decide what kind of DNA (nuclear or mitochondrial?) and how much DNA (how many base-pairs? A whole chromosome?) to look at. The scientist has to decide how much variation between horse, donkey, and onager DNA is ‘enough’: sufficient to categorize them into discrete species. The scientist has to decide how to analyze the sequenced DNA: which statistical test gives the ‘right’ result (reflects objective reality)? The scientist has to visually inspect the sequenced DNA for errors based on what he or she already knows the sequence should look like. The scientist has to use his or her judgment based on what he or she knows about the statistical tests to decide if the test has given the ‘right’ answer. In other words, the data still has to be interpreted. Choices made by the scientist during the course of the analysis dictate the nature of conclusions about species, to which category each unknown animal belongs. Most importantly, lines must still be drawn around the data: horse vs. donkey vs. onager. Neither a computer nor a genetic sequencer can draw those lines: on a human, the scientist, can. Therefore, judgment still has to be exercised. ‘Human error’ – the interpretive role of the scientist/observer – can never be removed from the process.

In this particular project, molecular data may have a distinct quantitative advantage over the morphological data. This does not necessarily mean that molecular analysis will always give a ‘better’ answer than morphological analysis. While quantity and quality of data are important, data are only as accurate and informative as their interpretation. The strengths and weaknesses of the scientist/observer’s interpretation exist in both morphological and molecular analyses. Unlike a tooth, DNA is not visible to the naked eye. Unlike a tooth, DNA cannot be assessed or interpreted by just looking at it. However, like a tooth and its visible features, DNA is eventually assessed and
interpreted by the scientist/observer. The difference is not that ‘human error’ is removed during molecular analysis. ‘Human error’, or the interpretation of the scientist/observer, merely occurs at a different stage. The point at which the scientist/observer interprets the data is the major difference between the morphological and molecular analyses discussed above. Both sets of data require human interpretation. This is a necessary lens through which objective reality is filtered. How else would the information be made understandable and meaningful?

The scientist/observer’s interpretation is a necessary and inescapable process. The ‘human error’ that improvements in science seek to remove could never be fully removed. Nor should it be. It is important to realize that even the most advanced scientific technologies are designed for human understanding. Science does not magically reveal truth and objective reality. Even if it did, we would be unable to understand it. In practicing science, we create categories in order to understand the natural world and other complex phenomena outside of our making. This does not mean that science is just another cultural construct to be ranked with all other cultural constructs. This examination of how we know what we know shows us that we practice science and create categories to make things understandable to us. The practice of science and creation of categories is not about objective reality. It is about human beings trying to understand that objective reality.

The relatively straightforward study discussed above led to the consideration of two major questions seemingly outside the scope of the project. First, is “species” a strictly scientific construct or is it also informed by the cultural context? When confronted with the subjects of this project – the horse, the donkey, and the onager – the
relationship between scientific species concept and cultural understanding of an animal comes under scrutiny. This scrutinization suggests that the scientific concept of species is most useful when it confirms our cultural understanding of an animal (arrived at ‘unscientifically’).

Second, is molecular analysis actually a better way of arriving at objective reality than morphological analysis? The primary task of this project – species assignment – forced an examination of whether the view that molecular analysis and results trumped morphological analysis and results was justified. Other researchers have similarly wrestled with this issue in a zooarchaeological context (Jung et al., 2002). While the tentative conclusion is that molecular data has advantages over morphological data in this project, conclusions about the two types of analyses were less cut-and-dry. Both morphological and molecular analyses involve the interpretation of the scientist/observer. The major difference between morphological and molecular analysis is the point at which the scientist/observer interprets the data. Neither type of analysis – nor science in general – holds up a mirror to objective reality. Rather, that object reality is filtered through a necessary lens: the scientist/observer’s interpretation. The exploration of these two questions returns to categorization and how it relates to what we think we know.

The process of categorization and how it relates to what we think we know is related to the question of whether the scientific inquiry in this project holds a mirror up to objective truth or reality, or whether it operates as a necessary lens that categorizes that information into comprehensible categories of thought. The horse-donkey-onager project illustrates that categorization in archaeology and other sciences reflect the cultural acts of drawing lines around and making discrete entities of the natural world. This is done to
make objective reality understandable. This does not mean to imply that there is no objective reality; rather, this highlights the interactive nature of our perception and our attempts to ‘know’ what is real and objective. These categories lie on the interface between cultural and scientific knowledge. We have constructed the categories and types that we seek to learn about, using differences between things to inform us of their essential identity, reality.

This is not to say that archaeologists and other scientists should stop attempting to assign teeth or other materials to species. Nor does it mean that science is just like any other cultural construct and plays no important role. It means that we need to pay more attention to what we are doing when we practice science. It means that how we know what we know influences what we do and, therefore, what we know. This is generally acknowledged to be true of our work in making sense of human-created archaeological materials. We recognize that our interpretations are filtered through our own understandings and, therefore, attempt to place the artifacts in a cultural context in addition to the physical one. This project and its implications suggest that there are ways in which our understanding of biological remains poses the same kind of epistemological problem.

CONCLUSIONS

Forty-two equid teeth from the faunal assemblage recovered at Godin, Iran, were analyzed using morphological and molecular methods in order to identify and assign species. Three equid species – *E. asinus*, *E. caballus*, and *E. hemionus* – were identified
using the morphological methods of Eisenmann (1986) in 14 teeth. Molecular methods resulted in three different mtDNA sequences from three of the 14 teeth, although these preliminary results can neither confirm nor negate the morphological findings at this state. At a minimum, it appears that the three species of equid coexisted in various periods at Godin, which adds to current understanding of the faunal assemblage at the site. Epistemological considerations regarding the justification of species identification and assignment, taxonomy, and terminology, as well as morphological and molecular data and species concept, are well-served by a project examining the extant equids at Godin: the donkey (*E. asinus*), the horse (*E. caballus*), and the onager (*E. hemionus*).

**ACKNOWLEDGMENTS**

I would like to thank the numerous individuals whose input and assistance made this research project possible. I thank J. Hodgson, K. Sterner, T. Tosi, and the rest of the New York University Molecular Anthropology lab for their technical assistance during the molecular portion of this project. I also thank my advisors, P. Crabtree and T. Disotell, for their support, guidance, and kindly shared expertise.

**LITERATURE CITED**


Meadow RH. A note on the distribution of faunal remains during the later periods of Mehrgarh (Baluchistan, Pakistan).


APPENDIX A - Protocols followed during molecular analysis.

Extraction

*Fresh tissue.* Tissues samples were collected from three living equids: hair and buccal swab from one horse (*E. caballus*), a veterinary blood sample from a second horse (*E. caballus*), and a buccal swab, hair, and tissue/blood sample from a donkey (*E. asinus*; see Table 2). Both horses were males; the donkey was a female. A total of nine samples were extracted following the subprotocols in the QIAGEN DNeasy DNA extraction kit.

*Teeth.* The ten unknown teeth from the Godin archaeological context were drilled with a handheld drill in a UV filtration hood in an isolated extraction room. Each tooth was surfaced cleaned with 1:10 bleach before commencement. Each of the ten teeth yielded over 100 mg of powdered material. DNA was extracted from the powdered material following a modified version of the GENECLEAN Ancient DNA extraction protocol.

PCR

An optimal PCR protocol was designed with the equid-specific primers and target region (amplicon) in mind. The PCR cocktail with a total volume of 25.0 μL is found below:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume (μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>dH₂O</td>
<td>12.88</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>1.5</td>
</tr>
<tr>
<td>5 X buffer</td>
<td>5.0</td>
</tr>
</tbody>
</table>
The PCR regime on a MyCycler machine is found below:

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<th>Temp</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>95°C</td>
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</tr>
<tr>
<td>94°C</td>
<td>15 sec</td>
</tr>
<tr>
<td>59°C</td>
<td>20 sec</td>
</tr>
<tr>
<td>72°C</td>
<td>30 sec</td>
</tr>
<tr>
<td>72°C</td>
<td>2 min</td>
</tr>
<tr>
<td>22°C</td>
<td>∞</td>
</tr>
<tr>
<td></td>
<td>repeat 50 times</td>
</tr>
</tbody>
</table>

Sequencing

Successful PCR products were identified by UV light as thick bright bands. Both known fresh tissue samples and unknown archaeological samples were subjected to three reactions – exoSAP, cycle sequencing, and ethanol precipitation – before being analyzed by a 3730 sequencer.

**ExoSAP.** 7.0 μL of the successful PCR product was mixed with 1.0 μL SAP buffer and 2.0 μL enzyme mix (taken from a mixture of 10.0 μL SAP and 1.0 μL Exo I at 10 μg/μL). This 10.0 μL reaction was then subjected to the following MyCycler protocol:

<table>
<thead>
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<th>Temp</th>
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</thead>
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<td>15 min</td>
</tr>
<tr>
<td>22°C</td>
<td>∞</td>
</tr>
</tbody>
</table>
Cycle sequencing. After the potential target sequence has progressed through the exoSAP reaction, it progresses to the cycle sequencing reaction. The cycle sequencing cocktail for a 10.0 μL reaction can be found below:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume (μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ExoSAP product</td>
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</tr>
<tr>
<td>5X buffer</td>
<td>1.5</td>
</tr>
<tr>
<td>Big Dye</td>
<td>1.0</td>
</tr>
<tr>
<td>Primer (F or R)</td>
<td>1.0</td>
</tr>
</tbody>
</table>

It is important to note that each PCR/exoSAP product undergoes two cycle sequencing reactions, one for the forward primer and one for the reverse primer. These reagents are subjected to the following MyCycler protocol:

96°C | 96°C | 50°C | 60°C | 22°C
10 sec | 10 sec | 5 sec | 4 min | ∞

____repeat 35 times

Ethanol precipitation. The final reaction in the sequencing stage of molecular analysis is ethanol precipitation. 10.0 μL of each cycle sequencing product (two cycle sequencing products per PCR/exoSAP product) is added to 1.0 μL 125 mM EDTA, 1.0 μL 3M sodium acetate, and 29.0 μL 100% ethanol in a single well of a plate. The septa is fitted to the plate and mixed by inversion, then left to sit at room temperature for 15 minutes. The septa-sealed plate is loaded into a balanced centrifuge at run at 37000 rpm for 30 minutes. After this, the septa is removed and the plate is placed upside-down on a Kimwipe in the balanced centrifuge, then run at 550 rpm for 2 minutes. 35 μL of 70% ethanol is then added to each well of the plate containing cycle sequencing product. The septa is fitted to the plate and mixed by inversion, then loaded into a balanced centrifuge...
and run at 2400 rpm for 15 minutes. After this, the septa is removed and the plate is placed upside-down on a Kimwipe in the balanced centrifuge, then run at 550 rpm for 4 minutes. 10.0 μL Hi-Dye formamide is added to each well containing cycle sequencing product; 10.0 μL 3730 buffer is added to any empty wells. The plate is resealed with the septa and is thus ready to be running through a 3730 sequencer.