Original Research

MITOCHONDRIAL DNA VARIATION OF FERAL HONEY BEES (*APIS MELLIFERA* L.) FROM UTAH (USA)

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Abstract

A study was conducted on the mitochondrial DNA genetic diversity of feral colonies and swarms of *Apis mellifera* from ten counties in Utah by sequencing the intergenic region of the cytochrome oxidase (COI-COII) gene region. A total of 20 haplotypes were found from 174 honey bee colony samples collected from 2008 to 2017. Samples belonged to the A (African) (48%); C (Eastern Europe) (43%); M (Western Europe) (4%); and O (Oriental) lineages (5%). Ten African A lineage haplotypes were observed with two unique to Utah among A lineage haplotypes recorded in the US. Haplotypes belonging to the A lineage were observed from six Utah counties located in the southern portion of the State, from elevations as high as 1357 m. All five C lineage haplotypes that were found have been observed from queen breeders in the US. Three haplotypes of the M lineage (n=7) and two of the O lineage (n=9) were also observed. This study provides evidence that honey bees of African descent are both common and diverse in wild populations of honey bees in southern Utah. The high levels of genetic diversity of A lineage honey bee colonies in Utah provide evidence that the lineage may have been established in Utah before the introduction of A lineage honey bees from Brazil to Texas in 1990.

Keywords: Africanized honey bees, *Apis mellifera*, feral colonies, genetic variation, mitochondrial DNA

INTRODUCTION

Colonies of honey bees, *Apis mellifera* L., were first brought to Utah, USA in covered wagons in 1848, and by 1872 there were approximately 2000 honey bee colonies in Utah (Nye, 1976). The Africanized honey bee (AHB) was first detected in Texas, USA in 1990 (Sugden & Williams, 1990), and by 2008 it was discovered in southern Utah (Hodgeson et al., 2010). By 2010 AHB had spread to three counties in Utah (Szalanski & Magnus, 2010). The Africanized honey bee in the United States is virtually indistinguishable in the field from the European honey bee (EHB) and requires a morphometric analysis for morphological identification (Rinderer et al., 1993). Mitochondrial DNA (mtDNA) can be used as a genetic marker for identifying colonies that have an Africanized queen since a single worker honey bee can represent the entire honey bee colony (Sheppard & Smith, 2000). Introgression of AHB genes using a mitochondrial DNA marker is, however, not detectable if an EHB queen has mated with AHB drones. Besides the concerns about the aggressive nature of AHB to humans, it has been recently shown that AHB can outcompete native pollinator species in southern Utah, resulting in the local extinction of the Andrenid bee *Perdita meconis* (Griswold) (Portman et al., 2017).

Honey bees have more than 26 subspecies which have been placed into six evolutionary lineages based primarily on morphometrics and their historical geographic distribution (Ruttner,

1988; Sheppard et al., 1997; Franck et al., 2001; Sheppard & Meixner, 2003; Ferreira et al., 2008; Alburaki et al., 2013). These lineages include the A (African group), M (North and Western Europe), C (Southeastern Europe), O (Near East and Middle East) (Ruttner et al., 1978; Ruttner, 1988; Franck et al., 2007; Kandemir et al., 2006; Ferreira et al., 2008; Shaibi et al., 2009; Rortais et al. 2011), Y (Ethiopia) (Franck et al., 2001), and Z (Syria and Lebanon) (Alburaki et al., 2011). Eight subspecies from four lineages (A, C, M and O) were introduced to the United States (Pellett 1938; Sheppard, 1989a, 1989b) before the enactment of the 1922 Honey Bee Act which ended all importation of adult honey bees due to the mite, *Acarapis woodi* (Rennie), which was responsible for the Isle of Wight disease (Phillips, 1923).

DNA sequence analysis of a portion of the mitochondrial DNA (mtDNA) COI-COII genome can provide information on the mitochondrial lineage of the queen honey bee in a colony. Unlike nuclear genotypes which can be altered during segregation or recombination during reproduction, mtDNA markers are maternally inherited, thus allowing mtDNA analysis to focus on the genealogies of individual lineages (Lansman et al., 1981). This also allows the use of a single individual to genetically characterize a honey bee colony (Sheppard & Smith, 2000). Studies on the genetic variation of the mtDNA COI-COII region of queen breeder honey bee populations in the United States have revealed that the vast majority of queens have only the C lineage (Delaney et al., 2009; Magnus et al., 2011). However, several studies on mtDNA diversity of feral honey bee colonies and swarms from the continental United States and Hawaii (Magnus & Szalanski, 2010; Magnus, 2015; Szalanski et al., 2016) have revealed a number of M and O lineage haplotypes that have not been observed in previous studies of queen breeders (Delaney et al., 2009; Magnus, 2011).

It is believed that feral colonies of European honey bees in the United States are rare in natural areas due to parasites and pathogens, especially *Varroa destructor* Anderson and Trueman, which greatly reduced feral bee populations after its introduction (Seeley, 2015). Several studies have shown that remaining feral colonies of European honey bees have persisted for at least 10 years with infections of *V. destructor* in New York, USA (Seeley, 2007) and in Europe (Fries et al., 2006, Le Conte et al., 2007). Also, beekeepers in the United States have become interested in unmanaged feral colonies for their breeding programs, dubbed 'survivor stock' due to their ability to persevere despite the presence of pathogens and parasites (McNeil, 2009a, 2009b; Jacobson, 2010). These feral colonies may not have been manipulated by beekeepers for a long period of time and may have adapted to the various stresses that threaten their survival (Loper & Sammataro, 2006; Seeley, 2007; Villa et al., 2008).

The objective of this study was to characterize the genetic diversity of honey bees from feral colonies and swarms in Utah, USA using DNA sequence data of the COI-COII mtDNA region of *A. mellifera*.

MATERIAL AND METHODS

Adult worker honey bees were collected from feral colonies and swarms, from 10 Utah counties, into 70% ethanol from 2008 to 2017 (Fig. 1, Tab. 1). Following Schiff et al. (1994), feral colonies are defined as established colonies occurring in unmanaged homesites (e.g. trees, caves, buildings). Samples collected from masses of bees discovered in the open, without comb, are defined as swarms. Swarms are commonly derived from unmanaged colonies, but their origin can be difficult to determine with certainty. DNA was extracted from individual honey bees using a salting-out protocol with in-house reagents (Sambrook & Russell, 2001). PCR Primers E2 and H2 (Garnery et al., 1993) were used to amplify extracted DNA via PCR. These primers will amplify an approximately 530 bp to 1230 bp portion of the mtDNA COI-COII genes. A total of 2 µL of extracted DNA was used for PCR. The PCR reaction, following Taylor et al. (1996), consisted of holding the samples for 5 min at 94°C, then 40 cycles of 94°C for 45s, 46°C for 1 min, and 72°C for 1 min,

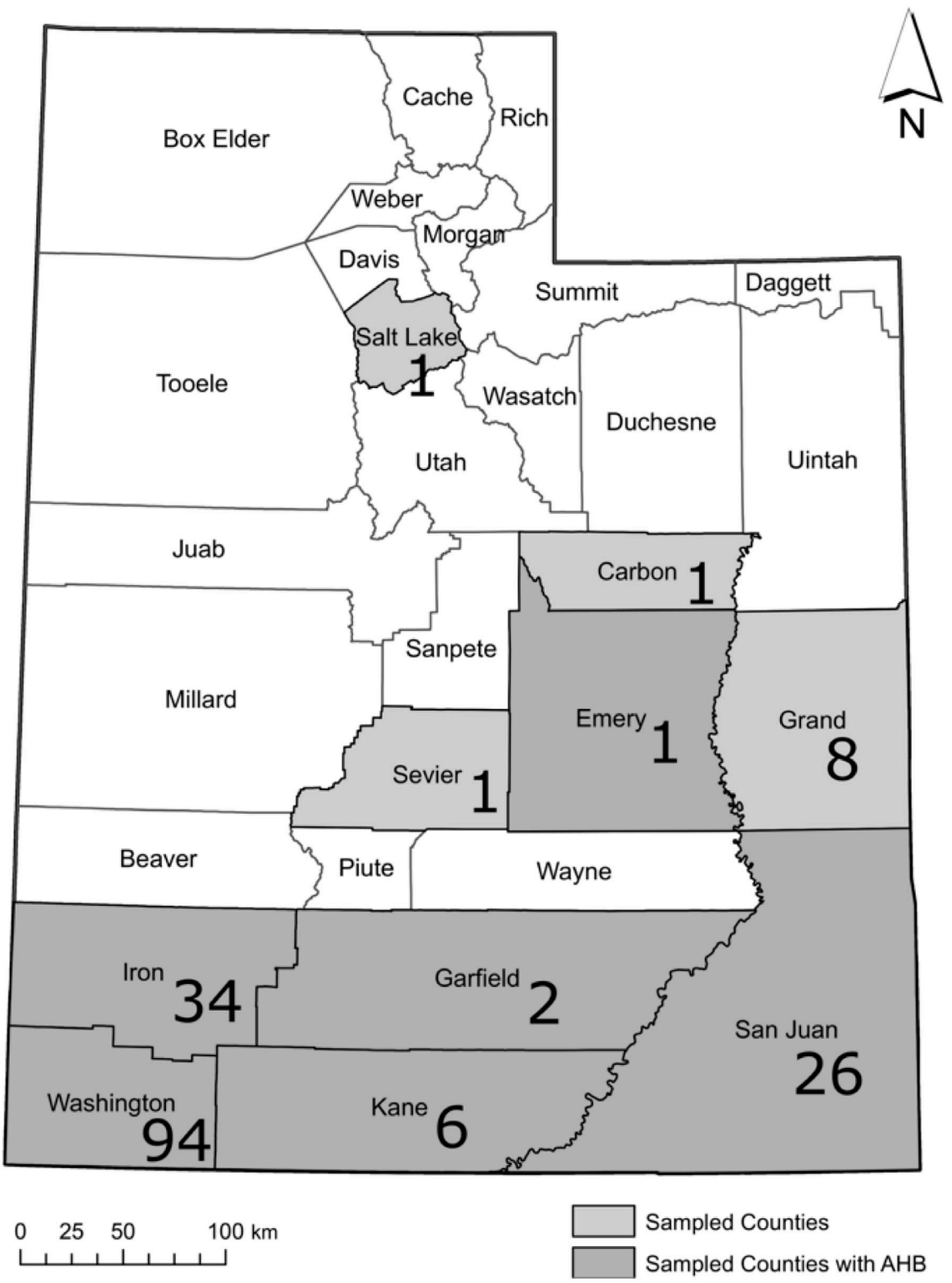

Fig. 1. Counties sampled in Utah for *Apis mellifera* feral colonies and swarms. Numbers indicate sample sizes. Counties with Africanized honey bees (AHB) are shown in dark grey; counties that were sampled, but in which no AHB were observed are shown in light grey.

Table 1.
Mitochondrial DNA haplotypes, for feral and swarm honey bees collected in Utah 2008–2017 by county.

County	A1	A1b	A1d	A1e	A26	A26a	A26c	A4	A4a'	A4''	C1	C2	C11	C12	C31	M3	M7	M3a	O2	O5	Total
Carbon													1								1
Emery								1													1
Garfield				1				1													2
Grand											6	2									8
Iron	3	3		7	3	4	1		1	1	3	1	2		1		1		3		34
Kane			3									1			1				1		6
Salt Lake															1						1
San Juan	4			6			6			1	9		1		1	1		3			26
Sevier													1								1
Washington	5	6	2	15	1	6	2	1		5	22	3	18	1		2			3	2	94
Total	12	9	5	29	4	10	3	3	1	7	40	7	23	1	4	3	1	3	7	2	174

followed by a final extension of 72°C for 5 min. Amplicon verification was conducted by gel electrophoresis using 1% agarose gels and visualizing PCR products using a BioDoc-it™ Imaging System (UVP, Inc., Upland, CA). Samples were purified and concentrated with VWR centrifugal devices (VWR, Radnor, PA) and sent to Eurofins Genomics (Huntsville, AL) for direct sequencing in both directions. Voucher specimens are deposited at the University of Arkansas Insect Genetics Laboratory in Fayetteville, Arkansas, USA. Consensus sequences with the primer ends removed were obtained using Geneious v6.1.6 (Biomatters Ltd., Auckland, New Zealand). Assignment and comparison of haplotypes was conducted by an NIH BLAST search of DNA sequences available on GenBank and our own database (ALS unpublished data). Genetic similarity of haplotypes among shared nucleotide sites was constructed using Geneious v6.1.6 (Biomatters Ltd., Auckland, New Zealand). An unrooted maximum likelihood tree using the observed haplotypes was constructed using Geneious v6.1.6 using the PHYLIP plugin (Felsenstein 1989) with 1,000 resamplings using the HYY 85 substitution model and NNI topology search.

RESULTS

A total of 174 samples of feral honey bee colonies and swarms were collected from 10 Utah counties (Fig. 1) and successfully sequenced (Tab. 1 and 2). Genetic similarity of shared nucleotide sites among the observed haplotypes ranged from 92.1 to 99.8% (Tab. 3). The majority of the samples were from Washington (n=94), Iron (n=34), and San Juan (n=26) counties located in southern Utah. A total of 20 COI-COII haplotypes were observed (Tab. 1), with 50% belonging to the A lineage, 25% to the C lineage, 15% to the M lineage, and 10% to the O lineage (Fig. 2). Samples with A lineage haplotypes (n=83) were observed from six Utah counties (Emery,

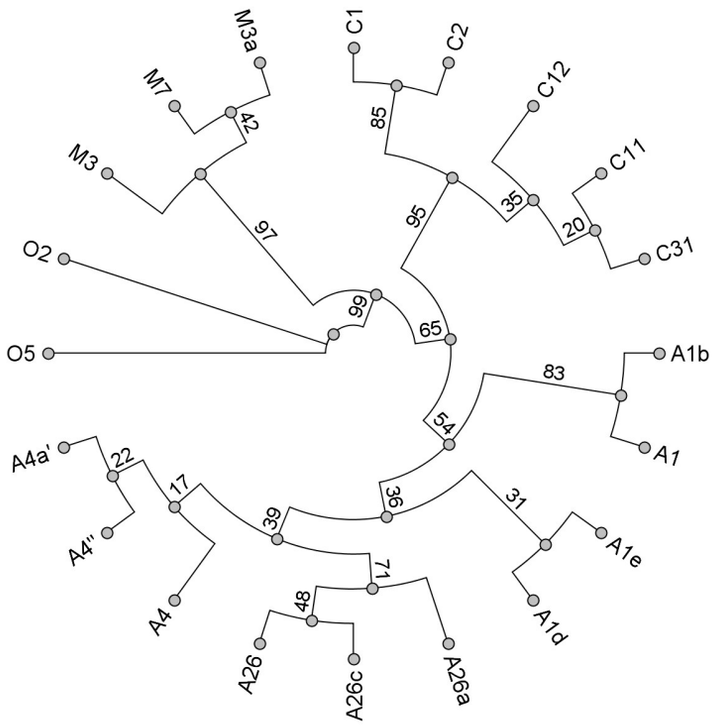

Fig. 2. Maximum likelihood phylogenetic tree showing the relationship among observed haplotypes.

Table 2.

Observed haplotypes, percent match to GenBank Accessions, and matching GenBank Accession Number

Haplotype	Haplotype	Percent Match
A1	EF033649	100
A1b	FJ477985	100
A1d	FJ743639	100
A1e	GU326335	100
A26	KJ661742	100
A26a	FJ743640	99.9
A26c	FJ890929	100
A4	EF033650	100
A4a'	KX463808	100
A4''	FJ478009	99.7
C1	EF033655	100
C2	JF934704	100
C11	FJ037776	100
C12	FJ037777	100
C31	HQ287900	100
M3	FJ743636	100
M7	KX463911	99.7
M3a	KX463884	100
O2	FJ477996	100
O5	FJ743633	100

Table 3.

Genetic similarity among observed haplotypes.

	A26a	C11	A1	A1e	C1	A26c	A26	O5	A1b	O2	A4''	C31	A4	M7	M3a	M3	A1d	A4a'	C2	C12
A26a		94.7	97.6	97.6	94.3	98.9	97.5	95.6	97.6	94.3	95.8	94	99.3	95.1	95.5	93.2	95.3	96.8	93.6	93.9
C11	94.7		94.7	94.8	99.3	94.5	94.5	93.1	94.7	93.2	94.7	99.6	94.7	94	94.9	93.8	94.8	94.5	99.1	99.5
A1	97.6	94.7		99.8	94.3	97.6	97.4	95.6	100	93.5	99.7	94	97.6	96.1	95.9	95.7	99.7	97.2	93.6	93.9
A1e	97.6	94.8	99.8		94.5	97.6	97.4	95.3	99.8	93.4	99.8	94.1	97.6	95.8	95.9	95.4	99.8	97.2	93.7	94.1
C1	94.3	99.3	94.3	94.5		94.3	94.1	93.1	94.3	93.2	94.3	99.3	94.3	93.7	94.5	93.5	94.5	94.1	99.5	99.1
A26c	98.9	94.5	97.6	97.6	94.3		98.1	96.2	97.6	94.8	96	93.8	99.6	95.7	95.5	93.6	95.3	97.4	93.4	93.7
A26	97.5	94.5	97.4	97.4	94.1	98.1		94.9	97.4	93.6	95.8	93.8	98.3	96.9	95.3	93.1	95.1	96.3	93.4	93.7
O5	95.6	93.1	95.6	95.3	93.1	96.2	94.9		95.6	97.8	94.1	92.5	96.2	95.2	95.1	93.6	92.7	94.5	92.4	92.4
A1b	97.6	94.7	99.4	99.8	94.3	97.6	97.4	95.6		93.5	99.7	94	97.6	96.1	95.9	95.7	99.7	97.2	93.6	93.9
O2	94.3	93.2	93.5	93.4	93.2	94.8	93.6	97.8	93.5		92.2	92.6	94.9	93.3	93.2	91.7	90.9	91.7	92.1	92.1
A4''	95.8	94.7	99.7	99.8	94.3	96	95.8	94.1	99.7	92.2		94	96	94.5	95.7	94.1	98.4	95.7	93.6	93.9
C31	94	99.6	94	94.1	99.3	93.8	93.8	92.5	94	92.6	94		94	93.3	94.2	93.2	94.1	93.8	98.6	99
A4	99.3	94.7	97.6	97.6	94.3	99.6	98.3	96.2	97.6	94.9	96	94		95.8	95.5	93.7	95.3	97.5	93.6	93.9
M7	95.1	94	96.1	95.8	93.7	95.7	96.9	95.2	96.1	93.3	94.5	93.3	95.8		96.7	96	93.6	92.8	92.9	93.3
M3a	95.5	94.9	95.9	95.9	94.5	95.5	95.3	95.1	95.9	93.2	95.7	94.2	95.5	96.7		96.5	95.7	95.4	93.8	94.1
M3	93.2	93.8	95.7	95.4	93.5	93.6	93.1	93.6	95.7	91.7	94.1	93.2	93.7	96	96.5		93.2	93.5	92.8	93.1
A1d	95.3	94.8	99.7	99.8	94.5	95.3	95.1	92.7	99.7	90.9	98.4	94.1	95.3	93.6	95.7	93.2		95	93.6	93.9
A4a'	96.8	94.5	97.2	97.2	94.1	97.4	96.3	94.5	97.2	91.7	95.7	93.8	97.5	92.8	95.4	93.5	95		93.4	93.8
C2	93.6	99.1	93.6	93.7	99.5	93.4	93.4	92.4	93.6	92.1	93.6	98.6	93.6	92.9	93.8	92.8	93.6	93.4		99.6
C12	93.9	99.5	93.9	94.1	99.1	93.7	93.7	92.4	93.9	92.1	93.9	99	93.9	93.3	94.1	93.1	93.9	93.8	99.6	

Iron, Kane, San Juan, Washington, and Garfield). Ten haplotypes of the African A lineage were observed with A1e (n=29), A26a (n=10), and A1b (n=9) being the most common. A total of five C lineage haplotypes were observed (n=75), with C1 (n=40) being most common. For the M lineage, three haplotypes were observed, M3 (n=3), M7 (n=1), and M3a (n=3). Two haplotypes of the O lineage were also observed (n=9) (O2 and O5), with O2 (n=7) being the most common. The maximum likelihood cladogram (Fig. 2) revealed the C, M, and O lineages forming single clades among their haplotypes, while the A lineage haplotypes formed three distinct clades. This consisted of haplotype A4a' forming a sister group with haplotypes A26, A26a, A26c, A4 and A4", while the A1 haplotypes (A1, A1b, A1d, and A1e) formed another distinct clade.

DISCUSSION

All of the M lineage haplotypes (M3, M7, and M3a) observed in this study have been previously observed in other western states and in the southern United States (Delaney et al., 2009; Magnus et al., 2014). Haplotype O2 has been observed from California (Kono & Kohn, 2015), and O5 from Oklahoma, New Mexico, and Texas (Magnus et al., 2014). All C lineage haplotypes observed in this study have been observed from queen breeders throughout the United States (Delaney et al., 2009; Magnus et al., 2011).

Among the 10 A lineage haplotypes, only two (A4a' and A4") are unique to Utah when compared to the rest of the United States. Five of the A haplotypes observed in Utah (A1, A1e, A26, A26c, and A4) have also been found in the bordering state, Arizona (Darger, 2013). Although Darger (2013) observed a total of 22 A lineage samples in Arizona, only these five A haplotypes were found in Utah in the present study. Also, three of the Utah A lineage haplotypes observed (A1, A1d, and A26a) have been documented in New Mexico, which also borders Utah, although only A1 was observed in all three states (Szalanski & Magnus, 2010). As New Mexico and Arizona are both south of Utah, the currently accepted AHB expansion scenario would suggest that AHB spread northward into Utah from these states. The diversity of haplotypes found throughout the southwest, and in Utah particularly, is surprising, given this expansion scenario. A recent study of genetic diversity of feral and managed honey bee colonies from San Diego County, California found 60% of the 48 worker honey bees sequenced for the COI-COII mtDNA region belonged to the A lineage with 10%, 17%, and 13% belonging to the C, O, and M lineages, respectively (Kono & Kohn, 2015). Only three (A2b, A4a, and A26) of the 10 A lineage haplotypes observed in Utah occurred among the 10 A lineage haplotypes from San Diego County, California, none of these were also observed in Arizona or New Mexico. This level of genetic diversity is interesting given the close regional proximity between the San Diego samples and those from this study and the proposed patterns of AHB expansion throughout the region.

Most A lineage bees in Utah belong to the A1 haplotype group (55 of 83) as reported in a smaller survey in the past (Szalanski & Magnus, 2010). However, the overall diversity of A lineage haplotypes in Utah is quite high given the limited geographic range sampled. The high number of haplotypes of A lineage honey bees, 10, observed in this study from southern Utah is on par with a previous study of AHB genetic diversity from seven southern U.S. states, which found a total of 12 A lineage haplotypes (Szalanski & Magnus, 2010). In addition, A lineage haplotypes were far more common in this sample (55%) than haplotypes from the other lineages, and two haplotypes have only been observed in Utah thus far. Also, the high proportion of AHB from the feral Utah honey bee samples is of importance given the ability of AHB to outcompete native pollinator species in southern Utah, resulting in the local extinction of the Andrenid bee *Perdita meconis* (Portman et al., 2017). This brings a new component to the importance of documenting the occurrence of AHB in Utah and throughout the southern United States.

In addition, given the high diversity of mtDNA haplotypes observed among feral honey bees in Utah, this gives evidence that these feral honey bees were not exposed to *Varroa* mites. Popu-

lations of feral honey bees found in a forest in New York, USA had a dramatic loss of mtDNA genetic diversity after exposure to *Varroa* mites (Mikheyev et al., 2015). Also, from our samples for our study, usually consisting of 20-40 worker honey bees, we have not observed any *Varroa* mites. This sample size was not ideal for *Varroa* mite surveillance, but it does provide evidence of a lack of exposure to *Varroa* mites in these populations.

The commonness of the A lineage in Utah, Arizona (Darger, 2013), New Mexico (Szalanski & Magnus, 2010) and San Diego County, California (Kono & Kohn, 2015) provides evidence that A lineage honey bees may be well adapted to the arid climate in the southwestern United States. The adaptiveness to this climate is also evident by A lineage samples in our study being recovered from elevations as high as change to 1357 m. The highest elevation that samples were found in our study was 1868 m for a colony of O lineage honey bees. A recent study by Wallberg et al. (2017) on honey bees from Eastern Africa found that *A. m. scutellata* honey bees were only recovered from locations where elevations remained below 1100 m. The presence of A lineage honey bees at elevations greater than 1300 m in Utah could be due to hybridization between Africanized honey bees and European honey bees allowing them to adapt to higher elevations. Another possible reason is that the A lineage samples collected in our study from Utah, which were found at elevations as high as 1357 m, are not *A. m. scutellata*, but a different A lineage subspecies. This could provide evidence that some of the A lineage honey bees from Utah, are not *A. m. scutellata* that entered the United States in 1990 from Brazil (Sugden & Williams, 1990), and were introduced to Utah before 1990.

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