Nuances of the Nochtli

Key Words: Aztec, Craft, Central America, Insects, Molecular Analyses

Lauren Clark

Department of Anthropology

University of Montana

Introduction

The domestication of insects in order to create dye for textiles, food, and cosmetics has been practiced all over the world for much of human history. For instance, the scale insect called kermes, native to coastal Mediterranean regions and parts of the Near East, was removed from the kermes oak tree to also provide a red dye, with evidence traced back as early as 300 BC (Donkin 1977). Additionally, other red dyes produced by the Polish cochineal of eastern Europe and the female lac insect of southern and southeastern Asia also have a deep history in the art of their respective culture (Donkin 1977). All these examples, though deeply rooted in their respective geographic region, have scant early archaeological evidence of organic dyed textiles and even less evidence of full-bodied insect remains. This assumption of poor archaeological data remains holds true in Mesoamerica and South America - both of which are areas that the cochineal beetle and its favored prickly pear host (Opuntiaficus-indica) currently call home.

Cochineal beetles, or nochtli, as they were originally called by the Nahuatl, a Uto-Aztecan people of Mexico and El Salvador (Donkin 1977), are currently categorized into nine distinct species, most of which are still wild. Very few species, including the focus of this study (Dactylopius coccus or D. coccus), became domesticates of early Americans (Chavez-Moreno, Tecante, and Casas 2009). Eventually, female D. coccus beetles were and continue to be exclusively reared on the prickly pear (Opuntia spp.) plant in which they produce all of the dye. Meanwhile, the male beetles are much smaller in size, winged, and only reside on the prickly pear during the mating season (Donkin 1977). Dactylopius coccus is the single remaining domesticated species of cochineal in which dye is still found in textiles and food today throughout the Americas and across the world. D. coccus is particularly identifiable in that it is double the size of wild cochineal beetles, lacks an exterior waxy coating, and females have a life cycle nearly twice as long as all other members of the Dactylopius genera (Donkin 1977). The loss of the waxy layer decreases the insect's protection from adverse weather conditions and predation, allowing for an increase in cochineals' defense mechanism of producing carminic acid (Campana et al. 2015). The production of this chemical, rich in anthraquinones, increases the production of red dye, serving as a human-mediated adaptation with little to no ecological benefit for the insect. Such an adaptation means that many cochineal beetles, especially those farmed today in Oaxaca, are unable to live and reproduce without the help of human support and a sufficient prickly pear host (Van Dam et al. 2015, Donkin 1977, Chavez-Moreno et al 2009).

In regard to the ecological specificity in rearing the cochineal beetle, the most common prickly pear species used as a host in pre-Columbian times up until present-day is Opuntia ficus-indica. This species is the victim of a parasitic relationship with D. coccus. Stationary female

cochineal beetles live their entire lives on the plant, draining the plant of its nutrients while producing carminic acid (Chavez-Moreno, Tecante, and Casas 2009). This plant is incredibly efficient in converting water into biomass and, like D. coccus, is not found at all in the wild today (Griffith 2004, Donkin 1977). The domestication of the O.ficus-indica has very early origins, potentially spanning back as far as 12,000 years ago, as noted in association with coprolites from a Paleoindian site in the Tehuacan Valley of central Mexico (Callen 1965, Griffith 2004). Therefore, it can be inferred that the prickly pear must have been domesticated much earlier than the cochineal beetle based on existing evidence (Donkin 1977).

From extensive ecological research and subsequent molecular analysis of this plant, the domestication of 0. ficus-indica occurred within the modem-day Oaxaca region of Mexico (Griffith 2004). Based on these findings, Van Dam et al. (2013), (2015) makes the claim that for the cochineal to be as successful as it has, while almost exclusively reared upon 0. ficus-indica, the domesticated D. coccus must have also originated within the Oaxaca region. This claim could be upheld by the additional molecular studies referred to below, but from a purely ecological perspective, such an argument carries little weight. In fact, there are several confounding ecological variables that could interfere with this assumption. First, the prickly pear can grow almost anywhere, as it is drought-resistant and can live in nutrient-poor soil (Griffith 2004). This could infer that the highlands of Peru are not an unreasonable location for early distribution and cultivation of the cochineal even though 0. ficus-indica is found only sparsely within the region today. Also, any sort of obligate, parasitic relationship with this kind of potential for human exploitation is very rarely found in an unexploited, natural environment. This ecological interaction could be characteristic of human introduction of one species into an environment in which there are no natural predators. Therefore, it could still be possible that the ancestral Dactylopius coccus organisms originated and later differentiated in a region outside or away from the domestication of Opuntia ficus-indica in Oaxaca. Yet, any sort of ecological claim of this magnitude is most stable with supporting evidence from molecular data, and additionally archaeological evidence.

Cultural Background

The earliest remains of textiles in the Americas with a red dye appear to be from the Chavin culture of Peru from 900 to 200 BCE and were used to dye cotton (Phipps and Shibayama 2010). Though the type of dye from these sites cannot be affiliated with a type of pigment, it can be assumed that it was not cochineal because these textiles were produced from cotton. The cochineal beetle was almost exclusively used to add pigmentation to animal fibers (Phipps and Shibayama 2010). However, by 300 BCE, the wool fibers from camelids specific to coastal sites, may have remnants of cochineal dye (Phipps and Shibayama 2010). Similar animal fibers from the Paracas mantles around 300-200 BCE also appear to use cochineal, but it appears from mass spectrometry analysis that use of cochineal dye here is only supplementary to the relbunium dye produced from the Madder plant of this region (Saltzman 1992, Phipps and Shibayama 2010). This is odd, however, as the dye produced from the Madder plant is much more difficult to produce and requires a prolonged boiling process of the plant, as compared to a simple crushing process of the cochineal (Saltzman 1992). Yet, peoples of early South America do not appear to use exclusively cochineal dye until 600 CE within the Moche Culture, also of Peru (Saltzman 1992). Peruvian evidence of the widespread use of the cochineal persists within

the record of written accounts among the Incan empire starting in the late 13th century (Phipps 2003). Such widespread written evidence as late as the Columbian period during European contact could infer that early use of the cochineal beetle within textiles may have been just as prevalent in the highlands as what is found in coastal sites. However, it is impossible for archaeologists to truly confirm this idea as the environmental conditions of these high-elevation locales are not suitable for protecting organic archaeological material.

It is true that early archaeological evidence of cochineal among coastal Peruvian sites is much more highly concentrated, but it also should be noted that the earliest, most clear examples of domestication of the D. coccus are found much farther north. The trade and distribution of cochineal became such an instrumental part of life among the Mixtec people and the rest of Mesoamerica during the colonial period that it was believed that the cochineal could not have come from anywhere but Mexico. It is also agreed upon that the earliest example of widespread cochineal cultivation and processing is found at a Tolteca settlement in Mexico (Pelham 1963). Yet, this site dates to around 1000 CE and other archaeological evidence of domestication of the cochineal as far north as Oaxaca remain few and far between. Therefore, it is molecular data that may give archaeologists and ecologists, alike, the clearest picture of the story of the domestication of the cochineal.

From theories developed by Dr. Steve Lekson and others of extensive trade and commerce throughout southern Mexico, Mesoamerica, and the American Southwest/Northwest, one would think that cochineal could even be found as far north as the United States. However, very few textiles with evidence of specifically cochineal dye this far north have been found. In one example of Casas Grandes, the red textiles that remain in the archaeological record in association with the Medio Period (1200-1450) are charred to the point in which identification of a pigment source is improbable, if not impossible (King 1974). Yet, evidence of other animal and plant dyes that could produce a similar color (like that of shellfish and madder) also remain absent. The madder and plant dyes in general, prior to high-performance liquid chromatography (HPLC) analysis, were thought to be the only source of pigment-producing substances this far north. However, it is now known that shellfish was used in addition to, and not as a substitute of, dye produced from the indigo plant, which completely alters this ideology. Such determination of the presence of the cochineal this far north must therefore depend on trade and commerce throughout this region based on findings of other goods in archaeological context.

It is apparent from the prior paragraphs that the archaeological evidence of the cochineal throughout South America and Mesoamerica is limited. Limited archaeological analysis does not have to limit all inquiries of domestication, however. The capabilities of using molecular analyses, including that of mitochondrial and nuclear genomic sequencing, may provide additional avenues of determining a lineage. Some of these analyses taken from information obtained from the mitochondrial genome may also hint at the time of domestication by determining a mitochondrial clock. With access to this technology, in addition to a wide range of samples that span Mesoamerica and South America, it may possible for archaeologists and molecular anthropologists to determine the time and place of the domestication of the cochineal beetle.

Molecular Background

Throughout the field of molecular anthropology's short academic history, there have been very few examples of insect domestication studies. As I make the claim above, the cochineal is not the only widespread insect to be domesticated in the world. Therefore, the quantity or availability of insects is not the limiting factor. It is instead the quality of samples and the ability to find an appropriate primer, or short piece of single-stranded RNA, to amplify the DNA for sequencing that makes these studies so difficult. To further complicate this matter, much of insect DNA is unknown, so constructing one's own primers is often necessary. Yet, once the DNA from these insect samples is successfully extracted, amplified, and sequenced, anthropologists and ecologists alike will be much closer to identifying a definitive location(s) of cochineal domestication. To analyze the existing variation between and among samples from Peru, Oaxaca, and elsewhere, it is critical to recognize the strengths and weaknesses of mitochondrial, nuclear, and endosymbiont genetic material outlined below.

The mitochondria is an organelle within all plant, animal, and fungi (eukaryotic) cells that formed initially around 1.45 billion years ago when a bacterial, or prokaryotic, cell was consumed by a larger eukaryotic cell and slowly became integrated into the function of the cell as a whole (Martin and Mentel 2010). This relationship allowed eukaryotic cells to take on additional genetic information and subsequent additional functionality that allowed the mitochondria, better known as the "powerhouse of the cell," to become what it is today. The mitochondrial genome of all eukaryotes is much shorter than of the nuclear genome, maternally inherited in all animals, and is highly conserved, meaning, it provides the perfect opportunity in identifying small changes in the genome as two populations diverge. These small changes are referred to as substitutions, or more specifically, single nucleotide polymorphisms (SNPs), and provide detailed information about human ancestry as well as the phylogenetic organization of multiple other eukaryotic species. For these reasons, mitochondrial analysis may be the best determinant in finding the time and place of cochineal domestication.

There are multiple regions of the mitochondrial genome that may be suitable for the analysis. These include the 12S, the COl (or COI-11), and in some cases the hypervariable, or control, region. The hypervariable region remains a common and useful approach in analyzing much of human mtDNA, but unfortunately the length of this region is highly variable across arthropods and is not well understood (Simon et al. 2006). Therefore, both research groups referred to below have chosen to amplify and sequence the CO1 region of the mitochondrial genome instead because this region has played a particularly robust role in the barcoding of multiple vertebrate and arthropod species (Allendorf, Luikart, and Aitken 2012).

Other potential sources of variation among cochineal beetles may be found in the nuclear genome. The nuclear DNA of any organism is often much more conserved, or similar if not the same across species, because there is much greater pressure than that of mitochondrial DNA to produce consistent, functional genes that retain the reproductive success of the organism. However, there continue to be several fragments of arthropod genomes that mutate at a fast-enough rate to determine a lineage.

As discussed below, the 18S rRNA region of nuclear DNA, used by Campana et al. (2015) is a very common region to amplify among nuclear samples. Yet, it is still unknown if this sequence truly has enough variation among beetles, much less within the Hemiptera family to which the cochineal beetles belong or throughout the entire phylum of Arthropoda. Additional studies, like that of Regier et al. (2008) have attempted to improve upon the collective understanding of arthropod's genetic structure by identifying the ten fastest mutating regions of nuclear DNA among this phylum. However, the sampling of this study remains limited in the context of this study as no close relatives to the cochineal beetles were included. Nevertheless, Van Dam et al. (2013) and (2015) still attempted to amplify all of these regions, but only two (E+P-tRNA synthase and GTP-binding protein) amplified. This in addition to the amplification of the popular phylogeographic nuclear marker, EFl -u, also from Van Dam et al. (2013), still provide little nuclear variation among an animal in which only female, or mitochondrial, markers are critical. From review of this and other analyses, it remains debatable whether mitochondrial or nuclear DNA is the preferred method in determining close arthropod lineages.

An additional potential difficulty of targeting and amplifying the correct sequence in insects is avoiding the other various sequences that may come from an insect's bacteriome or from a bacteriocyte (Vera-Ponce de Leon et al. 2017). Both of these elements contain genetic information that is not unique to an insect in which the bacteriome is an entire region composed of bacteria, while the bacteriocyte is a bacterial cell that has integrated itself and is able to reproduce among the insect's own cells. This factor alone can make the process of DNA extraction, namely mitochondrial DNA extraction, quite difficult (Hurst and Jiggins 2005). Yet, this problem is not impossible to overcome. It can be as simple as targeting the correct, distinctive mitochondrial sequence that will lead to targeted sequencing capable of producing enough diversification that discovering a lineage or origin may become possible.

Molecular Analyses

Past studies in regard to this topic have been conducted with sample sizes from few geographical regions. One such analysis from Campana et al. (2015), tracked the 12S rRNA and cytochrome c oxidase 1 (COl) regions of the mitochondrial genome. The researchers targeted these sequences in 40 samples from a small-scale farm in Oaxaca; 75 samples from large commercial farms in Mexico, Peru, and Chile; and 41 wild samples from Oaxaca. The results of this study only obtained nine distinctive SNPs between both regions and was therefore not able to determine enough differences between the domesticated and wild beetles. This study was also not able to create an effective mitochondrial clock because aphids, which are the closest relative to the cochineal genome and that were going to serve as their paleontological calibration point, had low coverage. To elab, the aphids, or the genetically dissimilar outgroup that would be expected to have a similar rate of evolutionary change, had poor quality DNA and, therefore, could not reliably point to a time of a most recent common ancestor among the cochineal beetles. In this case, their nuclear genomic analysis was more successful because they were able to ascertain 82 high-confidence SNPs (Campana et al. 2015). Yet, this relationship only allowed the researchers to ascertain that the large-scale, domesticated samples from Peru and Mexico more closely related to one another than the Oaxaca sample is related to either.

An additional study attempted to improve upon the findings and analyses of this study by using a larger variety of samples from Peru to give less biased information than that of Campana et al. (2015). However, the authors of this research admitted that the samples pulled from the Oaxaca sites were in fact all originally from the same farmed source. Even with this in mind, from the results of their study, it was still quite evident that the large number of samples obtained, amplified, and sequenced from Oaxaca did, in fact, have much greater genetic variation (Van Dam et al. 2015).

The increased presence of variation among local sources could point to a cultivation or domestication event in Mexico. This biological evidence is backed up by historical records, since, as they note, a large bottleneck event occurred in Mexico immediately after European contact in this region. As noted by Van Dam et al. (2015), domestication events, whether they occur all at once or as multiple events are often marked, by one or multiple bottleneck events. This could represent a secondary domestication event or simply a significant decrease in reproduction of cochineal as an effect of colonization. This would have biased the data in a way that would demonstrate increased variation in Mexico more so than other areas (Van Dam et al. 2015). These ecological arguments put forth by Van Dam et al. (2015) in regards to domestication locale appear to be valid upon first glance, but more data that is not included in these studies is necessary to back up these statements.

In regards to data collection alone, both Campana et al. (2015) and Van Dam et al. (2013) both struggle in identifying previously known phylogenetic structure of cochineal beetles as well as recognizing and producing primers that will accumulate greater variation . For instance, the study initially produced by the Van Dam et al. (2013) dissertation research chose to sequence the common mitochondrial genome (COi-Ii), while Campana et al. (2015) only built upon the mitochondrial study by including the 12S region. The Campana et al. (2015) authors realized this lack of variation and went so far to even request that future researchers pursue the control region. However, no such work, even by the subsequently published Van Dam et al. (2015) article has completed this work. Therefore, holes within the molecular data of cochineal domestication still exist.

Table 1. *Dactylopius coccus* mitochondrial primer sequences used by previous studies.

Marker	Forward primer	Reverse primer	Reference
12S	5-AAGAGTGACGGGCRATTTGTACATA-3	5-GTGCCAGCAGTWGCGGTTA-3	Campana (2015)

COl	5-TCCTTATCAGAAATGGAAAAC-3 (Fl) 5-TTTATGCAATAATCTCTATCG GAGTT-3 (F2)	5-CCATTCGTTGTTGAATGATTTT-3 (Rl and R2)	Van Dam (2013)
COl	5-TCCGRATAGAACTWATAAAYACYAA-3	5-TAAACTTCAGGGTGACCAAAAAATCA-3	Campana (2015)

Table 2. *Dactylopius coccus* nuclear primer sequences used by previous studies:

Marker	Forward primer	Reverse primer	Reference
18S	5-CTGGTTGATCCTGCCAGTAG-3	5-CCGCGGCTGCTGGCACCAGA-3	Campana (2015)
GIP-binding protein	TAGRGT ACCTGTTCCCGATG (FI) TGTTGAAGGGGAAGTTGACC (F2 mid)	GGTCCGGCAGTGAAGAAATA (Rl and 3) ATGACAGCACCAGGGTCATT (R2)	Van Dam (2013)
E+P-tRNA synthase	CGGAGATTTTACTACCACYG (Fl)	ATTTCCAACCGGAAGAATAG (Rl)	Van Dam (2013)
EFI- *a*	AGCTGAACGTGAACGTGGTA (Fl)	CAGTTGGCCGGGT AGGAG (RI)	Van Dam (2013)

Discussion

To consider ways to improve upon the data analysis collected on behalf of both of these research teams, one should repeat the work of Campana et al. (2015) in analyzing the COl, 12S rRNA, and 18S rRNA sequences. Once successfully amplified, future researchers should make further attempts in building primers that will effectively analyze the control region of the mitochondrial genome. As previously described, the hypervariable region is an excellent set of alleles to examine very recent changes within diverging populations in humans and many other animals. However, in arthropods, the control region is not only often variable in content, but also in length, making targeting of this region quite difficult (Simon et al. 2006).

The multiple lines of ecological, archaeological, written, and molecular evidence can be extremely influential in determining the history of the domestication of the cochineal beetle. The domestication of this small insect played such a large role in developing social stratification among pre-contact communities of the Americas. Therefore, the information gathered here may give future generation of researchers of multiple fields an improved understanding of several ecological and social implications of domesticating an animal on this scale. The field of

Molecular Anthropology is growing quickly and the drive to use rapidly improving methods and equipment will also benefit from the results of this study. The information provided by future studies has the power to improve understanding of the cultivation and development among past peoples.

Literature Cited

Allendorf, Fred W., Gordon H. Luikart & Aitken, Sally N.
2012. *Conservation and the genetics of populations, second edition*. Translated by Anonymous. 2nd ed. Wiley.

Callen, E. O.
1965. Food Habits of Some Pre-Columbian Mexican Indians. *Economic Botany* 19(4):335-343.

Campana, Michael G., Garcia, Nelly M. Robles & Tuross, Noreen
2015. America's red gold: multiple lineages of cultivated cochineal in Mexico. *Ecology and Evolution* 5(3):607-617.

Chavez-Moreno, C. K., Tecante, A. & Casas, A.
2009. The Opuntia (Cactaceae) and Dactylopius (Hemiptera: Dactylopiidae) in Mexico: a historical perspective of use, interaction and distribution. *Biodiversity and Conservation* 18(13):3337-3355.

Donkin, Robin A.
1977. *Spanish Red: An Ethnogeographical Study of Cochineal and the Opuntia Cactus*. Vol. 67,5, American Philosophical Society, Philadelphia.

Griffith, M. P.
2004. The origins of an important cactus crop, Opuntia ficus-indica (Cactaceae): new molecular evidence. *American Journal of Botany* 91(11):1915-1921.

Hurst, G.D. D. & Jiggins, F. M.
2005. Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: the effects of inherited symbionts. Proceed. *Royal Soc. B: Biol. Sci*. 272:1525- 1534.

King, Mary E.
1974. Medio Period Perishable Artifacts. *In Casas Grandes*, Vol. 8, pp. 76-81. Amerind Foundation [u.a.], Dragoon [u.a.].

Mentel, Marek & Martin, William
2010. Anaerobic animals from an ancient, anoxic ecological niche. *BMC biology* 8(1):32.

Phipps, Elena
2003. Color in the Andes: Inca Garments and 17th Century Colonial Documents. *Dyes in History and Archaeology* 19:51-59.

Phipps, Elena & Shibayama, Nobuko
2010. Cochineal red: tracing history through colour. *Hali: The International Magazine of Antique Carpet and Textile Art* (164):76-85.

Ramirez-Puebla, S.T., Rosenblueth, M., Chavez-Moreno, C. K., Catanho Pereira De Lyra, M. C., Tecante, A. & Martinez-Romero, E.
2010. Molecular Phylogeny of the Genus Dactylopius (Hemiptera: Dactylopiidae) and Identification of the Symbiotic Bacteria. *Environmental Entomology* 39(4):1178-1183.

Rodnguez, L. C., Mendez, M. A. & Niemeyer, H. M.
2001. Direction of dispersion of cochineal (Dactylopius coccus Costa) within the Americas. *Antiquity* 75:73- 77.

Saltzman, Max
1992. Identifying Dyes in Textiles. *American Scientist* 80(5):474-481.

Simon, Chris, Buckley, Thomas R., Frati, Francesco, Stewart, James B. & Beckenbach, Andrew T.
2006. Incorporating Molecular Evolution into Phylogenetic Analysis, and a New Compilation of Conserved Polymerase Chain Reaction Primers for Animal Mitochondrial DNA. *Annual Review of Ecology, Evolution, and Systematics* 37(1):545-579.

Van Dam, Alex R., Martinez, Liberato Portillo, Chavez, Antonio Jeri & May, Bernie P.
2015. Range Wide Phylogeography of Dactylopius coccus (Hemiptera: Dactylopiidae). *Annals of the Entomological Society of America* 108(3):299-310.

Vera-Ponce de Leon, Arturo, Ormefio-Orrillo, Ernesto, Ramirez-Puebla, Shamayim T., Rosenblueth, Monica, Esposti, Mauro Degli, Martinez-Romero, Julio & Martinez-Romero, Esperanza
2017. Candidatus Dactylopiibacterium carminicum, a Nitrogen-Fixing Symbiont of Dactylopius Cochineal Insects (Hemiptera: Coccoidea: Dactylopiidae). *Genome Biology and Evolution* 9(9):2237-2250.