Range Wide Phylogeography of *Dactylopius coccus* (Hemiptera: Dactylopiidae)

ALEX R. VAN DAM,[1,2,3] LIBERATO PORTILLO MARTINEZ,[4] ANTONIO JERI CHAVEZ,[5] AND BERNIE P. MAY[1]

Ann. Entomol. Soc. Am. 108(3): 299–310 (2015); DOI: 10.1093/aesa/sav017

ABSTRACT The process of domestication and geographic origins of the cochineal insect (*Dactylopius coccus* Costa) has remained largely unstudied despite its importance as a global food colorant commodity. Ecological evidence supports Oaxaca Mexico as the geographic origin of this species. Other recent genetic studies have been inconclusive. Here, we fill in the remaining gaps in the ecological record and look for corroboration from mtDNA markers as to the origin of this species. We use three mtDNA genes (CO1, tRNA-Leucine, and CO2) spanning 1294 bp, along with climate niche modeling of Holocene and Pleistocene cochineal distributions. We find the center of origin of *D. coccus* to be Oaxaca Mexico based on mtDNA data and climate niche modeling. Further meta-genomic data are needed to rule out selective sweeps from past and present endosymbionts for these results to be definitive.

KEY WORDS *Dactylopius coccus,* geographic origin, cochineal, domestic origin

Understanding the processes of domestication facilitates improving domesticates, and thereby human living conditions (Borlaug 2000, Diamond 2002, Purugganan and Fuller 2009). In plant and animal domesticates the tempo and mode of the domestication process can greatly affect genetic diversity (Cornille et al. 2012). In plant crops, population bottlenecks resulting from domestication have often been used as a signature of domestication (Diamond 2002, Innan and Kim 2004, Wright et al. 2005, Zeder et al. 2006, Ross-Ibarra et al. 2007, Cornille et al. 2012, Harpur et al. 2012). However, this view is changing as genetic data on nonslelfing crops becomes available.

Annual selfing crops (e.g., wheat, oats, rice, and soybeans) tend to show severe bottlenecks, losing 70-90% of total genetic diversity during domestication (Phillips and Murphy 1993, Diamond 2002, Hyten et al. 2006, Ross-Ibarra et al. 2007, Zhu et al. 2007, Zhang et al. 2009, Harpur et al. 2012, Hufford et al. 2012). In perennials with multi-year generation times, it is not possible to cull successive generations in a human life span. To solve this problem, our ancestors turned to outcrossing and introgression to domesticate perennials. Outcrossing buffers loss of genetic diversity and introgression actually increases genetic diversity (Griffith 2004, Coart et al. 2006, Besnard et al. 2007, Lubinsky et al. 2008, Myles et al. 2011, Cornille et al. 2012, Delplancke et al. 2012).

Although the domestication process in some animals results in an initially small effective population size (N_e), population bottlenecks may occur through highly controlled selective breeding and a single domestication event (e.g., rabbits, carp, and possibly goldfish) (Komiyama et al. 2009, Rylková et al. 2010, Carneiro et al. 2011). Many of our most prominent animal domesticates (e.g., cows, sheep, pigs) result from multiple domestication events; these examples include animals with formerly widespread ancestors and large N_e (Bruford et al. 2003, Wiener and Wilkinson 2011). Domestication that involves hybridization results in large N_e (e.g., cows, goats, pigs, chickens) (Bruford et al. 2003, Eriksson et al. 2008, Kanginakudru et al. 2008, Berthouly-Salazar et al. 2010, Rubin et al. 2010, Wiener and Wilkinson 2011, Lawler 2012). Even introgression between multiple wild ancestral species has led to domestication events, e.g., chickens, pigs, goats, sheep, horses, lamas, and alpacas (Bruford et al. 2003, Rubin et al. 2010).

Domestication events in insects are no less complex than in crops and vertebrates. Number of domestication events differs, from a single event in silkworms (Xia et al. 2009) and at least three in honeybees (Whitfield et al. 2006), followed by introgression in some cases (Harpur et al. 2012). Mechanisms of insect domestication typically involve introgression and hybridization with different subspecies and populations, but does not seem to have been between multiple species (Whitfield et al. 2006, Xia et al. 2009, Harpur et al. 2012). In lac insects *Kerria* spp., there are variable modes of domestication from single inbred lines to

[1] Department of Animal Science, University of California Davis, One Shields Avenue, Davis, CA 95616, USA.
[2] Current address: Denmark Technical University, Department of Systems Biology, Søltofts plads, Building 223, Lyngby, 2800 Kgs., Denmark.
[3] Corresponding author, e-mail: vandama01@gmail.com.
[4] Departamento de Departamento de Botánica y Zoología, Universidad de Guadalajara Centro Universitario de Ciencias Biológicas y Agropecuarias, Jalisco, C.P.44171, Mexico.
[5] Depertamento de Ciencias Agrarias, Universidad Nacional de San Cristobal de Huamanga, Ayacucho, 05000, Peru.

introgression in lac insects (Ranjan et al. 2011). In this article, we use genetic tools to characterize a third possible insect domesticate and global resource, the cochineal insect (*Dactylopius coccus* Costa).

D. coccus belongs to a group of parasitic scale insects (Dactylopiidae) that are specialists on *Cactaceae*, especially *Opuntioid cacti*, in the genus *Opuntia* (De Lotto 1974, Perez-Guerra and Kosztarab 1992, Van Dam and May 2012). *D. coccus* is unique among Dactylopiidae living in both Andean Mountains and southern Mexico (Perez-Guerra and Kosztarab 1992, Van Dam and May 2012). This unusual and highly disjunct amphitropic distribution, coupled with its use as a prominent red dye in archeological records has long raised suspicions that *D. coccus* was traded between pre-Columbian West Mexican and Andean societies (Donkin 1977, Chávez-Moreno et al. 2009).

D. coccus prefers *Opuntia ficus-indica* (L.) Mill. as its host plant over other cactus species, and *O. ficus-indica* has its origins centered around current day Oaxaca, Mexico (Griffith 2004, Griffith and Porter 2009), pointing to a Mexican origin of *D. coccus* (Portillo 2005, Rodríguez et al. 2006). Alternative hosts are also Mexican in origin (Griffith 2004, Portillo 2005, Chávez-Moreno et al. 2009, 2011, Griffith and Porter 2009, Majure et al. 2012). Other than *O. ficus-indica* no other hosts are known from Peru (Portillo 2005). Others have argued that despite the geographic origins of its preferred host plant, cochineal has its origins in South America as it can be found reproducing there without human assistance, unlike cochineals in Oaxaca Mexico where they are only found in protected farms (Donkin 1977, Portillo 2005, Chávez-Moreno et al. 2009). Additionally, an argument could be made that wild cochineal insects switched from an ancestral host to *O. ficus-indica* in South America, and then spread northward following introduction of *O. ficus-indica* across continents. Currently, *D. coccus* is used by humans as a cheap source of carminic acid as food coloring, which is extracted from the adult females and used in products across the world (Rodríguez and Pascual 2004, Portillo 2005, Rodríguez et al. 2006, Van Dam and May 2012). This modern trade in *D. coccus* highlights the importance of provenance for samples obtained in Mexico and Peru.

Cochineal's ubiquitous appearance as a modern food coloring belies its ancient roots where it was used as a brilliant red dye by pre-Columbian societies in Mexico and Peru (Donkin 1977, Chávez-Moreno et al. 2009). *D. coccus* has been cultivated or gathered for use as a brilliant red dye for tapestries or as a paint for ceramics by Mixtec and Zapotec societies in Oaxaca Mexico, and by Incas in southern Peru, for >1,000 years before present based on archeological evidence. (Donkin 1977, Portillo 2005, Peggie et al. 2008, Deveoglu et al. 2010, Phipps 2010, Serrano et al. 2011, Wouters and Rosario-Chirinos 2011). Pre-Columbian archeological evidence points to Peru as geographic origin of *D. coccus*, but there are also significant Pre-Columbian artifacts pertaining to cochineal production and usage in Mexico (Donkin 1977, Portillo 2005, Feinman et al. 2007, Chávez-Moreno et al. 2009). Archeological evidence is inconclusive as to the origins of *D. coccus*, but ecological evidence seems to point to Mexico as the geographic origin of this species.

Recently, genetic data on *D. coccus* were added to the discussion of its origins (Campana et al. 2015). However, despite having a robust set of nuclear and mitochondrial genetic markers the results were inconclusive, in part due to a lack of sampling in Peru. Only a single Peruvian geographic replicate was included and only two Mexican geographic replicates (Campana et al. 2015).

Ecological evidence supports *D. coccus* originating in Oaxaca, Mexico. In this article, we attempt to corroborate the already robust ecological data on *D. coccus* by filling in gaps through increased geographic replication of genetic data, and more rigorous field research. We survey for alternative host plants in Peru, and second use climate data to delimit the putative ancestral range of this species. Our study differs from past efforts to include genetic data in this discussion (Campana et al. 2015) by including many more geographic replicates from Peru and a key additional geographic replicate from Mexico. Finally to test the ecological evidence supporting the origins of *D. coccus*, we add comprehensive sampling of mtDNA and estimate divergence times of haplotypes across the full geographic range of *D. coccus* adding many new locations from Peru, Argentina, Madeira Island, and Mexico.

Materials and Methods

Specimen Collection and Field Survey. Collections were made from three different farms in Oaxaca, Madeira Island in the Atlantic Ocean, and from a total of 38 locations from Lima province and Sierra Central region of Peru (Figs. 1 and 4; Table 1).

A subset of seven localities from Peru were sampled for the genetic analyses (Fig. 1), as these represent the most disjunct Peruvian geographic regions. The Peruvian locations are comprised of representatives from both sides of the Andean cordillera. We chose localities in the Sierra Central and Lima Province from separate river valleys as we expected some level of geographic isolation based on other Andean taxa. We also wanted to survey the Sierra Central where cochineal occurs to try and find any alternative host-plants other than *O. ficus-indica* for *D. coccus*. We surveyed a total of 38 locations (Fig. 4; Table 1). Two specimens from a location in northern Argentina were also included (Table 1) as this location is geographically isolated far to the south of the main cochineal populations in Peru.

We discovered while surveying farms in Oaxaca that all of the farmed cochineal populations stem from a single location, the Aquilino farm. The Aquilino farm was the last remaining Zapotec farm propagating cochineal in the mid-twentieth century dating back prior to the collapse of the industry via an unbroken family tradition (Pelham Wright 1963), to the present day by Juan Aquilino Ramirez Ramirez. In the latter half of the twentieth century, the Tlapanochestli cochineal dispersion center lead by Manuel Loera has dispersed

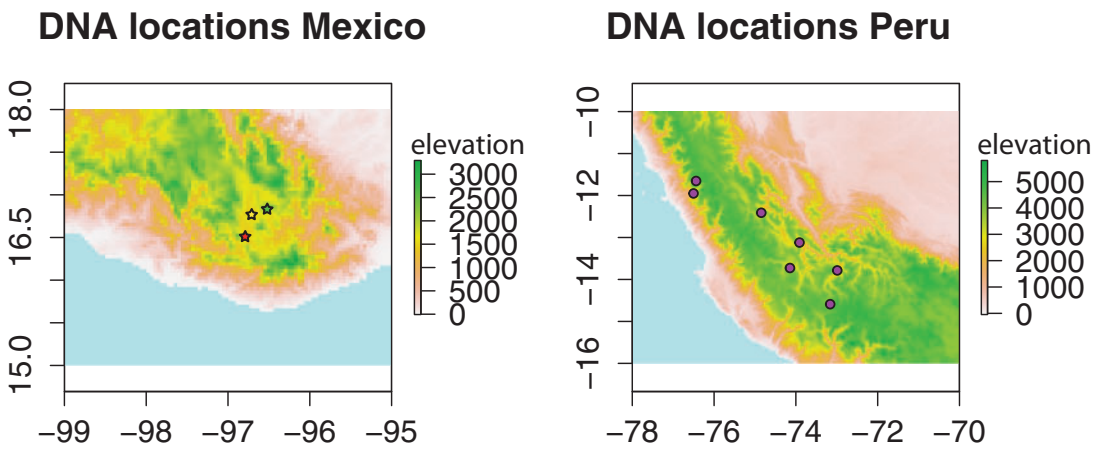

Fig. 1. Elevation map depicting geographic locations of *Dactylopius coccus* mtDNA sample collection localities. Left panel, stars indicate *D. coccus* mtDNA collection localities in Oaxaca, Mexico, with colors representing different farms, red online (furthest South print) = Aquilino farm, yellow online (middle print) = Tlapanochestli farm, green online (furthest North print) = Hernandez farm. In the right panel, purple (online) circles indicate Peruvian *D. coccus* mtDNA collection localities.

cochineal to other farms in Oaxaca via stock obtained from the Aquilino farm. We then chose to collect from farms that we knew had obtained stocks from Tlapanochestli such as farms in the town of Teotitlan del Valle. We also found farmers in Teotitlan del Valle, such as the farm run by Benito Hernandez, who also vouched for independent ancestry of their cochineal, we chose to include that farm as well.

A collection from Madeira was obtained that represents a fragmented population from Oaxaca. It was distributed there >170 years ago (Donkin 1977), we chose to include this location as it may represent unique haplotypes.

DNA Extraction. Legs were removed from individual insects using forceps that were cleaned with 10% bleach between each sample. DNA was extracted from leg tissue using Wizard SV 96 Genomic DNA Purification System (Promega Corporation, Fitchburg, WI). We chose to extract 19 individuals from Aquilino farm, eight from Hernandez farm, eight from Tlapanochestli, eight from Madeira, and from seven localities eight individuals per location from Peru, and two individuals from Argentina (Table 1).

Initial PCR and Cloning. An initial attempt was made to amplify CO1–CO2 mtDNA region that had been successfully amplified in other Coccoidea (Burban et al. 1999, Philpott et al. 2009, Yokogawa and Yahara 2009, Andersen et al. 2010). Primers c1-J-2753ywr (Provencher et al. 2005) and c2-N-3662 (Simon et al. 1994) were used in initial PCR. Initial PCR conditions were as follows: 0.01675 µg/µl final PCR concentration of GoTaq. GoTaq Flexi Buffer (Promega Corporation), 2 mM concentration of each dNTP, and 2.5 mM $MgCl_2$. The following PCR step-down protocol yielded the best products: 94°C: 4 min initial denaturation step followed by 30 s thereafter, initial annealing temp at 54°C for the first four cycles at 2 min with 72°C extension for 2 min 30 s, followed by 52°C annealing for four cycles with 72°C extension for 2 min, with the subsequent 26 cycles at 48°C annealing for 30 s and 72°C extension for 2 min, and finally a 5 min 72°C extension step followed by a 10°C hold. One microliter of the PCR product was used to run a check gel (1.3% agarose) in TAE buffer. This was followed by pGEM-T (Promega Corporation) vector cloning following the manufacturers protocols to get initial sequences.

Primer Design of Species-Specific Primers. Based on initial sequences from cloned samples program Primer3 (Rozen and Skaletsky 2000, Untergasser et al. 2012) was used to design species-specific primers for CO1–CO2 as well as internal sequencing primers. Forward PCR primer: 5′-TCCTTATCAGAAATGGAAAAC-3′, and alternative forward primer: 5′-TTTATGCAATAATCTCTATCG GAGTT-3′. Then we designed reverse sequencing primer that is compatible with both forward primers: 5′-CCATTCGTTGTTGAATGATTTT-3′. The same PCR protocol was used as in initial PCR in subsequent amplifications. Additionally, three nuclear markers were used on a subset of the data to see if there was any genetic variation (E+P-tRNA synthetase, GTP-binding protein, and EF1α) as we have found population level genetic variation in all other *Dactylopius* species using these markers (Van Dam 2013). For the nuclear markers, we chose to examine the collection from Argentina and an individual from the Aquilino farm as this represented the range extremes of this species between North and South America. The population in Argentina was also the least likely to have secondary contact with populations from Mexico due to its isolation.

Sequencing. For PCR cleanup, we used Agencourt Ampure XP (Beckman Coulter, Brea, CA) magnetic beads following manufacturer's protocol and eluted to 30 µl. Following PCR cleanup, cycle sequencing protocol used 5.63 µl H_2O, 1.63 µl BigDye Sequencing Buffer (Life Technologies, Grand Island, NY), 0.5 µl

Table 1. Locality of *Dactylopius coccus* collections sampled in this study

Locality	Latitude	Longitude	DNA	MaxEnt	Management
Peru					
P_1	12° 01′527″ S	76° 40′538″ W	0	No	Irrigated
P_2	12° 04′538″ S	76° 30′433″ W	0	No	Irrigated
P_3	12° 05′092″ S	76° 30′259″ W	0	No	Irrigated
P_4	12° 05′282″ S	76° 30′111″ W	0	No	Irrigated
P_5	12° 05′501″ S	76° 30′096″ W	0	No	Irrigated
P_6	12° 05′491″ S	76° 30′144″ W	8	No	Irrigated
P_7	13° 08′905″ S	74° 13′184″ W	0	Yes	Feral
P_8	13° 03′869″ S	74° 11′905″ W	0	Yes	Feral
P_8	13° 03′889″ S	74° 11′932″ W	0	Yes	Feral
P_8	13° 03′867″ S	74° 11′961″ W	0	Yes	Feral
P_8	13° 03′854″ S	74° 11′980″ W	0	Yes	Feral
P_9	13° 03′950″ S	74° 14′147″ W	0	Yes	Feral
P_10	13° 04′578″ S	74° 13′578″ W	0	Yes	Feral
P_11	13° 08′190″ S	74° 11′321″ W	0	Yes	Feral
P_12	12° 54′616″ S	74° 15′378″ W	0	Yes	Feral
P_13	12° 54′211″ S	74° 15′500″ W	0	Yes	Feral
P_13	12° 54′260″ S	74° 15′472″ W	0	Yes	Feral
P_13	12° 54′265″ S	74° 15′503″ W	0	Yes	Feral
P_13	12° 54′213″ S	74° 15′528″ W	0	Yes	Feral
P_14	12° 54′694″ S	74° 17′317″ W	0	Yes	Feral
P_15	12° 55′236″ S	74° 17′218″ W	0	Yes	Feral
P_16	12° 54′332″ S	74° 18′883″ W	0	Yes	Feral
P_17	12° 52′011″ S	74° 19′661″ W	0	Yes	Feral
P_18	13° 09′904″ S	74° 09′904″ W	0	Yes	Feral
P_19	11° 53′53″″ S	76° 26′27″ W	8	No	Irrigated
P_20	12° 29′412″ S	74° 50′735″ W	6	Yes	Feral
P_21	12° 27′895″ S	74° 46′509″ W	0	Yes	Feral
P_22	12° 31′474″ S	74° 41′463″ W	0	Yes	Feral
P_23	12° 36′018″ S	74° 40′028″ W	0	Yes	Feral
P_24	12° 38′134″ S	74° 38′351″ W	0	Yes	Feral
P_25	12° 44′249″ S	74° 32′324″ W	0	Yes	Feral
P_26	12° 49′355″ S	74° 22′752″ W	0	Yes	Feral
P_27	13° 36′248″ S	74° 09′839″ W	4	Yes	Feral
P_28	13° 37′693″ S	74° 08′630″ W	0	Yes	Feral
P_29a	13° 05′568″ S	73° 55′273″ W	4	Yes	Feral
P_29	13° 06′264″ S	73° 54′699″ W	0	Yes	Feral
P_30	13° 25′767″ S	73° 54′194″ W	0	Yes	Feral
P_31	13° 30′507″ S	73° 48′521″ W	0	Yes	Feral
P_32	13° 39′119″ S	73° 26′837″ W	0	Yes	Feral
P_33	13° 37′917″ S	73° 11′815″ W	0	Yes	Feral
P_34	13° 40′720″ S	72° 59′285″ W	8	Yes	Feral
P_35	13° 32′469″ S	72° 39′852″ W	0	Yes	Feral
P_36	13° 47′020″ S	72° 56′599″ W	0	Yes	Feral
P_37	13° 59′873″ S	73° 10′720″ W	0	Yes	Feral
P_38	14° 22′446″ S	73° 09′708″ W	5	Yes	Feral
Madeira Island					
Madeira	16° 54′35″ N	32° 39′4″ W	8	No	Irrigated
Mexico					
MX_51_Tlapanochestli	16° 57′27″ N	96° 42′33″ W	7	No	Farmed
MX_52_Aquilino	16° 30′50″ N	96° 47′26″ W	18	No	Farmed
MX_53_Hernandez	17° 1′36″ N	96° 31′25″ W	7	No	Farmed
Argentina					
Field local no. 86	25° 05′54″ S	66° 11′06″ W	2	No	Irrigated

DNA refers to number of successful DNA extractions. MaxEnt refers to localities being used in ecological niche modeling. Management refers to how the *D. coccus* are managed: Irrigated, is meant to describe the irrigation of *Opuntia ficus-indica* plants that are used to grow *D. coccus*, Feral refers to no special management, and Farmed refers to human mediated propagation of *D. coccus*.

of primer, and 0.75 µl BigDye per reaction. Cycle sequencing cleanup was completed using ethanol precipitation. Sequencing was completed on an ABI 3730xl capillary sequencer (Life Technologies) at Genomic Variation Lab (UC Davis). Post processing total length of mtDNA fragment going into alignment was 1,294 bp. DNA data were deposited in the Dryad repository (Accession number doi:10.5061/dryad.d772v).

DNA Alignment and Partitioning Scheme. DNA alignment was conducted using MUSCLE (Edgar 2004). Codon positions were calculated in Mesquite v.1.74 (Maddison and Maddison 2008, 2015) and gene-specific partitions were deduced by alignments using BLAST against the NCBI nucleotide database (Johnson et al. 2008). Post alignment a parsimony analyses was conducted using the R package "pegas" (Paradis 2010) (Fig. 1) for a rough estimate of relationships among samples. Partitionfinder (Lanfear et al. 2012) was used to calculate BIC scores to determine the best substitution model for

Table 2. Summary statistics of genetic diversity of *D. coccus* collections

Location and n = sample size	Tajima's D (P-value normal)	Haplotypes (Nei's genetic diversity ± SE) and private alleles by location or farm	Watterson's haploid population mutation rate ± SE
Aquilino farm (MX) $n = 18$	−0.740 ($P = 0.459$)	3 (3.131e-3 ± 3.387e-3) 2	NE
Hernandez farm (MX) $n = 7$	2.227 ($P = 0.026$)	3 (7.507e-3 ± 4.491e-3) 0	NE
Tlapanochestli farm (MX) $n = 7$	−1.006 ($P = 0.314$)	2 (2.208e-4 ± 9.545e-5) 1	NE
Madeira $n = 8$	NaN	1 (0) 1	NE
Mexico total collections $n = 32$	2.443 ($P = 0.015$)	5 (6.248e-03 ± 3.312e-3) 5	4.718 ± 0.310
Mexico and Madeira total $n = 40$	1.954 ($P = 0.051$)	6 (5.780e-03 ± 3.064e-3) 6	NE
Peru total collections $n = 43$	NaN	1 (0) 1	0
All collections $n = 83$	2.999 ($P = 0.003$)	7 (6.520e-03 ± 3.376e-3)	4.208 ± 0.151

Private alleles, NE, not estimated; NaN, not able to estimate.

this dataset. We used the HKY with invariant sites model with four gamma rate categories and partitioned each gene separately.

Phylogenetic Analyses. We chose to reconstruct the data using a coalescent process using exponential growth via BEAST v.2.3.1 (Bouckaert et al. 2014). A lognormal relaxed clock was chosen as this best accommodates the rate change in our dataset as we have several outgroup taxa that probably have different rates of substitution than the population level sampling of *D. coccus*. As outgroups, we chose representatives from the two most closely related clades to *D. coccus*. *D. coccus* is sister to both the Cactoidea feeding *Dactylopius* and the primarily Opuntioidea feeding *Dactylopius* (Van Dam 2013). *D. confertus* represents the Cactoidea feeding *Dactylopius* and was included as an outgroup. Representatives of the Opuntioid feeding clades [*D. austrinus*, *D. ceylonicus*, *D. opuntiae* (*Nopalea* biotype), and *D. zimmermanni*] that also share a common ancestor with *D. coccus* were also included as outgroups. *D. tomentosus* was not included as an outgroup, as it does not share a recent common ancestor with *D. coccus* and the other outgroups chosen, and would cause artificial monophyly of the outgroups (Van Dam 2013).

To calibrate, the phylogeny and best approximate the rate variation found in CO1 we chose to use the method described in Marshall et al. (2012). Here, we used the "Brower" rate (Brower 1994) of 0.0115 substitutions per site per million years per lineage for CO1 and then linked that rate to the other genes (tRNALeu and CO2), at the same time we let the rate variation range across the other known insect substitution rates for CO1 (Marshall et al. 2012). We ran 20×10^7 mcmc generations as this resulted in mixing upon examining the log files in Tracer v1.5 (Rambaut and Drummond 2009).

Demographic History Summary Statistics. Genetic diversity estimates were obtained from R libraries "ape" and "pegas" (Paradis 2010; Table 2).

Migration Route Analyses. Bayes-factor comparisons of different migration route models were performed using Migrate-*n* v.3.3.1 (Beerli and Palczewski 2010). Initial parameter settings included a mutation rate calculation from data using an estimated Watterson's statistic in Migrate-*n*. Inheritance scalar of 0.25 was used. Finally, Bayes-factors and model probability were calculated using methods described in Beerli and Palczewski (2010) and Kass and Raftery (1995).

Species Distribution Niche Modeling. In order to test, if present day absence of cochineal insects outside of cultivation in Mexico was the result of Holocene climate change, a species distribution model was constructed using R package "dismo" (Hijmans et al. 2010) as outlined by Hijmans and Elith (2013). Raster files of postindustrial climate data (1950–2000 AD) and elevation data were downloaded from WorldClim database (Hijmans et al. 2008) at 2.5 arc min scale. Only presence training data from Sierra Central region in Peru (Table 1) was used to reconstruct distribution models for species because this is the only area sampled where cochineal insects occur without human assistance. Furthermore, it would be inappropriate to include the samples from Oaxaca here, as they need human care to exist. For example, captive animals in a zoological park would not be included when modeling their respective species distributions either.

Identical elevation data were used for Pleistocene species distribution models. We used raster data from Community Climate System Model (CCSM), Model for Interdisciplinary Research on Climate version 3.2 (MIROC), and WorldClim data for climate models of the last glacial maxima (21×10^3 ybp; Hijmans et al. 2008). Raster files were taken at 2.5 arc min precision level.

Results

Specimen Collection and Field Survey. No alternative host-plants were found in Peru despite surveying much of the Sierra Central. This solidifies what was already known about this species host-range in Peru.

In Oaxaca, we document the history of the farms sampled there. Our results indicate no secondary importation of Peruvian cochineal into Oaxacan farms sampled.

Demographic Summary Statistics. In total, 43 extractions were successful from Peru, 25 from Mexico, eight from Madeira, and two from Argentina (Table 1). Argentinian *D. coccus* had the same haplotype as the Peruvian samples and represents an introduced population from Peru. This confirms what we know about this regions cochineal form the literature (Chávez-Moreno et al. 2009). Argentinian *D. coccus* had the same nuclear haplotypes as the Mexican cochineal

subsampled. These markers were able to detect intraspecific differentiation in other *Dactylopius* spp. (Van Dam 2013). The nuclear markers are too slowly evolving to detect differentiation between these two locations, indicating that the Peru and Mexico divergence event is quite recent.

We expected to find differences in the mtDNA haplotypes between Peruvian collections given the geographic structure and numerous barriers. However, they were all identical. This is unusual as all other *Dactylopius* species show significant genetic structure, when geographic replicates are taken at different locations, especially in the Andean Mountains at both mtDNA and nuclear markers (Van Dam 2013). In Andean, *Dactylopius* species in particular they show high levels of differentiation that frequently leads to speciation (Van Dam 2013).

Summary statistics indicate that cochineal insect collections in Mexico have undergone a decline in mtDNA diversity over time as indicated by Tajima's D statistics >2 (Table 2) (Frankham et al. 2011). The Peruvian and Mexican cochineal clades were at most 0.7% divergent with 19 substitutions. The mean divergence was $0.5 \pm 0.03\%$ (mean $\pm$ standard deviation) between the Peruvian and Mexican haplotypes. The maximum divergence between Mexican clades was also 0.7% divergence with 19 substitutions. Intraspecific differences between Mexican *D. coccus* haplotypes is no more than the Peruvian *D. coccus*. Sister Peruvian and Mexican MX1 clades (Fig. 3) are only separated by a single substitution. Divergence among mtDNA haplotypes is at the low end of intraspecific divergence for insects, but within the range of what we would expect based on other mtDNA intraspecific Hexapod studies (Cognato 2006).

Table 3. Mean coalescent times for node ages followed by 95% highest posterior density (HPD) intervals for mtDNA *D. coccus* clades as described in Fig. 3

Clade	Mean node age in millions of years [95% HPD interval]
MX1	0.0173 [0.0057–0.0375]
Peru	0.0225 [0.0075–0.0519]
MX1.Peru	0.0357 [0.0121–0.0867]
(MX1.Peru),(MX2,MX3)	0.072 [0.034–0.2096]
Madeira	0.0024 [0.0013–0.0211]
MX2	0.0109 [0.0055–0.0333]
Madeira,MX2	0.0112 [0.0109–0.0434]
MX3	0.007 [0.0013–0.0219]
(Madeira,MX2),(MX3)	0.0252 [0.0091–0.0927]
D. coccus, outgroups	1.6664 [1.2885–6.5838]

Phylogenetic Analyses. The Peruvian haplotype was sister to Mexican haplotypes and not ancestral to the haplotypes from Mexico and Madeira Island (Fig. 3). The Peruvian haplotype diverged from the sister Mexican haplotypes $3.57 \times 10^4 \pm 1.21 \times 10^4 - 8.67 \times 10^4$ years before present. This would seem to indicate that the coalescence of these mtDNA clades was relatively recent. A full list of *D. coccus* coalescence ages and 95% highest posterior density intervals is given in Table 3. We report coalescent times for identical haplotypes for completeness only in Table 3 (e.g., Peru haplotype), and those specific values should not be used for inferences.

Migration Route Analyses. Migrate-*n* analyses clearly indicated that no mtDNA migration occurred between Mexican cochineals and Peruvian cochineals (Table 4). When constrained to a single population a migration model of gene flow from Mexico to Peru was significantly favored over a model in reverse direction or panmixia (Table 4).

Species Distribution Niche Modeling. MaxEnt species distribution model implemented in "dismo" accurately predicted the presence of cochineal insects based on sampled locations (Table 1), with an AUC = 1 (a random guess would give an AUC value of 0.5 (Hijmans et al. 2008).

Pleistocene reconstructions differed in prediction. CCSM model showed a stable pattern for availability of ecological niche space in the Andean Cordillera (Fig. 4). MIROC model showed a reduction of habitat niche space during last glacial maxima. CCSM model showed that there is little suitable niche space available in Oaxaca (Fig. 4). MIROC model indicated that there was substantial niche space available for *D. coccus* across present day Oaxaca and Puebla states (Fig. 4) during the last glacial maxima. The MIROC climate model predicts dryer conditions with a less pronounced monsoon season. This provides support to the hypothesis that *D. coccus* was once abundant in Oaxaca prior to the onset of a pronounced monsoon season during the Holocene that Oaxaca currently experiences.

Discussion

Where Did Cochineal Originate, Oaxaca or Peru? The pattern of mtDNA diversity corroborates ecological data pointing to the origins of *D. coccus*. The *D. coccus* mtDNA haplotypes from three small Oaxacan farms is five times more diverse than Peruvian samples found across a broad geographic range

Table 4. Migrate-*n* gene flow models comparing *D. coccus* mtDNA collections from Mexico and Peru under a full model of gene exchange with two migration rates compared with a model specifying no migration and two populations

Model	Ln[mL] Bezier	Ln[Bayes-factor] Bezier	Model probability	Model choice
Migration two location model	−3530	−1421	2.333e-309	2
No Migration two location model	−2577	0	1	1 (best)
One way Mexico –> Peru	−3719	−2283 (0)	0 (0.797)	3 (1)
One way Peru –> Mexico	−3720	−2287 (−3.590)	0 (0.133)	4 (2)
Single panmictic population	−3721	−2288 (−4.852)	0 (0.071)	5 (3)

Last three rows Migrate-*n* gene flow models of *D. coccus* mtDNA under a scenario where populations are constrained to one location to compare models of gene migration.

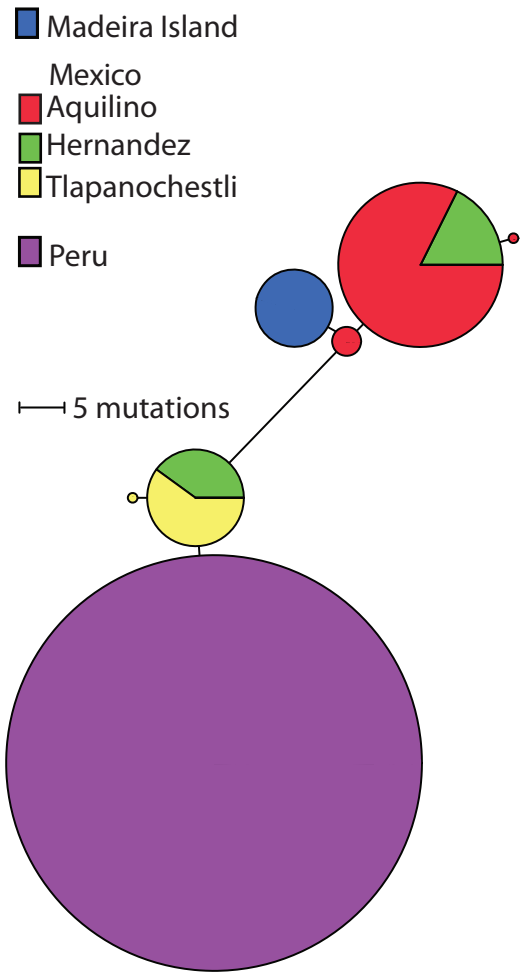

Fig. 2. *D. coccus* (cochineal insects) parsimony haplotype network reconstruction. Haplotype names are by location (name of cochineal farm in Oaxaca Mexico, Madeira, and Peru).

spanning hundreds of kilometers. Both the mtDNA (Figs. 2 and 3) and lack of variation in the three nuclear genes support a recent divergence date. In other *Dactylopius* species, similar levels of genetic diversity are found within geographically restricted lineages (Van Dam 2013). It would be helpful to have additional nuclear markers that are even more rapidly evolving than the mtDNA markers to corroborate the divergence times calculated here, as mtDNA data alone is not robust.

Alternative host plants were not found in Peru, making it highly unlikely that *D. coccus* switched hosts from a Peruvian cactus to *O. ficus-indica*, which originated in Oaxaca. Ecological niche modeling also explains why current populations of *D. coccus* are not found outside of farms in Mexico, and show that conditions during the Pleistocene were much more amenable to *D. coccus*.

Migrate-*n* analyses provide significantly higher support for a model of no migration between Mexico and Peru (Table 4). Such results clearly support dispersal as opposed to vicariance events where roughly equivalent amounts of genetic diversity are expected (Kropf et al. 2006). From the interviews of Oaxacan cochineal farmers, we find no evidence to support transport from Peru during the twentieth century. However, this has taken place in other regions of Mexico as Chávez-Moreno et al. (2009) outline. Campana et al. (2015) suggest that transport from Peru to Oaxaca has occurred, but one would certainly expect to find at least a few Peruvian haplotypes if this is the case. Nonetheless, admixture from Peru could be a serious problem in solving the origins of this species and should be considered carefully in future analyses.

To further undermine the Peruvian origins of *D. coccus*, endemic insect predators of *D. coccus* have not been documented from Peru while they are abundant in Oaxaca (Portillo 2005). In Peru, the insect predators of *D. coccus* that have been documented (*Sympherobius* sp.) are the result of purposeful introductions to control Psudococcidae crop pests (Portillo 2005). The lack of native host plants, endemic natural enemies, and low genetic diversity, makes the origins of *D. coccus* from Peru unlikely. In some ecological and genetic respects, Peruvian *D. coccus* seems more like an invasive species than an endemic insect. Additionally, Peruvian and Madeiran cochineals show a similar lack of genetic diversity. Tajima's D statistics (Table 2) indicate that there has been a decline in the Mexican *D. coccus*. This helps explain why the Peruvian and Madeiran haplotypes were not recovered from the Mexican samples. The complete lack of mtDNA haplotype diversity, native insect predators, and absence of alternative host-plants found in Peru, are replicated in Madeira where historical records provide clear evidence that it was introduced from Oaxaca.

We also try to resolve why despite plentiful host plants, of *D. coccus* cannot be found outside of cultivation in Mexico. *D. coccus* can be found in vacant lots in urban settings in Ayacucho, Peru, and other towns in the Sierra Central. *O. ficus-indica* also grows in urban environments in Oaxaca city and the surrounding areas. This indicates that urbanization in parts of the Valley of Oaxaca is only a partial explanation for its complete absence outside of farms. We provide evidence that climate change in addition to habitat loss (Chávez-Moreno et al. 2009) is a likely explanation for the necessity to grow *D. coccus* under protected shelters in Oaxaca (Fig. 4). From the results of our niche models, we show that adequate environmental conditions for *D. coccus* in Oaxaca are all but absent. The absence of a strong monsoon season in the Peruvian Andes is a contributing factor that allows them to survive there. Intense monsoon rainfall dislodges *D. coccus* from the *Opuntia* cladodes while the other species in Oaxaca are considerably smaller, harder to dislodge, and can hide under the bark of the *Opuntia*. The habitat where they occur in Peru is drier without the same monsoon conditions. In fact, *D. coccus* is grown without shelters during the dry season in Oaxaca at the Aquilino farm, again echoing that the pronounced monsoon season of present day Oaxaca is a major driver for it's absence

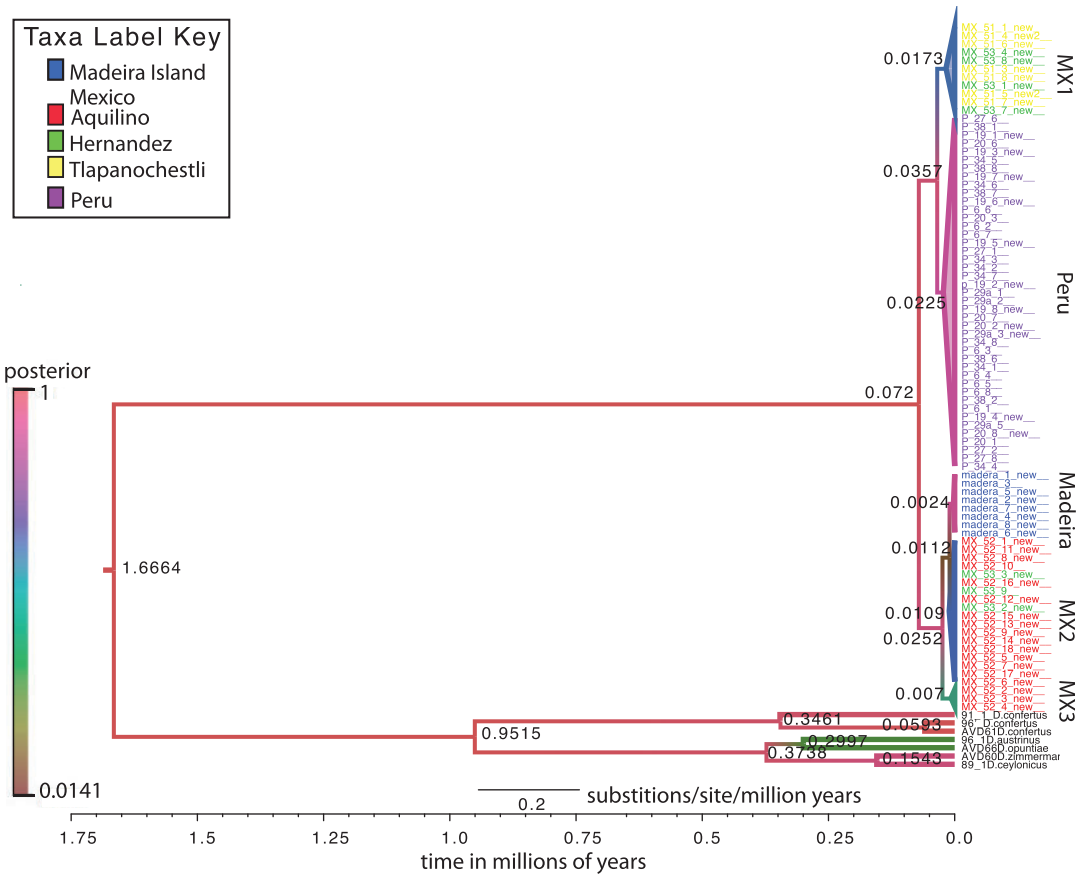

Fig. 3. Phylogenetic maximum clade credibility tree using mean branch lengths from mtDNA gene regions CO1–CO2 as calculate by BEAST v.2.3.1 for collections of *D. coccus*. Node labels are mean divergence time. Branch lengths represent mutations per site per millions of years. Taxa labels are shaded by location as in Fig. 1. Branches are colored (online only) by Bayesian posterior probability support as in the figure legend. Clade MX1, MX2, and MX3 are from cochineal insects collected in farms in Oaxaca Mexico, Maderia from Madeira Island in the Atlantic Ocean, and Peru clade are from cochineal insects collected from Peruvian localities.

outside of farms. Thus habitat loss alone cannot fully explain the lack of *D. coccus* outside of farms in Mexico, and it is probably a combination of both factors.

Although we provide strong mtDNA evidence in this study we are lacking in nuclear markers that capture detailed population structure. All that our nuclear markers allow us to indicate here is that *D. coccus* haplotypes are recently diverged. Thus we are limited in our ability to discredit the hypothesis that endosymbiont selective sweeps may have resulted in the absence of mtDNA diversity found in Peru (Hurst and Jiggins 2005, Ramírez-Puebla et al. 2010, Campana et al. 2015). Although endosymbiont driven selective sweeps has been proposed as a hypothesis in this system actual evidence for such an event is still lacking (Campana et al. 2015). If ecological and climate data were not taken into account and we didn't replicate this same lack of mtDNA diversity in Madeira and Peru it would seem like a good alternative hypothesis to explain the data. Fortunately, we have lots of natural history data for *D. coccus* as it's well studied in that respect.

Future Directions

In order to rule out bacterial driven selective sweeps a complex meta-genomic approach will need to be taken. Facultative endosymbionts can be short lived but leave behind important genes via lateral gene transfer. Such events will have to be taken into account before ruling out the endosymbiont sweep hypothesis. Additionally, uncommon or new endosymbionts might play a role in putative selective sweeps other than *Wolbachia* as other endosymbionts are known to drive selective sweeps in insects (Hurst and Jiggins 2005). Future work using nuclear markers will also have to take into account paternal genome elimination (PGE) which is the mode of inheritance in *D. coccus* (Normark 2003, Úbeda and Normark 2006, Ross et al. 2011). PGE increases the chance of missing a bacterial endosymbiont driven selective sweeps by eliminating the genomic contribution

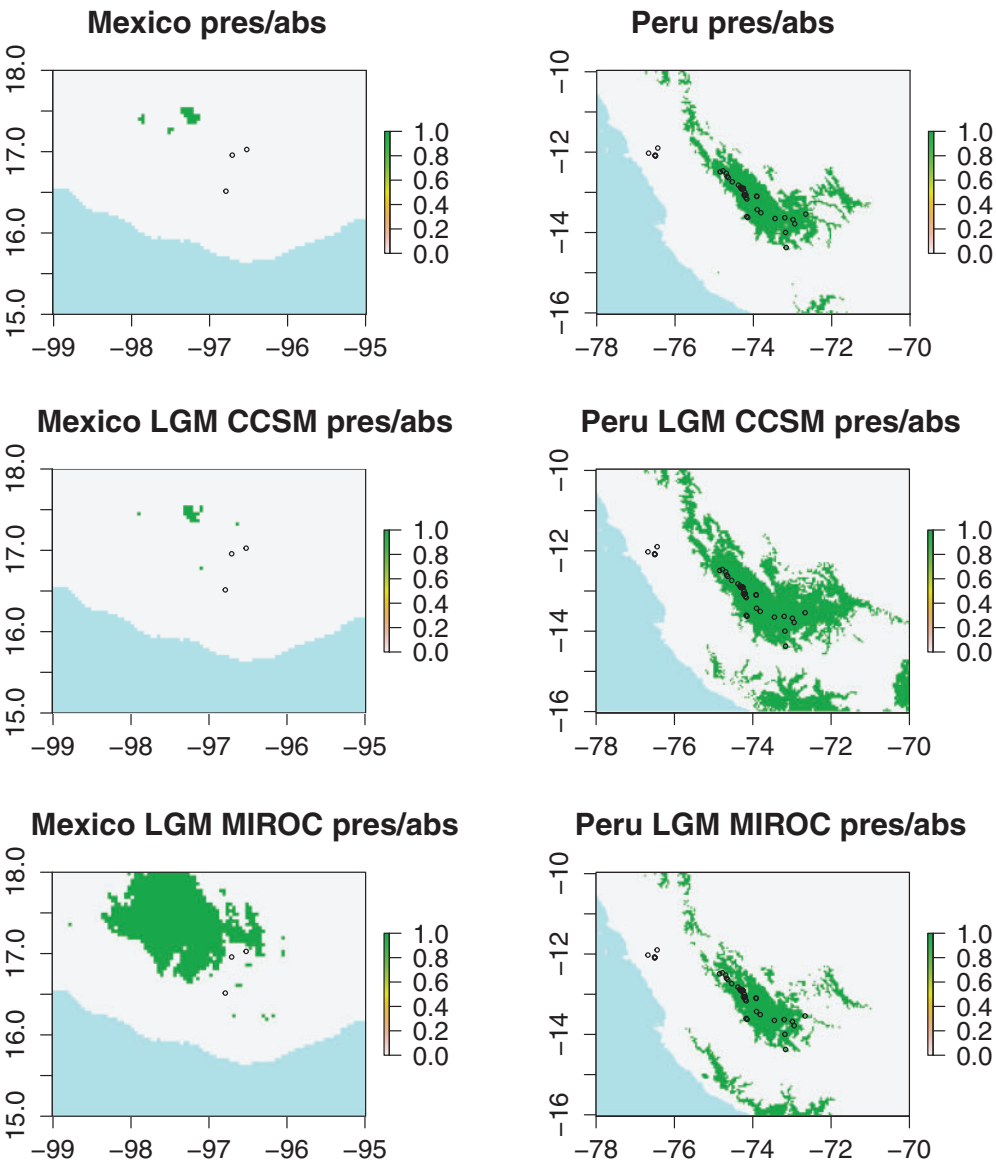

Fig. 4. Left column: above threshold values (pres per abs = presence–absence) of MaxEnt of species distribution models for cochineal insects found in present day Oaxaca with collection localities marked by open circles: top present day, center CCSM last glacial maxima reconstruction, bottom MIROC 3.2 last glacial maxima reconstruction. Right column: above threshold values of MaxEnt of species distribution models found in present day Peru marked by open circles: top present day, center CCSM last glacial maxima reconstruction, bottom MIROC 3.2 last glacial maxima reconstruction.

from the male paternal lineage concomitantly reducing the male N_e, and thereby reducing the coalescence time for nuclear markers.

Conclusions

Although we cannot completely rule out bacterial driven selective sweeps leading to only a single mtDNA haplotype across the entire Andean range of *D. coccus*, other lines of evidence in addition to the mtDNA data make the selective sweep explanation for the observed pattern less likely. Primarily there are no native South American alternative host-plants for *D. coccus*, and an absence of endemic insect predators and parasites. Climate change along with habitat loss in Oaxaca helps to explain why *D. coccus* isn't found outside of farms in Oaxaca. Our mtDNA data presented here is also reliable. We find no evidence of secondary admixture in Oaxacan cochineal from Peru based on field surveys and the genetic data itself. This adds to the credibility

of the Oaxacan farmers' stories. It is certainly possible that in other Oaxacan farms smuggled Peruvian cochineal into Oaxaca, but unlikely as there was already an available source of cochineal. We find that Campana et al. (2015) may have relied heavily on samples from different farms than we did in this study and don't clearly report which farms in Oaxaca they actually sampled from. Finally, we find a replicated lack of mtDNA genetic diversity in Madeira indicating a clear loss of past diversity in Oaxaca and low mtDNA diversity in regions of introduction.

Although we are not able to provide indisputable evidence to solve the geographic origins of *D. coccus* here, we do bring new data to light that indicates a geographic origin near present day Oaxaca. Future work will have to involve sampling multiple geographically replicated populations using meta-genomic sampling and careful attention to admixture in both directions to fully resolve this question.

Acknowledgments

We thank Matthew Van Dam (UC Berkeley) for collaborative field-work in Mexico. We thank Pesach Lubinsky (USDA) and Ana Lilia Viguerez (Universidad de Guadalajara Centro Universitario de Ciencias Biológicas y Agropecuarias, Jalisco Mexico) for advice on field-work in Mexico. We thank Victor Florez (Universidad Nacional de San Cristobal de Huamanga Peru), and Rosmarina Marin (Universidad Nacional de La Molina Peru) for advice on field-work in Peru. We thank Chris Pagan (UC Davis) for cloning and primer design advice. We thank Steve Nadler and Jay Rosenheim (UC Davis) and Matthew Van Dam for initial reviews of this manuscript. We thank our funding support from the UC Davis Entomology and Animal Science Department, Theodore Roosevelt Memorial Fund from the American Museum of Natural History, Lewis and Clark Scholarship, UC MEXUS mini-grant and DIG, Pacific Rim Research Program mini-grant and AGRF, Robert van den Bosch Scholarship in Biological Control.

References Cited

Andersen, J. C., M. E. Gruwell, G. E. Morse, and B. B. Normark. 2010. Cryptic diversity in the *Aspidiotus nerii* complex in Australia. Ann. Entomol. Soc. Am. 103: 844–854.

Beerli, P., and M. Palczewski. 2010. Unified framework to evaluate panmixia and migration direction among multiple sampling locations. Genetics 185: 313–326.

Berthouly-Salazar, C., X. Rognon, T. N. Van, M. Gély, C. V. Chi, M. Tixier-Boichard, B. Bed'Hom, N. Bruneau, E. Verrier, J. C. Maillard, et al. 2010. Vietnamese chickens: a gate towards Asian genetic diversity. BMC Genet. 11: 53.

Besnard, G., R. Rubio de Casas, and P. Vargas. 2007. Plastid and nuclear DNA polymorphism reveals historical processes of isolation and reticulation in the olive tree complex (Olea europaea). J. Biogeogr. 34: 736–752.

Borlaug, N. E. 2000. Ending world hunger. the promise of biotechnology and the threat of antiscience zealotry. Plant Physiol. 124: 487–490.

Bouckaert, R., J. Heled, D. Kühnert, T. Vaughan, C. H. Wu, D. Xie, M. A. Suchard, A. Rambaut, and A. J. Drummond. 2014. BEAST 2: a software platform for bayesian evolutionary analysis. PLoS Comput. Biol. 10: e1003537. doi:10.1371/journal.pcbi.1003537.

Brower, A. 1994. Rapid morphological radiation and convergence in geographical races of the butterfly, Heliconius erato, inferred from patterns of mitochondrial DNA evolution. Proc. Natl Acad. Sci. U. S. A. 91: 6491–6495.

Bruford, M. W., D. G. Bradley, and G. Luikart. 2003. DNA markers reveal the complexity of livestock domestication. Nat. Rev. Genet. 4: 900–910.

Burban, C., R. J. Petit, E. Carcreff, and H. Jactel. 1999. Rangewide variation of the maritime pine bast scale *Matsucoccus feytaudi* Duc (Homoptera: Matsucoccidae) in relation to the genetic structure of its host. Mol. Ecol. 8: 1593–1602.

Campana, M. G., N. M. Robles García, and N. Tuross. 2015. America's red gold: multiple lineages of cultivated cochineal in Mexico. Ecol. Evol. 5: 607–617.

Carneiro, M., S. Afonso, A. Geraldes, H. Garreau, G. Bolet, S. Boucher, A. Tircazes, G. Queney, M. W. Nachman, and N. Ferrand. 2011. The genetic structure of domestic rabbits. Mol. Biol. Evol. 28: 1801–1816.

Chávez-Moreno, C. K., A. Tecante, and A. Casas. 2009. The *Opuntia* (Cactaceae) and *Dactylopius* (Hemiptera: Dactylopiidae) in Mexico: a historical perspective of use, interaction and distribution. Biodivers. Conserv. 18: 3337–3355.

Chávez-Moreno, C. K., A. Tecante, A. Casas, and L. E. Claps. 2011. Distribution and habitat in Mexico of *Dactylopius* Costa (Hemiptera: Dactylopiidae) and their cacti hosts (Cactaceae: Opuntioideae). Neotrop. Entomol. 40: 62–71.

Coart, E., S. Van Glabeke, M. De Loose, A. S. Larsen, and I. Roldán-Ruiz. 2006. Chloroplast diversity in the genus *Malus*: new insights into the relationship between the European wild apple (*Malus sylvestris* (L.) Mill.) and the domesticated apple (*Malus domestica* Borkh.). Mol. Ecol. 15: 2171–2182.

Cognato, A. I. 2006. Standard percent DNA sequence difference for insects does not predict species boundaries. J. Econ. Entomol. 99: 1037–1045.

Cornille, A., P. Gladieux, M.J.M. Smulders, I. Roldán-Ruiz, F. Laurens, B. Le Cam, A. Nersesyan, J. Clavel, M. Olonova, L. Feugey, et al. 2012. New insight into the history of domesticated apple: secondary contribution of the European wild apple to the genome of cultivated varieties. PLoS Genet. 8: e1002703.

De Lotto, G. 1974. On the status and identity of the cochineal insects (Homoptera: Coccoidea: Dactylopiidae). J. Entomol. Soc. South. Afr. 37: 167–193.

Delplancke, M., N. Alvarez, A. Espíndola, H. Joly, L. Benoit, E. Brouck, and N. Arrigo. 2012. Gene flow among wild and domesticated almond species: insights from chloroplast and nuclear markers. Evol. Appl. 5: 317–329.

Deveoglu, O., E. Torgan, and R. Karadag. 2010. Identification of dyestuffs in the natural pigments produced with Al3+, Fe2+ and Sn2+ mordant metals from Cochineal (*Dactylopius coccus* Costa) and Walloon oak (Quercus ithaburensis Decaisne) by HPLC-DAD. Asian J. Chem. 22: 7021–7030.

Diamond, J. 2002. Evolution, consequences and future of plant and animal domestication. Nature 418: 700–707.

Donkin, R. A. 1977. Spanish red: an ethnographical study of cochineal and the *Opuntia* cactus. Trans. Am. Philisophical Soc. New Ser. 67: 1–84.

Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32: 1792–1797.

Eriksson, J., G. Larson, U. Gunnarsson, B. Bed'hom, M. Tixier-Boichard, L. Strömstedt, D. Wright, A. Jungerius, A. Vereijken, E. Randi, et al. 2008. Identification of the yellow skin gene reveals a hybrid origin of the domestic chicken. PLoS Genet. 4: e1000010.

Feinman, G. M., L. M. Nicholas, and H. R. Haines. 2007. Classic period agricultural intensificaiton and domestic life at

el Palmillo, Valley of Oaxaca, Mexico. *In* T. L. Thurston and C. T. Fisher (eds.), Seeking a richer harvest, Springer, NY.

Frankham, R., J. D. Ballou, and D. A. Briscoe. 2011. Introduction to conservation genetics. Cambridge University Press, NY

Griffith, M. P. 2004. The origins of an important cactus crop, *Opuntia ficus-indica* (Cactaceae): new molecular evidence. Am. J. Bot. 91: 1915–1921.

Griffith, M. P., and J. M. Porter. 2009. Phylogeny of Opuntioideae (Cactaceae). Int. J. Plant Sci. 170: 107–116.

Harpur, B. A., S. Minaei, C. F. Kent, and A. Zayed. 2012. Management increases genetic diversity of honey bees via admixture. Mol. Ecol. 21: 4414–4421.

Hijmans, R. J., and J. Elith. 2013. Species distribution modeling with R Introduction. (http://cran.r-project.org/web/packages/dismo/vignettes/sdm.pdf) (accessed June (2014)).

Hijmans, R. J., S. E. Cameron, J. L. Parra, P. Jones, A. Jarvis, and K. Richardson. 2008. WorldClim version 1.4. (http://www.worldclim.org) (accessed December (2008)).

Hijmans, R., S. Phillips, J. Leathwick, and J. Elith. 2010. Dismo: species distribution modeling. R package version 0.5-4 (http//CRAN. R-project. org/package= dismo) (accessed June (2014)).

Hufford, M. B., X. Xu, J. van Heerwaarden, T. Pyhäjärvi, J.-M. Chia, R. A. Cartwright, R. J. Elshire, J. C. Glaubitz, K. E. Guill, S. M. Kaeppler, et al. 2012. Comparative population genomics of maize domestication and improvement. Nat. Genet. 44: 808–811.

Hurst, G.D.D., and F. M. Jiggins. 2005. Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: the effects of inherited symbionts. Proc. Biol. Sci. 272: 1525–1534.

Hyten, D. L., Q. Song, Y. Zhu, I.-Y. Choi, R. L. Nelson, J. M. Costa, J. E. Specht, R. C. Shoemaker, and P. B. Cregan. 2006. Impacts of genetic bottlenecks on soybean genome diversity. Proc. Natl Acad. Sci. U. S. A. 103: 16666–16671.

Innan, H., and Y. Kim. 2004. Pattern of polymorphism after strong artificial selection in a domestication event. Proc. Natl Acad. Sci. U. S. A. 101: 10667–10672.

Johnson, M., I. Zaretskaya, Y. Raytselis, Y. Merezhuk, S. McGinnis, and T. L. Madden. 2008. NCBI BLAST: a better web interface. Nucleic Acids Res. 36: W5–W9.

Kanginakudru, S., M. Metta, R. D. Jakati, and J. Nagaraju. 2008. Genetic evidence from Indian red jungle fowl corroborates multiple domestication of modern day chicken. BMC Evol. Biol. 8: 174.

Kass, R. E., and A. E. Raftery. 1995. Bayes factors. J. Am. Stat. Assoc. 90: 773–795.

Komiyama, T., H. Kobayashi, Y. Tateno, H. Inoko, T. Gojobori, and K. Ikeo. 2009. An evolutionary origin and selection process of goldfish. Gene 430: 5–11.

Kropf, M., H. P. Comes, and J. W. Kadereit. 2006. Long-distance dispersal vs vicariance: the origin and genetic diversity of alpine plants in the Spanish Sierra Nevada. New Phytol. 172: 169–184.

Lanfear, R., B. Calcott, S.Y.W. Ho, and S. Guindon. 2012. Partitionfinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol. Biol. Evol. 29: 1695–1701.

Lawler, A. 2012. From farmyard to the lab. Science 338: 1022–1023.

Lubinsky, P., K. M. Cameron, M. C. Molina, M. Wong, S. Lepers-Andrzejewski, A. Gómez-Pompa, and S.-C. Kim. 2008. Neotropical roots of a polynesian spice: the hybrid origin of Tahitian vanilla, *Vanilla tahitensis* (Orchidaceae). Am. J. Bot. 95: 1040–1047.

Maddison, W. P., and D. R. Maddison. 2008. Mesquite: a modular system for evolutionary analysis. Evolution 62: 1103–1118.

Maddison, W. P., and D. R. Maddison. 2015. Mesquite: a modular system for evolutionary analysis. Version 3.02. https://mesquiteproject.wikispaces.com/ (accessed February 2015).

Majure, L. C., R. Puente, M. Patrick Griffith, W. S. Judd, P. S. Soltis, and D. E. Soltis. 2012. Phylogeny of *Opuntia* s.s. (Cactaceae): Clade delineation, geographic origins, reticulate evolution. Am. J. Bot. 99: 847–864.

Marshall, D. C., K.B.R. Hill, K. A. Marske, C. Chambers, T. R. Buckley, and C. Simon. 2012. Limited, episodic diversification and contrasting phylogeography in a New Zealand cicada radiation. BMC Evol. Biol. 12: 177.

Myles, S., A. R. Boyko, C. L. Owens, P. J. Brown, F. Grassi, M. K. Aradhya, B. Prins, A. Reynolds, J.-M. Chia, D. Ware, et al. 2011. Genetic structure and domestication history of the grape. Proc. Natl Acad. Sci. U. S. A. 108: 3530–3535.

Normark, B. B. 2003. The evolution of alternative genetic systems in insects. Annu. Rev. Entomol. 48: 397–423.

Paradis, E. 2010. pegas: an R package for population genetics with an integrated-modular approach. Bioinformatics 26: 419–420.

Peggie, D. A., A. N. Hulme, H. McNab, and A. Quye. 2008. Towards the identification of characteristic minor components from textiles dyed with weld (Reseda luteola L.) and those dyed with Mexican cochineal (*Dactylopius coccus* Costa). Microchim. Acta. 162: 371–380.

Pelham Wright, N. 1963. A thousand years of cochineal: a lost but traditional Mexican industry on its way back. Am. Dyest. Report. 635–639.

Perez-Guerra, G., and M. Kosztarab. 1992. Biosystematics of the family Dactylopiidae (Homoptera: Coccinea) with emphasis on the life cycle of *Dactylopius coccus* Costa. Bull. Virginia Agric. Exp. Stn. 92: 1–90.

Phillips, T. D., and J. P. Murphy. 1993. Distribution and analysis of isozyme polymorphism in north-american cultivated oat germplasm. Crop Sci. 33: 460–469.

Philpott, D. E., S. H. Berlocher, R. F. Mitchell, and L. M. Hanks. 2009. Molecular validation of a morphological character for distinguishing between the armored scale insects *Chionaspis pinifoliae* and *Chionaspis heterophyllae* (Hemiptera: Diaspididae). Ann. Entomol. Soc. Am. 102: 381–385.

Phipps, E. 2010. Tracing cochineal through the collection of the metropolitan museum. Textile Society of America Symposium Proceedings. Paper 44. http://digitalcommons.unl.edu/tsaconf/44. (accessed February (2015)).

Portillo, L. 2005. Origen de *Dactylopius coccus* Costa (Hemiptera: Dactylopiidae): Norte o Sudamérica? Dugesiana 12: 1–8.

Provencher, L. M., G. E. Morse, A. R. Weeks, and B. J. Normark. 2005. Parthenogenesis in the *Aspidiotus nerii* Complex (Hemiptera: Diaspididae): a single origin of a worldwide, polyphagous lineage associated with cardinium bacteria. Ann. Entomol. Soc. Am. 98: 629–635.

Purugganan, M. D., and D. Q. Fuller. 2009. The nature of selection during plant domestication. Nature 457: 843–848.

Rambaut, A., and A. J. Drummond. 2009. Tracer V1.5. (http//beast.bio.ed.ac.uk/Tracer) (accessed June (2014)).

Ramírez-Puebla, S. T., M. Rosenblueth, C. K. Chávez-Moreno, M. C. Catanho Pereira De Lyra, A. Tecante, and E. Martínez-Romero. 2010. Molecular phylogeny of the genus *Dactylopius* (Hemiptera: Dactylopiidae) and identification of the symbiotic bacteria. Environ. Entomol. 39: 1178–1183.

Ranjan, S. K., C. B. Mallick, D. Saha, A. S. Vidyarthi, and R. Ramani. 2011. Genetic variation among species, races, forms and inbred lines of lac insects belonging to the genus *Kerria* (Homoptera, Tachardiidae). Genet. Mol. Biol. 34: 511–519.

Rodríguez, L. C., and U. Pascual. 2004. Land clearance and social capital in mountain agro–ecosystems: the case of *Opuntia* scrubland in Ayacucho, Peru. Ecol. Econ. 49: 243–252.

Rodríguez, L. C., Ú. Pascual, and H. M. Niemeyer. 2006. Local identification and valuation of ecosystem goods and services from *Opuntia* scrublands of Ayacucho, Peru. Ecol. Econ. 57: 30–44.

Ross, L., D. M. Shuker, and I. Pen. 2011. The evolution and suppression of male suicide under paternal genome elimination. Evolution 65: 554–563.

Ross-Ibarra, J., P. L. Morrell, and B. S. Gaut. 2007. Plant domestication, a unique opportunity to identify the genetic basis of adaptation. Proc. Natl Acad. Sci. U. S. A. 104: 8641–8648.

Rozen, S., and H. Skaletsky. 2000. Primer3 on the WWW for general users and for biologist programmers. Methods Mol. Biol. 132: 365–386.

Rubin, C.-J., M. C. Zody, J. Eriksson, J.R.S. Meadows, E. Sherwood, M. T. Webster, L. Jiang, M. Ingman, T. Sharpe, S. Ka, F. Hallböök, et al. 2010. Whole-genome resequencing reveals loci under selection during chicken domestication. Nature 464: 587–591.

Rylková, K., L. Kalous, V. Šlechtová, and J. Bohlen. 2010. Many branches, one root: first evidence for a monophyly of the morphologically highly diverse goldfish (*Carassius auratus*). Aquaculture 302: 36–41.

Serrano, A., M. M. Sousa, J. Hallett, J. A. Lopes, and M. C. Oliveira. 2011. Analysis of natural red dyes (cochineal) in textiles of historical importance using HPLC and multivariate data analysis. Anal. Bioanal. Chem. 401: 735–743.

Simon, C., F. Frati, A. Beckenbach, B. Crespi, H. Liu, and P. Flook. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entomol. Soc. Am. 87: 651–701.

Úbeda, F., and B. B. Normark. 2006. Male killers and the origins of paternal genome elimination. Theor. Popul. Biol. 70: 511–526.

Untergasser, A., I. Cutcutache, T. Koressaar, J. Ye, B. C. Faircloth, M. Remm, and S. G. Rozen. 2012. Primer3—new capabilities and interfaces. Nucleic Acids Res. 40: 1–12.

Van Dam, A. R., and B. May. 2012. A new species of Dactylopius Costa (*Dactylopius gracilipilus* sp. nov.) (Hemiptera: Coccoidea: Dactylopiidae) from the Chihuahuan Desert, Texas, U.S.A. Zootaxa 3573: 33–39.

Van Dam, A. R. 2013. Species delimitation of the cochineal insects (*Dactylopius*) using molecular phylogenetics and morphology. Ph.D. dissertation, University of California. Davis.

Whitfield, C. W., S. K. Behura, S. H. Berlocher, A. G. Clark, J. S. Johnston, W. S. Sheppard, D. R. Smith, A. V. Suarez, D. Weaver, and N. D. Tsutsui. 2006. Thrice out of Africa: ancient and recent expansions of the honey bee, *Apis mellifera*. Science 314: 642–645.

Wiener, P., and S. Wilkinson. 2011. Deciphering the genetic basis of animal domestication. Proc. Biol. Sci. 278: 3161–3170.

Wouters, J.A.N., and N. Rosario-Chirinos. 2011. Dye analysis of pre-columbian peruvian textiles with high-performance liquid chromatography and diode-array detection. J. Am. Inst. Conserv. 31: 237–255.

Wright, S. I., B. S. Gaut, I. V. Bi, S. C. Schroeder, M. Yamasaki, M. D. McMullen, and J. F. Doebley. 2005. The effects of artificial selection on the maize genome. Science 308: 1310–1314.

Xia, Q., Y. Guo, Z. Zhang, D. D. Li, Z. Xuan, Z. Li, F. Dai, Y. Li, D. Cheng, R. Li, et al. 2009. Complete resequencing of 40 genomes reveals domestication events and genes in silkworm (*Bombyx*). Science 326: 433–436.

Yokogawa, T., and T. Yahara. 2009. Mitochondrial phylogeny certified PGL (Paternal Genome Loss) is of single origin and haplodiploidy sensu stricto (arrhenotoky) did not evolve from PGL in the scale insects (Hemiptera: Coccoidea). Genes Genet. Syst. 84: 57–66.

Zeder, M. A., E. Emshwiller, B. D. Smith, and D. G. Bradley. 2006. Documenting domestication: the intersection of genetics and archaeology. Trends Genet. 22: 139–155.

Zhang, L.-B., Q. Zhu, Z.-Q. Wu, J. Ross-Ibarra, B. S. Gaut, S. Ge, and T. Sang. 2009. Selection on grain shattering genes and rates of rice domestication. New Phytol. 184: 708–720.

Zhu, Q., X. Zheng, J. Luo, B. S. Gaut, and S. Ge. 2007. Multilocus analysis of nucleotide variation of *Oryza sativa* and its wild relatives: severe bottleneck during domestication of rice. Mol. Biol. Evol. 24: 875–888.

Received 29 June 2014; accepted 12 February 2015.