UNIVERSITY OF CALIFORNIA SAN DIEGO

Genomic Admixture and Nest Defense Behavior in the Africanized Honey Bee of the American Continents

A dissertation submitted in partial satisfaction of the
requirements for the degree Doctor of Philosophy

in

Biology

by

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The Dissertation of Daniela Zarate is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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DEDICATION

I dedicate this doctoral thesis to my mother, Carmen Martinez, who supported me through the seven years it took to complete this journey. We have gone through such a journey together and she remains my best friend and biggest champion. Te quiero mamí.

I also would like to thank my brother and sister, Ricardo and Ariana Zarate for their constant support and love through my entire educational journey. They both set the path for me to walk on and follow in their footsteps to success. I would also like to express my love for my baby boy, Bugzz the pug who was there for me through it all.

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ABSTRACT OF THE DISSERTATION

Genomic Admixture and Nest Defense Behavior in the Africanized Honey Bee of the American Continents

by

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Professor Joshua Kohn, Chair

Genomic admixture, the mixture of two or more distinct gene pools, is a common and widespread biological phenomenon of significant evolutionary importance. The African-hybrid honey bee (AHB) represents one of the most impressive and ecologically successful cases of admixture in a social insect. While honey bees are now a common feature of the American landscape and an indispensable part of commercial agriculture, their origins are rooted in importations from Eurasia and Africa that began in the 1500s. The African-hybrid honey bee (AHB) is a New World amalgamation of several subspecies of the western honey bee (*Apis mellifera*). *Apis mellifera* is a taxonomically diverse species, comprised of more than 30 subspecies historically grouped into four major biogeographic lineages: African (A), Western European (M),

Eastern European (C), and Eastern Mediterranean (O). In 1956, honey bee biologists in Brazil imported honey bee queens of the African subspecies *Apis mellifera scutellata* for experimental breeding with pre-existing European stock. Researchers hoped to forge a honey bee that combined the tropical hardiness of *A. m. scutellata* with the honey production capabilities and gentleness of the popular European subspecies currently in use. In a now infamous incident, these experimental "Africanized" hybrids were accidently released from their research apiaries, initiating a spectacular hybrid species expansion that now extends from northern Argentina to northern California (U.S.A.). The heightened degree of territorial nest defense characteristic of African-hybrid honey bees spurred a large degree of public concern over the expansion and success of this invasive insect—gaining it substantial attention from popular press who dubbed it the "killer bee". To this end, this dissertation seeks to characterize genomic admixture dynamics and nest defense behavior in the African-hybrid honey bee. I hope my work serves to inform adaptive honey bee breeding practices that will aid in the preservation of a robust population of honey bees for commercial pollination and help combat world-wide honey bee decline

INTRODUCTION

Hybridization, the interbreeding of distinct genetic lineages, has long complicated taxonomic boundaries and challenged the perception of species as discrete taxonomic and evolutionary units. Many early evolutionary biologists considered hybridization an infrequent and abnormal process of limited evolutionary importance, resulting from the breakdown of natural isolating mechanisms (Dobzhansky 1936; Mayr 1942; Barton 2001). This was an attitude largely espoused by animal researchers—plant biologists, conscious of the high frequency of hybridization leading to viable offspring in plants, recognized introgression as a creative source of genetic novelty (Lotsy 1916; Suarez-Gonzalez *et al*. 2018).

Hybridization is now recognized as a common and creative evolutionary force in processes of adaptation and diversification, producing mosaic genomes on which selection can act (Anderson & Stebbins 1954; Barton 2001; Abbott *et al.* 2013; Dittrich-Reed & Fitzpatrick 201; Hedrick 2013). Advances in sequencing technology and ancestry estimation have facilitated the identification of introgression, exposing heretofore undiscovered hybridization with surprising frequency. Admixture as a driver of adaptation and diversification has been widely studied across diverse taxonomic groups. Sunflower (*Helianthus)* hybrids can colonize and flourish in habitats that neither parental species could exist in (Rieseberg *et al.* 2003; Whitney *et al.* 2015). Admixture jumpstarted the spectacular diversification and adaptive radiation recognized in African cichlids (Seehausen 2004). Admixture between extinct Denisovans and ancient *Homo Sapiens* facilitated the transfer of high-altitude adaptation genes found in contemporary Tibetan peoples (Huerta-Sanchez *et al.* 2014).

The Africanized honey bee (AHB) is one of the most well-documented examples of human-mediated hybridization (HMH) in a social insect. HMH is a phenomenon of increasing frequency, resulting from either accidental or intentional introductions of exotic biota to geographical areas beyond their native ranges (Grabenstein and Taylor 2018). The western honey bee (*Apis mellifera*), well-known for its critical role as a pollinator in commercial agriculture, was first introduced from the Old World during the colonization of the American continents in the early 1500s. *Apis mellifera* is a diverse taxon, comprised of over two dozen recognized subspecies which cluster into four major lineages based on genetic, geographic, and morphometric data: A (African), M (western European), C (eastern European), and O (Middle Eastern) (Ruttner 1988). Substantial variation in genetics and behavior exists within and among the clades and new subspecies continue to be recognized.

Early importations of honey bees to the Americas were mainly from the western European (M) and eastern European (C) clades; the former dominating the 16th to 18th century introductions while the latter dominated later introductions (Sheppard 1989; Schiff *et al*. 1994; Schneider *et al.* 2004). Due to their gentle nature, Eastern European (Clade C) honey bees are now the variety of choice in commercial agriculture in the U.S.A., where pollination services of honey bees are valued at an estimated $14.5 billion (Crane *et al.* 1999; Morse & Calderone 2000). Middle Eastern honey bees (Clade O) were introduced in the late 1880s and 1890s in limited quantities but their importations were phased out by the end of the century in favor of other subspecies (Sheppard 1989). African (A) subspecies were largely excluded from importation with the exception of the Egyptian subspecies *A. m. lamarkii* which was introduced to North America in low frequency (Schiff & Sheppard 1993).

Temperate-adapted European honey bees struggled to thrive in the tropics of the New World, surviving only under intense management with meager honey production. Honey bee researchers believed the influx of tropic-derived African honey bee genetic material would produce a hybrid better adapted to the environment (Spivak et al. 1991; Schneider et al. 2004). In 1956, honey bee biologists imported 47 queens of the African subspecies (*A. m. scutellata*) to Sao Paulo, Brazil for experimental breeding. In a now infamous incident, these experimental admixed "Africanized" honey bees (AHB) were accidently released from their apiaries and quickly spread into the surrounding countryside (Kent 1988; Schneider *et al*. 2004). Their subsequent expansion across the American continents over the past 60+ years is considered one of the "most spectacular biological invasions of all time" (Pinto *et al*. 2005). Africanized honey bees spread across South and Central America, hybridizing with and displacing pre-existing populations of European honey bees. They reached their southern range limit in Argentina in the 1970s; apparently precluded from advancing further by the colder climate (Kerr *et al*. 1982; Taylor & Spivak 1984). The AHB reached Panamá by 1982, Costa Rica by 1986, Mexico by 1989, Texas by 1990, and California by 1994 (Winston 1992). The current northern range limit of the AHB lies in the Napa and Sacramento counties of northern California (Kono & Kohn 2015; Lin *et al*. 2017).

The replacement of pre-existing feral populations of European honey bees by hybrids with predominately African genomes suggests that Africanization affords strong ecological advantages. While the AHB continues to possess some European ancestry, the behavior and biology of the hybrid is largely consistent with that of its African ancestor. Compared to European honey bees, AHB exhibit heightened levels of territorial defense, higher frequencies of swarming and absconding (when an entire colony abandons its nest to establish itself elsewhere), rapid rates of colony growth and reproduction, and heightened resistance to the parasitic mite *Varroa destructor*

(Collins et al. 1982; Guzman-Novoa et al. 1996; McNally & Schneider 1992, 1996; DeGrandi-Hoffman et al. 1997). Due to the popularity of honey bees in both commercial-scale agriculture and hobbyist beekeeping, the intensity of nest defense exhibited by the AHB is of particular public concern. The large-scale use of honey bees in pollination services has prompted the promotion of gentle honey bee strains in these industries and the use of management practices that prevent Africanization. In the United States, beekeeping with African strains is considered untenable and strict practices to prevent Africanization of hives are standard protocol. In contrast, beekeepers in Central and South America have learned to work and live with the AHB.

The AHB deposits more stings on a target, responds faster and in greater numbers, and pursues any perceived threat further compared to the gentle Italian or Carniolan honey bees, subspecies popularly used in apiculture (Collins *et al*. 1982). This formidable level of nest defense, one of the most distinguishable hallmarks of the hybrid, has earned the AHB the epithet "killer bee" in the popular press.

Some researchers have begun to move away from the well-accepted label of "Africanized" honey bee to the more taxonomic-specific term "*scutellata* hybrid" (Calfee *et al.* 2020). The existence of several different subspecies of African honey bees, many of which differ vastly from *Apis mellifera scutellata,* particularly in nest defense, complicate the idea of a general "African" honey bee. Indeed, there exist several African honey bees well known for their gentle characters (Ruttner 1988). Thus, the term "Africanized" is seen as frustratingly broad, conflating the various and diverse subspecies of the African continent. In fact, it can be argued that the use of the term "Africanized" is a reflection of Western public consciousness that perceives the African continent as a monolithic entity and associates negative characteristics (e.g. aggression, invasiveness) with African identity. While this discussion is barely in its inception, it raises valuable questions about

the use of language employed in scientific discourse and how scientific writing can reflect the social perceptions of overarching public consciousness, at times in detrimental ways.

This dissertation seeks a broad assessment of genomic admixture and nest defense behavior in the Africanized honey bee, a species of substantial cultural and economic value. It is divided into three chapters. The first is a quantitative assessment of nuclear and mitochondrial ancestry in 60 honey bees sampled across a broad geographic range (San Diego, CA to Panamá City, Panamá). The second chapter is a seasonal assessment of honey bee nest defense between feral-sourced colonies and those under standard management practices to examine the impacts of time and honey bee stock type on defensive behavior. The third chapter is a general review of Africanized honey bees in California synthesizing the literature published since the introduction of this insect and reviewing the impact the AHB has had on the state's agricultural and natural resources. Together, the work detailed here aims to contribute to our genetic and behavioral understanding of Africanized honey bees and inform our practices in engaging with this imported, hybrid, insect.

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Chapter 1: Admixture in Africanized honey bees (Apis mellifera) from Panamá to San Diego, California (U.S.A.)

ABSTRACT

The Africanized honey bee (AHB) is a New World amalgamation of several subspecies of the western honey bee (*Apis mellifera*), a diverse taxon historically grouped into four major biogeographic lineages: A (African), M (Western European), C (Eastern European), and O (Middle Eastern). In 1956, accidental release of experimentally bred "Africanized" hybrids from a research apiary in Sao Paulo, Brazil initiated a hybrid species expansion that now extends from northern Argentina to northern California (U.S.A.). Here, we assess nuclear admixture and mitochondrial ancestry in 60 bees from four countries (Panamá; Costa Rica, Mexico; U.S.A) across this expansive range to assess ancestry of AHB several decades following initial introduction and test the prediction that African ancestry decreases with increasing latitude. We find that AHB nuclear genomes from Central America and Mexico have predominately African genomes (76-89%) with smaller contributions from Western and Eastern European lineages. Similarly, nearly all honey bees from Central America and Mexico possess mitochondrial ancestry from the African lineage with few individuals having European mitochondria. In contrast, AHB from San Diego (CA) show markedly lower African ancestry (38%) with substantial genomic contributions from all four major honey bee lineages and mitochondrial ancestry from all four clades as well. Genetic diversity measures from all New World populations equal or exceed those of ancestral populations. Interestingly, the feral honey bee population of San Diego emerges as a reservoir of diverse admixture and high genetic diversity, making it a potentially rich source of genetic material for honey bee breeding.

INTRODUCTION

Hybridization, the interbreeding of distinct genetic lineages, has long complicated taxonomic boundaries and challenged the perception of species as discrete taxonomic and evolutionary units. Initially considered an evolutionary dead-end, hybridization is now recognized as a driver of adaptation and diversification across various evolutionary lineages. Sunflower (*Helianthus)* hybrids colonize habitats prohibitive to parental species (Rieseberg *et al.,* 2003; Whitney *et al.,* 2015). Admixture jumpstarted the spectacular diversification and adaptive radiation recognized in African cichlids (Seehausen, 2004). Interbreeding between extinct Denisovans and ancient *Homo sapiens* facilitated the transfer of high-altitude adaptation genes found in contemporary Tibetan peoples—an adaptation paralleled in highland wolves and their domesticated dog counterparts (Huerta-Sánchez *et al.,* 2014; VonHoldt, Fan, Vecchyo, & Wayne*,* 2017). Whether admixture generates creative evolutionary novelty (Barton 2001; Hedrick, 2013; Suarez *et al.,* 2018), or leads to destructive cellular incompatibilities (Dobzhansy 1935; Burton & Baretto 2015), hybridization dynamics are of great eco-evolutionary interest, particularly in the context of increasing rates of unnatural, human-mediated hybridization (HMH) (reviewed in Grabenstein & Taylor, 2018).

The Africanized honey bee (AHB) is a human-mediated hybrid of the American continents, created from the intercross between an African subspecies (*Apis mellifera scutellata*) and various European and Middle Eastern honey bee subspecies. The western honey bee (*Apis mellifera*) is taxonomically diverse, comprised of over thirty recognized subspecies traditionally clustered into four major, lineages based on genetic, geographic, and morphometric data: A (African), M (Western European), C (Eastern European), and O (Middle East and Anatolia) (Ruttner, 1988;

Whitfield *et al.,* 2006; Wallberg *et al.,* 2014; Cridland, Tsutsui, & Ramirez, 2017). Recently, new lineage groupings have been proposed: Y, for bees from the Arabian Peninsula (see, e.g. Cridland *et al.,* 2017b) and Z (Alburaki *et al.,* 2013) referring to bees of the traditional O clade from Syria. Here we are particularly interested in which honey bee lineages contribute to New World bee populations and we use the A, M, C, and O nomenclature because known importations of bees to the New World come from these clades (Ruttner, 1988; Carpenter & Harpur, 2021). Substantial variation in behavior, morphology, and genetics exists across honey bee subspecies, even within the overarching clades. The Eastern European subspecies (C clade) are particularly favored in commercial beekeeping due to their gentle nature and predilection for honey production. In contrast, African subspecies are largely disfavored for both commercial and hobbyist use due to the intensity of their nest defense and high propensity to abscond (abandon the nest *en masse* and move to another (Ruttner, 1988)).

Early honey bee importations to the Americas were largely Western European (M) and Eastern European (C) in origin (reviewed in Schneider, DeGrandi-Hoffman, & Smith, 2004). Generally, African (A) subspecies were excluded from importation with modest exceptions (see Schiff & Sheppard, 1993). However, temperate-adapted, non-native European honey bees (EHB) struggled to thrive in the Neotropics. In response, honey bee researchers in Sao Paolo, Brazil initialized a breeding program in 1956, importing 47 queens of the African subspecies (*A. m. scutellata*) for experimental crossing (reviewed in Schneider *et al*., 2004). Researchers bred this African subspecies with European races, hoping to forge a superior hybrid for tropical beekeeping. These hybrid "Africanized" honey bees (AHB) escaped from their experimental apiaries and spread into the surrounding countryside (reviewed in Schneider *et al*., 2004).

The expansion of the AHB across the American continents over the past 60+ years is considered one of the "most spectacular biological invasions of all time" (Pinto, Rubink, Patton, Coulsen, & Johnston, 2005). From their original Brazilian epicenter, AHB spread across South and Central America, rapidly hybridizing with and replacing the pre-existing European honey bee population with one of predominantly African ancestry (reviewed in Schneider *et al.,* 2004 and references therein; Whitfield *et al.,* 2006; Nelson, Wallberg, Simoes, Lawson, & Webster, 2017; Cridland *et al.,* 2017b). On the southern front, AHBs reached their range limit in Argentina in the 1970s at approximately 34° south latitude; presumably stopped from advancing further by the colder climate (Taylor & Spivak, 1984). In their northern expansion, AHB reached Panamá by 1982, Costa Rica by 1986, Mexico by 1989, Texas by 1990, and California by 1994 (Kim & Oguro, 1999). Currently, African mitochondria and nuclear markers are present in feral honey bees in California as far as 38° north latitude (Calfee, Agra, Palacio, Ramirez, & Coop, 2020; Kono & Kohn, 2015; Lin, McBroome, Rehman, & Johnson, 2017). While the current northern range limit may not be stable in the face of climate change, northward range expansion has clearly slowed in comparison to its explosive (160-500 km/year) neotropical expansion (Schneider *et al.,* 2004 and references therein).

The dramatic shift from European to predominantly African ancestry throughout the majority of the New World, suggests that Africanization provides ecological advantages in the areas where it dominates. Several behavioral and physiological traits of the Africanized honey bee are thought to drive AHB success: high reproductive rates; intense nest defense (McNally & Schneider, 1992a,b, 1996; Fewell & Bertram, 2002; see also Breed, Guzmán-Novoa, & Hunt, 2004); and higher resistance to infestation from the mite (*Varroa destructor*), a parasite and disease vector implicated in honey bee nest failure (Guzman-Novoa, Sanchez, Page Jr., & Garcia, 1996;

Goulson, Nicholls, Botías, & Rotheray, 2015). The advantages, if any, AHB derives from its remaining European ancestry are less clear although Nelson *et al.,* 2017 identified European genes underlying ovary size inferred to be selected for and Harpur, Kadri, Orsi, Whitfield, & Zayed, (2020) showed that both African and European ancestry underlies AHB nest defense.

In this study, we characterize the admixture and genetic diversity of AHB in the Neotropics and the southwestern United States using a dataset of 60 high depth (~25X) AHB whole genome sequences (WGS) collected from four regions spanning ~6,000 km. Each sampling site reflects a distinct time since initial contact between resident European and advancing Africanized forms: the isthmus of Panamá; Guanacaste NP, Costa Rica; Chiapas, Mexico; and San Diego County, CA, U.S.A. We leverage two existing sequencing projects (Harpur *et al.,* 2014; Wallberg *et al.,* 2014) and the recently published honey bee reference genome with chromosome-length scaffolds (Wallberg *et al.,* 2019) for our analyses. This is the first genomic study to assess ancestry in AHB samples from Central America and Mexico as well as the first to assay the contribution of the O lineage in the regions sampled. The contribution of this lineage to the California honey bee population was not evaluated in previous genomic studies (Calfee *et al.,* 2020, Cridland *et al.,* 2017a) and is of interest because mitochondria from this lineage are known to be present at considerable frequency in southern California's feral honey bees (Kono & Kohn, 2015) and occasionally elsewhere in the USA (Magnus & Szalanski, 2010). Ultimately, our study aims to broaden our understanding of the admixture dynamics of a massive hybrid takeover of an invasive social insect of great agricultural importance.

MATERIALS AND METHODS

Sample Collection

We collected 60 Western honey bees (n = 15/country) from sites in each of four countries: the isthmus of Panamá; Guanacaste National Park, Costa Rica; Chiapas, Mexico; San Diego County, California, U.S.A. (Table 1.1). All samples were collected in June 2015 – August 2016 by hand-netting. Honey bees in Panamá were collected with an insect net while they foraged either on natural vegetation in rural areas, or on street vendor syrup dispensers in urban areas. Honey bees were collected across the isthmus of Panamá from five sites, each separated by > 5 km: Panamá City, Gamboa, Barro Colorado Island (BCI), Santa Rita Arriba, and Cólon. Individuals from Costa Rica were collected from the Santa Rosa sector of Guanacaste National Park in northwestern Costa Rica. These bees were collected from a localized region and likely originate from a small number of feral colonies. Honey bees from Mexico were collected from an apiary in the southern state of Chiapas, with each bee collected from a different hive. Honey bees from San Diego County, California, U.S.A. were workers collected while foraging on flowers. San Diego bees were collected across 15 sites each separated by > 5 km so that each likely represents a worker from a different colony. The furthest collection sites were separated by 65 km. Collection sites ranged from urban to rural settings. Due to the presence of hobbyist and agricultural beekeeping we do not rule out the possibility that the captured honey bees were from managed rather than feral hives. However, most honey bee foragers in San Diego are from feral hives (Kono & Kohn, 2015, and see results).

Reference Honey Bee Genomes

Reference honey bee genomes were obtained by downloading 89 whole genomes sequenced by two previously published sequencing projects and made available on NCBI: Wallberg *et al*., (2014) (Project ID: PRJNA236426) and Harpur *et al.,* (2014) (accession no. SRP029219). The reference genomes sequenced by Wallberg *et al.,* 2014 were generated by whole genome sequencing on a SOLiD 5500xl platform to produce 75-bp reads with an average coverage of 4.4X ±1.5X per individual (Wallberg *et al.,* 2014). The reference genomes sequenced by Harpur *et al.,* (2014) were sequenced using Illumina Hi-Seq to produce 50-bp reads with an average coverage of 38X. Between both sequencing projects, we obtained 21 African (A) genomes of the subspecies *Apis mellifera scutellata*; 29 genomes from the Western (M) clade, comprising two subspecies: *Apis mellifera mellifera* (n = 14) and *Apis mellifera iberiensis* (n = 15); 29 genomes of the Eastern European clade (C) represented by the subspecies: *Apis mellifera carnica* (n = 19) and *Apis mellifera ligustica* (n = 10) and 20 genomes from the Middle Eastern (O) clade, including the subspecies: *Apis mellifera anatoliaca* (n = 10) and *Apis mellifera syriaca* (n = 10) (see Table 1.1). In total we used a panel of 89 reference honey bee genomes representing the four major honey bee clades and spanning 7 subspecies.

DNA Extraction & Sequencing

We extracted DNA from crushed heads of the 60 sampled honey bees using the standard protocol of the Qiagen DNAeasy Blood & Tissue extraction kit. DNA purity and appropriate concentration for sequencing were validated with a Qubit fluorometer prior to submission for library preparation. The DNA was submitted for DNA KAPA library construction and whole-genome sequencing at the Institute for Genomic Medicine (IGM), UC San Diego. All 60

individuals were multiplexed and sequenced across three lanes of an Illumina HiSeq4000 platform to produce 100-bp paired end reads. Average genomic coverage per individual was 29±1.2X.

Sequence Filtering & Alignment

Raw reads generated from sequencing, and those downloaded from NCBI, were trimmed and filtered for quality and length using a PoPoolation (Kofler *et al*., 2011) perl script (trim-fastq.pl) (settings: –fastq-type sanger --quality-threshold 25 --min-length 40). Filtered reads were aligned to the most recently assembled honey bee reference genome Amel_HAv3.1 with chromosome-length scaffolds (Wallberg *et al.,* 2019 using the BWA v0.7.12 bwa mem algorithm under default settings (Li & Durbin, 2009). Reads were then sorted, merged, and filtered again for mapping quality (quality score < 20 were discarded) using Samtools (Li, 2011). Read duplicates were removed using GATK Picard Tools' Remove Duplicates function (Picard Tools).

Variant Calling and Genotype Likelihood Estimation

We used the program ANGSD v0.930 (Korneliussen, Albrechtson, & Nielson, 2014) to call variant sites and estimate genotype likelihoods (--doGlf 2) across all 159 honey bee genomes. The major and minor alleles were inferred (--doMajorMinor 1) as follows. A threshold likelihood ratio for SNP calling was set (--SNP_pval 1e-6) and allele frequencies were calculated using inferred major and minor alleles (--doMaf 1). In addition, we discarded reads with a mapping quality below 30 and a base quality below 20. We removed tri-allelic sites and only included sites in which we had at least 63% of individuals reporting information with a depth of coverage of at least 3X. We chose a genotype likelihood approach over a called genotype approach such as that used by other ancestry software progams like ADMIXTURE (Alexander, Novembre, & Lange, 2009) as

genotype likelihoods have been shown to be robust to low-coverage sequencing data (Skotte, Korneliussen, & Albrechtsen, 2013; Kornliussen *et al.,* 2014). This SNP set was then thinned for linkage disequilibrium, keeping 1 in every 100th SNP for an average spacing of 689 bp distance between SNPs.

Admixture and Principal Components Analysis (PCA)

For admixture analysis we used the program NGSadmix (Skotte *et al*., 2013), which uses a genotype-likelihood approach that factors in uncertainty associated with next-generation sequencing and has been shown to have good performance even with low-coverage data. We ran NGSadmix using the BEAGLE genotype likelihood files created by ANGSD with K values ranging from 2 to 6 (K = number of assumed genetic clusters). We included only SNPs that were present in at least 94% of all individuals and had a minimum minor allele frequency of 5%. Here we focus on the results from K = 4 genetic clusters because we are interested in assessing the contributions of the four ancestral lineages (A, M, C, and O) historically imported into the Americas. We used R (R Core Team 2014) to graph admixture estimates. We used PCAngsd (Kornliussen *et al.,* 2014) to conduct a principal components analysis of all SNPs, and graphed the resulting PCA with R (R Core Team, 2014).

Mitochondrial Sequence Assembly and Phylogenetic Analysis

Filtered reads of all 60 New World honey bees were aligned to a mitochondrial reference genome from an individual of subspecies *Apis mellifera ligustica* sequenced by Crozier & Crozier (1993) using the BWA v0.7.12 bwa mem algorithm under default settings (Li & Durbin, 2009). We then called variants using samtools v1.10v (mpileup function) and used bcftools v1.10.2 (Li &

Durbin, 2009; Li *et al.,* 2009; Li, 2011) to extract the consensus sequence and convert to FASTQ with the vcfutils.pl script. We downloaded 12 previously assembled full mitochondrial sequences from *A. mellifera* subspecies representing all four major lineages from NCBI to compare with our samples (listed in Table 1.2).

FASTQ files of mitochondrial sequences from all 73 honey bees (13 reference honey bees and 60 AHB samples) were aligned using MAFFT (Katoh, Rozewicki, & Yamada, 2019), on the XSEDE via Cipres 2.0 Science Gateway. We used MEGAX (Kumar, Stecher, Li, Knyaz, & Tamura, 2018) and complete deletion of gaps and missing data to create a neighbor-joining phylogeny under a Kimura 2-parameter model to compute evolutionary distances. We then ran 2000 bootstrap replicates to estimate confidence in the resulting phylogeny.

Measures of Genetic Diversity

To assess allelic diversity, we calculated estimates of both pairwise theta ($\hat{\theta}_\pi$)**,** based on the number of mean pairwise differences between sequences, and Watterson's theta ($\hat{\theta}_w$), based on the measure of segregating sites for each sampled and reference population using ANGSD v.928 (Kornliussen *et al.,* 2014). Our reference populations were created by including honey bees from both the Wallberg *et al.,* (2014) and *Harpur et al.,* (2014) sequencing projects. However, the Middle Eastern (Clade O) population contained only honey bees from Wallberg *et al.,* (2014) because *Harpur et al.,* (2014) did not sequence bees from this lineage. Using only sites in which at least 50% of individuals in a population provided data, we estimated the folded site frequency spectrum (SFS) across the entire genome using the reference honey bee genome as the ancestral state. We then calculated and averaged thetas per site, including invariant sites, using ANGSD's

realSFS program. To ensure that our diversity estimates were not overly affected by the difference in coverage between our reference and newly-sequenced genomes, we calculated an additional measure of pairwise nucleotide diversity ($\hat{\theta}_\pi$) using only higher-confidence SNPs with >5% minor allele frequency (MAF) in the total sample, following a pipeline described in Calfee *et al.,* (2020). Using ANGSD, we first identified a set of SNPs with > 5% minor allele frequency in the total sample and inferred the major and minor alleles at those SNPs using observed base counts (-doMajorMinor 2 -doCounts 1 -doMaf 8 –minMAF 0.05). We excluded SNPs where more than half of individuals in the total sample did not have coverage. Using this list of SNPs (n = 5,666,586) as a reference, we calculated allele frequencies for each population based on observed base counts in ANGSD (-doMajorMinor 3 -doCounts 1 -doMaf 8). From these population allele frequencies, we calculated the average pairwise diversity per SNP, correcting for small sample sizes. To account for invariant sites in our estimate of nucleotide diversity (π) we weighted our measure of π per-SNP by the genome SNP density (total number of SNPs / total positions in the genome). For each measure of genome-wide nucleotide diversity, we estimated standard errors using a block-jackknife procedure, treating each chromosome as a block and re-computing nucleotide diversity with sequential exclusion of each chromosome.

RESULTS

Global genomic ancestry in Africanized honey bee samples

At K = 4 clustering, the 99 reference honey bees from Wallberg *et al.,* (2014) and Harpur *et al.,* (2014) resolve into four groups representing the four recognized honey bee lineages (A, C, M & O), largely agreeing with previous genomic analyses (Whitfield *et al.,* 2006; Han *et al.,* 2012;

Wallberg *et al.,* 2014; Chen *et al.,* 2016; Cridland *et al.,* 2017b, 2017b; Nelson *et al.,* 2017). Generally, there is limited evidence of admixture between these groups (Figure 1.1). However, honey bees of the subspecies *Apis mellifera syriaca* (Clade O) are an exception, with ~20% of its ancestry attributed to the African clade, consistent with results found by Wallberg *et al.,* (2014). Two individuals from the Western clade (M) (one from subspecies *A. m. mellifera* and one from subspecies *A. m. iberiensis*) showed significant ancestry from other clades (Clades C and O, respectively), a finding also consistent with Wallberg *et al.,* (2014) (Figure 1.1). We also observed a small proportion (~1%) of Middle Eastern (O) ancestry across all the sampled honey bees from the African lineage *A. m. scutellata*.

The nuclear genomes of honey bees from Central America and Mexico were heavily Africanized. Honey bees from the isthmus of Panamá averaged 89% (SE 0.17%) African (A) ancestry with the remaining 11% (SE 0.12%) of their genomes derived from the Western European (M) lineage. Honey bees from Guanacaste, Costa Rica, averaged 85% (SE 0.07%) African (A), 11% (SE 0.42%) Western (M) and 4% (SE 0.03%) Eastern European (C). In Chiapas, Mexico, honey bees averaged 77% (SE 0.61%) African (A), 15% (SE 0.36%) Western (M) and 8% (SE 0.34%) Eastern European (C) (Figure 1.1, Table 1.2).

In contrast to the honey bees of Central America and Mexico, genomes of all 15 honeybees sampled from San Diego (California, U.S.A.) exhibited a diverse admixture of all four major clades (A, M, C, and O). Ancestry of San Diego bees averaged 37% (SE 1.2%) African (A), 19% (SE 0.41%) Western European (M), 35% (SE 1.1%) Eastern European (C) and 9% (SE 0.22%) Middle Eastern (O) (Figure 1). African (A) ancestry of San Diego bees was far lower than that found in bees from any of the other sampled sites and contributions from the Eastern European (C) lineage were higher than all other populations sampled. All San Diego bees possessed substantial Middle

Eastern (O) ancestry while all other sites sampled had negligible or no ancestry from this clade (Figure 1.1, Table 1.2).

Principial Component Analysis (PCA)

The principal components analysis of the 99 reference honey bees representing the four major honey bee clades (A, M, C, O) and the 60 honey bees we sampled from Panamá to San Diego separated populations by clade and sampling site (Figure 1.2). The ancestral honey bee lineages were widely separated from each other on the first two principal component axes. Bees from the four sampled sites (Panamá; Costa Rica; Mexico; San Diego, CA, U.S.A.) separated into distinct clusters with the exception of partial overlap among the bees from Panamá and Costa Rica. Bees from Mexico, Costa Rica, and Panamá clustered near African (A clade) honey bees. San Diego bees formed a more distant cluster relative to bees from Mexico, Costa Rica, and Panamá, falling more equidistantly between the A, M, C, and O groups, consistent with their ancestry drawing substantially from all four groups.

Mitochondrial Ancestry in Africanized honey bee samples

Each mitochondrial sequence from New World honey bees grouped strongly with reference mitochondria from one of the four ancestral lineages (A, M, C, O) in a midpoint rooted phylogeny (Figure 1.3, Table 1.3). Notably, mitochondrial sequences from subspecies *A. m. anatoliaca* (Clade O) grouped loosely with subspecies *A. m. ligustica* and *A. m. carnica* (both C). *A. m. anatoliaca* has previously been shown to possess C type mitochondria although it remains characterized as an O clade honey bee due to similarities of morphological and nuclear markers (Smith, Slaymaker, Palmer, & Kaftanoglu, 1997; Palmer, Smith, & Kaftanoglu, 2000; Wallberg *et al.,* 2014).

Genetic Diversity

All four sampled AHB populations have similar levels of genetic diversity for all three estimators which use both genome-wide sites and a smaller number of high-quality SNPs (Table 5). Genetic diversity estimates in the admixed AHB populations are consistently higher than those estimated for the European and Middle Eastern populations (clades M, C, and O) (Table 1.5). The admixed populations have similar genetic diversity as African honey bees (Clade A) for estimators calculated from genome-wide sites. In contrast, the pairwise estimation of genetic diversity for admixed populations exceeds the genetic diversity of African honey bees when using only high-quality SNPs in the analysis. Among ancestral lineages, for all measures the African lineage is the most diverse, followed by the Middle Eastern (O) lineage, the Western European (M) lineage and lastly, the Eastern European lineage (C).

DISCUSSION

Africanized honey bee (AHB) populations exhibit distinct genomic admixture profiles across their Central and North American range with African ancestry decreasing with increasing latitude (Figures 1.1 & 1.2; Table 1.2). Despite considerable differences in ancestral composition between populations, each sampled region exhibits little variation amongst individual honey bees. This is true whether honey bees were sampled over many tens of kilometers (Isthmus of Panama, San Diego, California) or from geographically restricted sampling points (Chiapas, Mexico and Guanacaste, Costa Rica). At the scales sampled, AHB populations appear to be well-mixed hybrid swarms. Honey bees from Panama, Costa Rica, and Mexico all possess substantial amounts of African ancestry (76-89%), similar to that reported in Brazil (Wallberg *et al.,* 2014; Nelson *et al.,*

2017). This extensive Africanization is perhaps reflective of the longstanding presence of AHB in these regions and that little is done to prevent Africanization of managed hives for both agricultural and hobbyist use. Beekeepers in Central America and Mexico have adapted to working with the AHB and gene flow between feral and managed hives is largely uninhibited (Ratnieks & Visscher, 1996; Guzman-Novoa & Page, 1999).

The substantial amount of Eastern European (C) ancestry persisting in the honey bees sampled from Mexico suggests that insufficient time may have passed since the arrival of AHB for honey bees to reach the high levels of African ancestry seen in lower latitudes. However, AHB first arrived in southern Mexico in the late 1980s, and studies have shown that levels of African ancestry can reach high, apparently stable, levels in less than a decade (Pinto *et al.*, 2005). Alternatively, the substantial European honey bee (EHB) population that existed throughout Mexico prior to AHB arrival might have served as a genetic buffer, allowing for the persistence of C ancestry despite ample time since contact with AHB (Clarke, Rinderer, Franck, Quezada-Euán, & Oldroyd, 2002). Beekeeping with C-lineage honey bees was widespread across Mexico prior to the arrival of AHB, with an estimated 1.5 million managed colonies present throughout the country and Mexico remains one of the largest exporters of honey on the global market (Winston, 1979; Guoda, Chun, & Fuliang, 2001). In contrast, Costa Rica and Panamá both had modest managed beekeeping activity prior to AHB arrival, and feral EHB colonies were quite rare, particularly in the rainy lowlands (Roubik & Boreham, 1990; Lobo, 1995). Additionally, many beekeepers in Central America abandoned the trade after AHB arrival (van Veen, Fallas, Murillo, & Arce, 1998). Thus, AHB likely encountered a much smaller population of EHB in Central America than in Mexico, allowing for a rapid and extensive Africanization of the honey bee gene pool.

In striking contrast to the honey bees from Mexico and Central America, African ancestry in honey bees collected in San Diego County, California (U.S.A.) is relatively low ($\bar{x}$ = 37.5% ± 1.12%) and San Diego bees feature substantial genomic contributions from all four major honey bee lineages (Figure 1.1 & 1.2). Eastern European (C) ancestry ($\bar{x}$ = 34.8% ± 1.14%) in San Diego honey bees is substantially higher than the other three sampled sites while the contribution of the Western European (M) lineage ($\bar{x}$ = 19.1% ± 0.41%) is also somewhat elevated (Figure 1, Table 2). Honey bees from San Diego also have lower African genomic content in comparison to those from Texas and Arizona (~75% A) (Whitfield *et al*., 2006; Bozek *et al.,* 2018).

Notably, all honey bees from the San Diego sample possessed considerable Middle Eastern (O) ancestry ($\bar{x}$ = 8.5% ± 0.24%). Honey bees from Middle Eastern lineages were imported to the United States during the last two decades of the 19th century after which time these limited importations stopped (Magnus & Szalanski, 2010 and references therein; Carpenter & Harpur, 2021). Nevertheless, surveys of feral honey bees in the United States have reported the continued presence of O-clade mitochondria (Magnus & Szalanski, 2010; Kono & Kohn, 2015), and Whitfield *et al.*, (2006) found evidence of some O nuclear genomic content in AHB in Texas. Our findings concerning relatively low levels of African ancestry in San Diego largely agree with other recent genomic studies of feral honey bees in southern California (Cridland *et al.*, 2017a; Calfee *et al*., 2020). However, this is the first assessment of Middle Eastern (O) ancestry in southern California honey bees.

Why is African genomic content in southern California bees much lower than elsewhere? We explore three hypotheses that might account for this. First, models built from climate data fitted to the southern AHB range limit predict that colder winter weather plays a considerable role in halting AHB expansion. (Taylor & Spivak, 1984; Southwick, Roubik, & Williams, 1990; Harrison,

Fewell, Anderson, & Loper, 2006). Nearer the northern (California, USA) and southern (Buenos Aires, Argentina) range limits of AHB, African ancestry is notably reduced in favor of European ancestry (Calfee *et al.,* 2020). Western San Diego County has a mild Mediterranean climate featuring dry summers with a mean high temperature of 25°C (August) and mild winters with a mean minimum of 8°C (January) (NOAA - National Weather Service Forecast Office). South Texas, where African genomic content is much higher than in San Diego, has a hot and humid climate, on average reaching 35°C in the hottest summer month but possesses similarly cool winters to those in San Diego, reaching an average minimum of 7°C in January) (Rangel *et al.,* 2016; NOAA - National Weather Service Forecast Office). Thus, while climate is likely important in limiting the penetrance of African genomic material, simple measures of winter cold temperatures are unlikely to be the only determining factor.

Second, gene flow from managed European honey bee populations could restrain the introgression of genes of African origin in San Diego County. In the United States, AHB are generally considered unfit for apiculture and commercial agriculture due to undesirable characteristics such as a higher propensity to sting and to abandon their nests (reviewed in Schneider *et al*., 2004). The desired lineages of European clades are actively maintained via consistent requeening of colonies with mated queens of European origin (Schiff & Sheppard, 1995, 1996). However, such beekeeping practices have failed to noticeably inhibit the introgression of high levels of African genes into feral Texas and Arizona bee populations (Pinto *et al.,* 2005; Whitfield *et al.,* 2006, Bozek *et al*., 2018). One potential mitigating factor preventing excessive Africanization of San Diego honey bees may be the large agricultural presence in the county with ~230,000 acres of planted crops, many of which (e.g. avocados and citrus) use honey bees for pollination services (San Diego County Crop Statistics Annual Report, 2019). Gene flow from

high-density European managed hives could counter Africanization. However, genetic swamping by managed honey bees would require that a substantial fraction of the honey bees in San Diego County come from managed, genetically European hives. Our finding that all 15 foraging workers examined here had substantial African and Middle Eastern ancestry—lineages not used in managed colonies—argues against this. This finding is consistent with the hypothesis, supported by previous (Kono & Kohn, 2015) and current mitochondrial data, that most bees foraging in San Diego County, whether in urban or non-agricultural rural settings, derive from feral, Africanized colonies.

Finally, insufficient time may have elapsed since the introduction of the AHB to San Diego County for African ancestry to reach levels comparable to those seen elsewhere in the southwestern U.S. (Pinto *et al.,* 2005; Whitfield *et al.,* 2005). AHB arrived in San Diego County in 1994 and our bees were sampled more than two decades later, suggesting either that Africanization is taking much longer than in Texas, or differences in conditions in San Diego relative to Texas lead to reduced penetration of the African genome.

Notably, all four sampled regions report a significant amount of Western European (M) ancestry. Studies that have tracked the process of Africanization elsewhere have shown that African genetic material largely or completely replaces genomic content from the Eastern European (C) lineage, while the contribution from the M lineage to genomes of AHB remains substantial and is never completely eliminated (Clarke *et al*., 2002; Pinto *et al*., 2005; Whitfield *et al*., 2006; Cridland *et al*., 2017a; Nelson *et al*., 2017). All of our sampled honey bee genomes from San Diego to Panamá possess moderate levels of M ancestry while C ancestry content declines precipitously from north to south and is nearly totally absent in samples from Costa Rica and Panamá. This pattern suggests that the M-lineage content that persists in highly Africanized populations may be selected for while C-lineage content is selected against except where A-lineage contribution

declines at higher latitudes (Whitfield *et al*., 2006; Nelson *et al*., 2017). Previous studies have identified some regions of Western European (M) ancestry that appear to be under positive selection, in particular a region on Chromosome 13 which is associated with a QTL for worker ovary size (Calfee *et al*., 2020; Nelson *et al*., 2017). In addition, genes of both M and A ancestry appear to underly nest defense behavior in AHB (Harpur *et al.,* 2020). Further work is needed to determine whether these regions of M ancestry are under selection in our sampled populations. Alternatively, small amounts of M ancestry may be hitchhiking within predominantly African genomes.

Mitochondrial analysis of our four sampled populations is largely consistent with findings from nuclear genomes (Figure 1.3; Table 1.4). All bees sampled from Panamá and Costa Rica, where nuclear genomes were predominantly African, carried mitochondria of African origin. In Mexico, the majority of honey bees carried the A mitotype while a few carried mitochondria of the C lineage. San Diego honey bees harbor all four mitochondrial lineages. While C-lineage mitochondria were absent in our current sample of 15 bees, Kono & Kohn (2015) assayed a larger sample and found mitotypes representing all four clades, with the African mitotype the most frequent (65%) and mitochondria from the other three lineages present in similar proportions. Failure to uncover any mitochondria from the C lineage in the present study likely resulted from the limited number of honey bees sampled.

Admixed populations from the four sampled sites report similar high levels of genetic diversity and these levels are higher than genetic diversity measures from Eastern European (C), Western European (M), and Middle Eastern (O) reference populations. Additionally, depending on the estimator, the genetic diversity of admixed populations exceeds or equals that of the African clade, the honey bee lineage previously found to have the highest genetic diversity (Harpur, Minaei,

Kent, & Zayed, *et al.,* 2012; Wallberg *et al.,* 2014; Calfee *et al.,* 2020; Espregueira-Themudo *et al.*, 2020). The high level of genetic diversity of admixed populations likely results from both substantial contributions from the genetically-diverse African lineage in addition to admixture bringing together variation found among ancestral lineages (Harpur *et al.,* 2012). For San Diego bees, the effect of admixture from multiple ancestral lineages appears to raise their genetic diversity to levels not different than those found in A lineage reference bees or in AHB populations with much higher proportions of A ancestry.

Wallberg *et al.,* (2014) employed a SNP-based measure to calculate Watterson's estimator and then divided the measure by total sites in the genome in order to obtain a per-bp estimate. Our pairwise estimator was calculated in a similar fashion and gave similar, though slightly lower, values. Two of our genetic diversity estimates (genome-wide pairwise and Watterson's estimators) were calculated from the majority of sites in the genome, including both high-quality SNPs and other variable and invariable sites. Those methods resulted in higher estimates of genetic diversity. However, the rank order of our diversity estimates among reference populations, with the A lineage having considerably higher diversity than the O lineage, followed by M and then C is consistent with previous studies (Harpur *et al.,* 2012; Wallberg *et al.,* 2014; Espregueira-Themudo *et al.*, 2020).

We find that the honey bees of the subspecies *Apis mellifera syriaca* are substantially (~ 23%) admixed with the African lineage, consistent with the analysis of Wallberg *et al.,* (2014). Cridland *et al.,* (2017b) ascribed a similar amount of admixture into the *A. m. syriaca* bees from a population of bees from the Arabian Peninsula (termed Clade Y). We have not included bees from the Y lineage in our analysis because they are not known to have been introduced to the American continents (Ruttner, 1988; Carpenter & Harpur, 2021). It is possible that the admixture in *A. m.*

syriaca reported here and in Wallberg *et al.,* (2014) represents intermixing with bees from the Arabian Peninsula rather than Africa.

Africanized honey bees in the New World represent one of the largest and best-documented biological invasions resulting from human-mediated hybridization. Feral European honey bee populations have been replaced by Africanized honey bees throughout most of the New World suggesting their genetic makeup provides strong ecological advantages except at higher latitudes. In San Diego County, feral AHB are super abundant, responsible for 75% of all floral visits to native plants and reaching greater dominance (> 90% of all pollinator visits) on the most abundantly blooming species (Hung *et al.,* 2018; 2019). This occurs in spite of carrying detrimental viral diseases at titers similar to those found in managed bees, suggesting they can resist negative viral affects for which managed hives receive mitigating treatments (Geffre *et al.,* 2021). Future work to determine local genomic ancestry could investigate selection on genomic regions that consistently come from African versus European lineages. Such regions, and the genes they contain, are critical to understanding the genetic changes that underlie the ecological success of Africanized honey bees. Such analyses could also shed light on the locations and origins of genomic regions useful for breeding managed honey bees to be more resistant to factors currently harming the honey bee industry.

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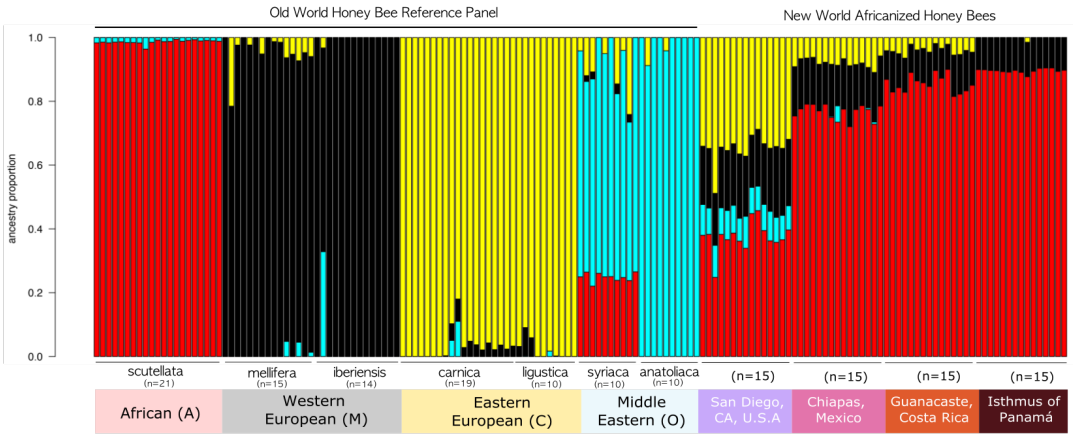

Figure 1.1: NGSadmix barplot of honey bee ancestry. Each vertical bar is one honey bee genome and colors represent the estimated proportion of ancestry derived from each genetic cluster (K=4). The 99 reference genomes belonging to the four major evolutionary lineages of *Apis mellifera* (A, M, C, O) are grouped and labeled beginning with the African clade. The 60 admixed AHB genomes are arranged north to south by geographic origin, beginning with San Diego, CA and followed by the honey bees from Mexico, Costa Rica, and Panamá.

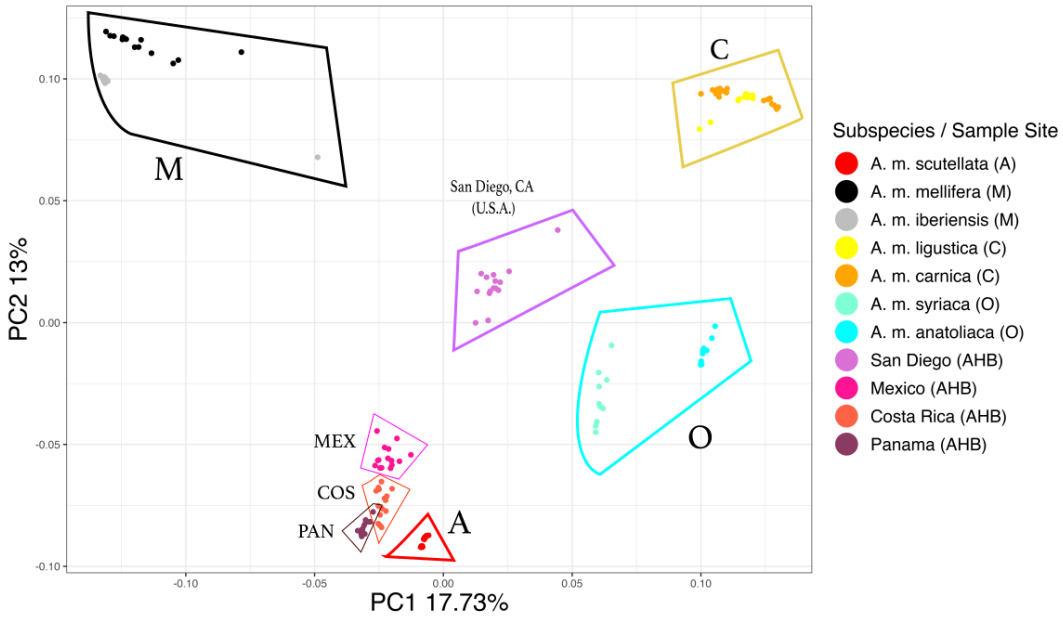

Figure 1.2: Principal Component Analysis (PCA) of the 99 reference honey bees and 60 admixed honey bee genomes.

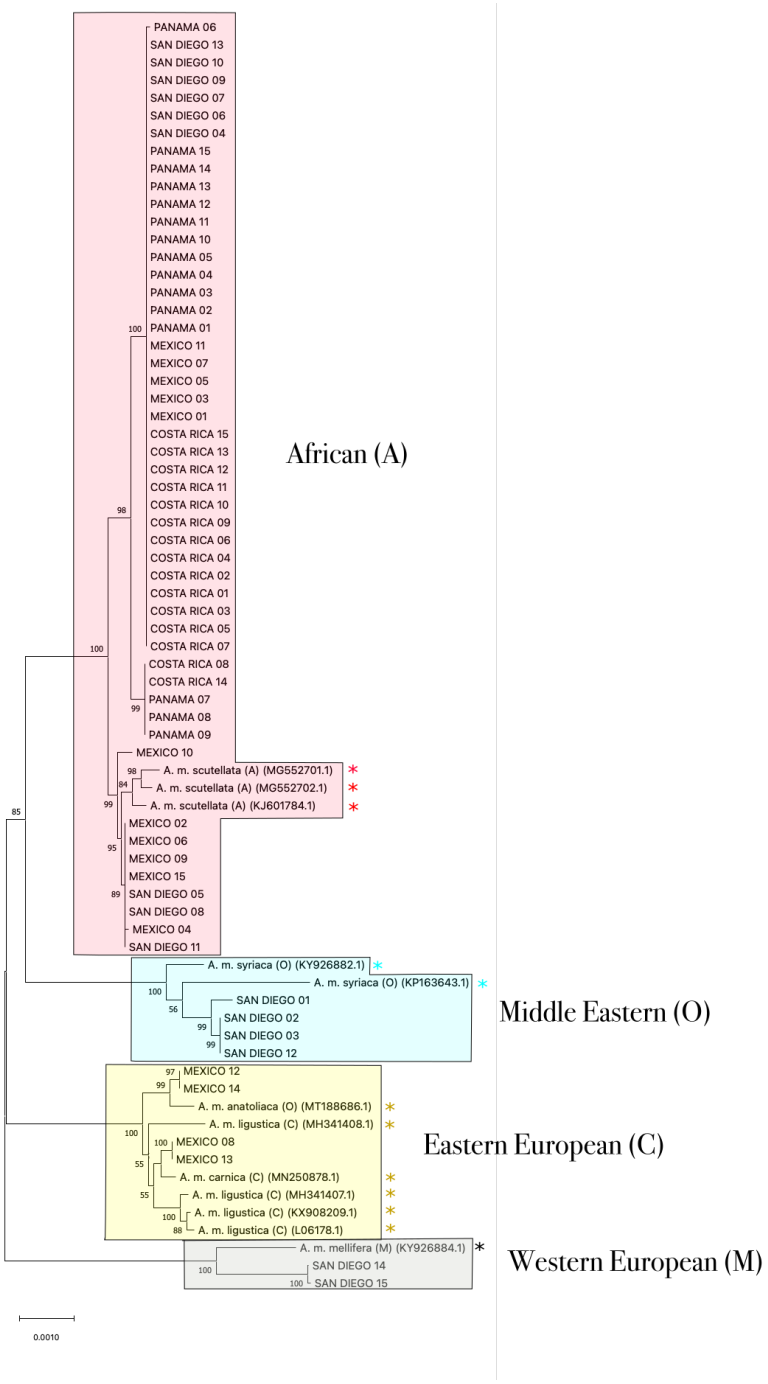

Figure 1.3: Midpoint-rooted neighbor joining phylogeny constructed from the mitochondrial genomes of 60 admixed honey bees collected from San Diego, Mexico, Costa Rica, and Panamá (n=15, per population) and 12 reference mitochondrial sequences obtained from NCBI: *A. m. mellifera* (n=1), *A. m. syriaca* (n=2), *A. m. carnica* (n=1), *A. m. scutellata* (n=3), *A. m. ligustica* (n=4), and *A. m. anatoliaca* (n=1). NCBI mitochondrial sequences are denoted by an asterisk (*). Values on each node represent the percent bootstrap support (n = 2000 bootstraps).

Table 1.1: Summary of all 159 genomes included in this ancestry analysis, including (A) 99 reference honey bee genomes downloaded from NCBI from Wallberg *et al.,* (2014) and Harpur *et al.,* (2014) (B) 60 admixed honey bee genomes collected from four distinct sampling sites.

A

Clade	Subspecies	(n)	Source Country	Sequencing Project
A	*A. m. scutellata*	21	South Africa	Wallberg *et al.,* (2014); Harpur *et al.,* (2014)
M	*A. m. mellifera*	15	England, Poland	
	A. m. iberiensis	14	Ireland, Spain	
C	*A. m. carnica*	19	Italy, Germany, Croatia, Slovenia	
	A. m. ligustica	10	Greece	Wallberg *et al.,* (2014)
O	*A. m. syriaca*	10	Syria	
	A. m. anatoliaca	10	Lebanon	

B

Location	(n)	Coordinates
San Diego, CA, U.S.A.	15	32.7157° N, 117.1611° W
Chiapas, Mexico	15	16.7569° N, 93.1292° W
Santa Rosa National Park, Costa Rica	15	10.8379° N, 85.7051° W
Panamá City, Panamá	15	9.1521° N, 79.8465° W

Table 1.2: Mean percentage (SE) of genomic contributions from the four major honey bee lineages in each sampled population (n = 15 bee genomes per sample).

Clade Ancestry	San Diego, CA	Mexico	Costa Rica	Panamá
African (A)	37.5 % ± 1.12 %	76.7 % ± 0.61 %	85.4 % ± 0.07 %	89.6 % ± 0.17 %
Western European (M)	19.1 % ± 0.41 %	14.9 %± 0.36 %	10.5 % ± 0.42 %	10.3 % ± 0.12 %
Eastern European (C)	34.8 % ± 1.14 %	8.07 % ± 0.34 %	4.05 % ± 0.03 %	0.09 % ± 0.01 %
Middle Eastern (O)	8.51 % ± 0.24 %	0.45 % ± 0.33 %	0.00 % ± 1.9e-21 %	0.00 % ± 6.72e-21 %

Table 1.3: Whole mitochondrial sequences representing A/C/M/O honey bee clades downloaded from NCBI and used in mtDNA haplotype analysis.

GenBank Accession Number	Subspecies	Clade
KJ601784.1	*A. m. scutellata*	A
MG552702.1	*A. m. scutellata*	A
MG552701.1	*A. m. scutellata*	A
KY926884.1	*A. m. mellifera*	M
MN250878.1	*A. m. carnica*	C
KX908209.1	*A. m. ligustica*	C
MH341408.1	*A. m. ligustica*	C
MH341407.1	*A. m. ligustica*	C
L06178.1	*A. m. ligustica*	C
MT188686.1	*A. m. anatoliaca*	O
KP163643.1	*A. m. syriaca*	O
KY926882.1	*A. m. syriaca*	O

Table 1.4: Number of honey bees sampled from each admixed population (San Diego (CA), Mexico, Costa Rica, Panamá) found to carry mitochondria from each of the four clades (A, M, C, O).

	San Diego, CA	Mexico	Costa Rica	Panamá
African (A)	9	11	15	15
Western European (M)	2	0	0	0
Eastern European (C)	0	4	0	0
Middle Eastern (O)	4	0	0	0

Table 1.5: Genetic diversity measures (mean ± SE) for admixed and reference populations

Population	Pairwise Estimator ($\hat{\theta}_\pi$)	Pairwise Estimator ($\hat{\theta}_\pi$) using called SNPs only (minor allele frequency > 0.05)	Watterson's Estimator ($\hat{\theta}_w$)
African (A)	0.0100 ± 0.0018	0.0047 ± 9.205e-05	0.0152 ± 0.0012
Western European (M)	0.0047 ± 0.0007	0.0028 ± 3.503e-05	0.0049 ± 0.0006
Eastern European (C)	0.0036 ± 0.0003	0.0023 ± 2.343e-05	0.0042 ± 0.0004
Middle Eastern (O)	0.0059 ± 0.0005	0.0037 ± 5.569e-05	0.0062 ± 0.0004
San Diego, CA, U.S.A.	0.0096 ± 0.0018	0.0061 ± 8.863e-05	0.0108 ± 0.0020
Chiapas, Mexico	0.0109 ± 0.0023	0.0063 ± 0.0001	0.0124 ± 0.0023
Guanacaste, Costa Rica	0.0105 ± 0.0024	0.0061± 0.0001	0.0110 ± 0.0021
Isthmus of Panamá	0.0106 ± 0.0024	0.0060 ± 0.0001	0.0114 ± 0.0020

Chapter 2: Nest Defense Behavior in European and Africanized Honey Bees (Apis mellifera) in Southern California: Effects of genetic composition and season

ABSTRACT

Colony defense in the Western honey bee (*Apis mellifera*) is mediated by complex social, environmental, and genetic factors that can result in the recruitment of thousands of individuals in a fearsome display of social aggression. We conducted a seasonal assessment (May through November) of colony nest defense behavior between feral Africanized (AHB) and managed European (EHB) honey bee hives using standardized field assays to examine the effect of season, genetics, and husbandry on defensive behavior. Measures of defensiveness were low in both AHB and EHB colonies during May. Defensiveness then increased from May through November in both types of bees but substantially more so in AHB colonies. Levels of nest defensiveness in AHB measured here appear lower than those previously documented in AHB colonies from Brazil. This lower level of defensiveness in feral AHB colonies from Southern California could be due to their lower levels of African ancestry. Finally, colonies of European honey bees often displayed considerable African genomic content, likely the result of introgression via AHB drone insemination or AHB usurpation, highlighting the difficulty of preventing Africanization in honey bees in a region where AHB is common.

INTRODUCTION

Many social insects possess an impressive capacity for nest defense due to their ability to orchestrate concerted attacks involving hundreds to thousands of individuals, all of which are

prepared to self-sacrifice in defense of the hive (Shorter & Rueppell, 2012). Such seemingly altruistic self-sacrifice may reflect the haplodiploid composition of the hive, composed of non-reproductive, highly-related workers receiving indirect fitness benefits via kin selection (Hamilton, 1964). The study of complex behaviors, such as nest defense, in social species poses special challenges as the behavior is a colony level trait determined by a number of social, genetic, and environmental factors that are not easily teased apart (Breed, Guzmán-Novoa, & Hunt, 2004).

The defensive behavior of the Western honey bee (*Apis mellifera*) is of interest because it affects husbandry in both commercial agriculture and hobbyist beekeeping which favor lower levels of nest defense behavior ("gentleness"). Western honey bees are taxonomically diverse, comprised of more than two dozen recognized subspecies falling within several broader biogeographic clades: Africa (A), Western Europe (M), Eastern Europe (C), Middle East (O), and Arabian Peninsula and Eastern Africa (Y) (reviewed in Carpenter & Harpur, 2021; Ruttner, 1988). The various honey bee subspecies differ markedly in the intensity of their nest defense, both within and among lineages. Certain subspecies are renowned for their gentleness (e.g. Italian honey bees – *Apis mellifera ligustica*) and others feared for their propensity to collectively sting and harass in response to a disturbance of their hive (e.g. the widespread African honey bee – *Apis mellifera scutellata* and the Syrian honey bee – *Apis mellifera syriaca*) (Kasangaki *et al.,* 2018; Ruttner 1988). The relatively gentle Italian honey bee has become the honey bee of choice, both for large-scale commercial and backyard beekeeping across North America and several European countries (Delaney *et al.,* 2009).

Honey bee nest defense differences between the various subspecies became widely apparent during the introduction and subsequent expansion of the Africanized honey bee (AHB; Reviewed in Ruttner, 1988). Prior to the introduction of African (*Apis mellifera scutellata*)

subspecies to the American continents, honey bees were largely of German (*Apis mellifera mellifera*), Italian (*Apis mellifera ligustica)* or Iberian (*Apis mellifera iberiensis*) origin (Whitfield *et. al.,* 2006). The introduction of African honey bees to Sao Paulo, Brazil in 1957 by honey bee research scientists brought this subspecies into contact with the European subspecies, with which they hybridized to form the AHB (Kerr, 1967). Beekeepers quickly realized that the Africanization of honey bees caused a concerning increase in defensiveness (Quezada-Euán & Paxton, 1999). In Mexico, the uptick in defensive behavior in addition to various other undesirable qualities of the ABH (e.g. higher rate of absconding, lower honey production) caused an initial decrease in the number of beekeepers willing to work with this new hybrid bee, although this decrease has since rebounded as management practices and people adapted to the AHB (Guzman-Novoa *et. al.,* 2011). However, the increase in AHB-caused fatalities following their arrival in Mexico, 480 fatalities out of more than 5,000 individual cases, caused widespread public fear, leading to the dubbing of the AHB as the "killer" bee (Becerril-Ángeles, *et. al.,* 2013).

Several assays have been developed to measure honey bee defensive behavior across individual, small group, and colony scales (reviewed in Nouvian, Reinhard, & Giurfa, 2016). Studies using a variety of these assays have documented the higher degree of defensiveness of the AHB compared to European honey bee lineages (*A. m. carnica* and *A. m. ligustica*) preferred by beekeepers for their docile natures. AHB in Mexico display a similar level of high defensiveness as pure African (*A. m. scutellata*) honey bee colonies (Guzmán-Novoa & Page, 1994). AHB have lower thresholds of response to a disturbance or alarm pheromone, respond with recruitment of more nestmates, and ultimately a higher deployment of defenders in proportion to the total hive (Stort, 1974; Collins *et. al.,* 1982; Guzmán-Novoa & Page, 1993; Guzmán-Novoa *et. al.,* 2002; Hunt *et. al.,* 2003). AHB sting a target up to 8.5x more than EHB in the first 90 seconds (Collins

et. al., 1982; Guzmán-Novoa *et. al.,* 2004). AHB assessed in Brazil pursued a target up to 7X greater distances than Italian honey bees (Stort, 1974). In a similar study conducted in Mexico, EHB colonies often failed to pursue to any distance and in co-fostered AHB/EHB colonies, AHB comprised nearly 70% of pursuing honey bees (Guzmán-Novoa *et. al.,* 2004). In Africanized colonies that contain both AHB and EHB offspring, the majority of the workers that respond in the first 10 seconds after disturbance are Africanized (Guzmán-Novoa *et. al.,* 2004). In addition, in colonies composed of both AHB and EHB, the presence of AHB will influence European workers to act more defensively (Guzmán-Novoa & Page, 1994; Guzmán-Novoa *et. al.,* 2004).

While fierce defensive behavior is strongly tied to African ancestry, the genetics underlying this complex behavior is still under examination. Nest defense is heritable, dominant, and in hybrids between AHB and EHB, is higher if AHB drones are mated with EHB queens than the reverse (Guzmán-Novoa & Page Jr., 1993; DeGrandi-Hoffman *et. al.,* 1997; Guzmán-Novoa *et. al.,* 2002; Guzmán-Novoa *et. al.,* 2005; Gibson *et. al.,* 2015). Quantitative trait loci (QTL) have been identified that correlate with increased measures of defensiveness (e.g. tendency to sting) on both the colony and individual level (Hunt *et. al.,* 1998; Arechavaleta-Velasco *et. al.,* 2003). Guzmán-Novoa *et. al.,* (2002) found that AHB backcrossed honey bees carrying the African allele for a marker linked to the QTL for stinging behavior (*sting*-1) responded more rapidly to a disturbance and were more likely to sting. However, in a whole-genome study of 116 AHB colonies, Harpur *et. al.,* (2020) found that for two out of 65 defense associated QTL, the variant from the European ancestor caused increased defensive behavior.

Africanization does not always lead to increased defensiveness as is apparent from the gentle AHB (gAHB) of Puerto Rico (Rivera-Marchand, Oskay, & Giray, 2012; Galindo-Cardona *et. al.,* 2013). The gAHB has lower African genomic content than AHB from Central and South America (~40%

in Puerto Rico in comparison to ~ 80% where defensive behavior of AHB has been measured previously; Rivera-Marchand, *et. al.,* 2012). Genomic analysis of gAHB populations in Puerto Rico provided evidence that gentleness arose via a soft selective sweep under the unique island conditions combining human pressure, predator absence, and a strong oceanic barrier to further AHB influx (Avalos *et. al.,* 2017). Although the gAHB population, like other mainland AHB populations, is a hybrid bee—the high frequency of haplotypes rare to either mainland AHB and pure parental lineages indicates that the gAHB is a unique population undergoing a fairly rapid and distinct evolutionary trajectory (Galindo-Cardona *et. al.,* 2013; Avalos *et. al.,* 2017).

Honey bee defense behavior is of particular interest to the general public and the beekeeping industry due to the widespread use of this insect in commercial pollination and backyard beekeeping. The increased concern for worldwide honey bee declines and honey bee conservation in the general public has led to rising interest in becoming a beekeeping hobbyist. In regions like southern California, where AHB arrived in 1994 and is now well-established in the feral honey bee population, there exists a growing need to provide a profile of defensive behavior in the two common honey bee types (EHB and AHB) to inform honey bee management and husbandry. While many beekeepers maintain requeening policies to prevent Africanization of their EHB hives, the extent to which requeening is successful in maintaining EHB purity and gentleness in an area where AHB is common is under question. Currently, no quantitative assessment of defensiveness in honey bees from southern California exists and few studies have examined defensiveness across a seasonal basis in any region. Here, we conducted a seasonal assessment of colony-level nest defense between feral Africanized honey bees and managed European honey bees utilizing previously established field-based assays to assess how season, genetics, and husbandry influence nest defense behavior in honey bees.

MATERIALS AND METHODS

Honey bee apiary settings & colony demographics

All honey bee hives assessed in this study were in two apiaries in San Diego, California (U.S.A.). The first apiary is located at the Biological Field Station on the University of California San Diego campus in La Jolla, CA, henceforth designated the BFS apiary. The second apiary is located at the Elliot Chaparral Reserve of the University of California (henceforth, the ECR apiary) in San Diego, CA, a largely undisturbed coastal sage scrub habitat. These two apiaries are located within 9 miles of each other, with the ECR apiary located farther inland than the BFS apiary. We designate the honey bee colonies within the BFS apiary as managed as they are subject to considerable human intervention for both breeding and parasite control. Colonies at our BFS apiary originate from queens of European ancestry (*Apis mellifera ligustica* queens obtained from Wildflower Meadow, a southern Californian apiary located in Vista, CA) and are regularly treated for the parasitic mite *Varroa destructor*, as well as other honey bee diseases as per standard methods. BFS colonies are regularly requeened to promote colony health and prevent Africanization. During the winter season, these hives are provided with sucrose solution *ad libitum* to assist with colony feeding. In contrast, honey bee colonies at the ECR apiary are completely unmanaged and originate from feral swarms captured throughout San Diego country, of putative African ancestry (Zarate *et. al.,* 2022). ECR colonies are not requeened nor treated for *Varroa*. No sucrose supplements are provided to these colonies nor are they manipulated or interfered with in any way. All colonies at both apiaries are contained in standard Langstroth box hives. Ten colonies were chosen from each apiary (total n = 20), based on comparable size and colony resource structure (i.e. similar honey stores, area dedicated to brood, etc.). However, as some colonies swarmed and absconded from their hives. In the case that a colony was lost due to absconding, we

chose another colony from the apiary to assess in order to maintain a sample size of 10 colonies evaluated per site.

Colony level nest defense behavior assays

Colony level assays were conducted on sunny or near-sunny days with negligible to no cloud clover to ensure environmental standardization. Temperature and climate conditions were recorded at the start of each round of examinations using a temperature data logger (Hobologger©, Onset, USA). We conducted a modified version of the ratings assay detailed in Guzman-Novoa *et. al.,* (2003) which assesses colony defense by rating the tendencies of various aspects of defensive behavior in honey bees. This assay was rated as the most reliable measure of assessing nest defense in comparison to three other assays (Guzmán-Novoa *et. al.,* 2003). The operator wore sterile latex gloves over standard leather beekeeper gloves that were disposed of and replaced after each assay. Hives were opened, two puffs of smoke were applied to the tops of the frames, and two brood frames were removed and closely observed simulating an inspection protocol. The operator ranked each colony on the tendency of honey bees to (1) run on the comb, (2) fly off the comb, and (3) fly off and hit operator's veil. We ranked all measures on a scale of 1-5 with 1 being the least defensive and 5 the most. The brood frames were returned to the Langstroth box hive and then a black leather flag (6 cm x 6 cm) was waved briskly over the brood frames at a consistent rate (~1 wave/second) for 15 seconds. The black flag was then deposited in a clear plastic bag and the number of stings deposited on it was counted at the conclusion of the trial. The top of the Langstroth hive was returned and then extent and intensity of honey bee pursuit was assayed. The observer retreated, to a distance of 25 meters and then to 50 meters. At each distance, the number of bees pursuing the operator was estimated and ranked on the same 1-5 scale. The operator then

removed themselves from the trial area and waited until all honey bees had ceased pursuit before counting the number of stings deposited on the latex gloves covering the leather gloves. Latex gloves were removed after each trial and leather gloves were subjected to a few puffs of smoke in order to dissipate any alarm pheromone that might have transferred to the leather. Colony assays were repeated three times over three consecutive days spanning the late morning to early afternoon (10:00 – 15:00) and all measures averaged over the three days. Colony assays were first conducted in late May 2021, then repeated in July, August, September, and November 2021.

Honey bee colony size quantification

In order to assess the effect of colony size on measures of defensiveness, we quantified colony size each month, a few weeks before conducting defense assays. We inspected each colony and photographed both sides of every frame for each colony. We then used the open-access image software GIMP (v. 2.10) to estimate the number of individual bees present on each frame using a protocol developed by Heather Broccard-Bell & Brandon Mukogawa (*pers. communication*). A standardized grid was mapped onto each frame in each photograph and all honey bees contained in one cell were counted and then this estimate was used to estimate the total honey bees contained across all cells. While we were able to quantify size for most colonies, we were unable to collect size data for the period of July 2021. In this case, we did not include size as an effect in the model. We also did not have size estimates for three colonies in October, these were excluded from preliminary analyses that included size. To assess differences in colony size between site, we ran a one-way analysis of variance (ANOVA) for each month we had data (all except July).

Honey Bee genomic sequencing

To assess the genetic composition of honey bee colonies from each apiary, we collected three honey bee workers from each colony (total, n = 60) from the brood frames selecting bees that exhibited characteristics indicative of younger castes (e.g. less wing tear, thicker blond hair on thorax) in order to ensure that these were workers from the hive and not non-nestmates (e.g. robbers). Honey bees from the ECR apiary were sampled in mid-November 2021, following the last defensiveness assay. Honey bees from the BFS apiary were sampled in late August, approximately two months since the last requeening. Honey bees were euthanized and preserved in 100% ethanol at -80 Celsius. We extracted DNA from crushed heads of the 60 sampled honey bees using the standard protocol of the Qiagen DNAeasy Blood & Tissue extraction kit. The DNA was submitted for DNA KAPA library construction and whole-genome sequencing at the Institute for Genomic Medicine (IGM), UC San Diego. All 60 individuals were multiplexed and sequenced across three lanes of an Illumina NovaSeqS4 platform to produce 150-bp paired end reads at 20X coverage.

Assessing differences in nest defense across site and calendar month

To assess differences in defensive behavior across site and time, we conducted a repeated measured analysis of variance using JMP v.16.1 for each defense measure separately, including site as a fixed effect (JMP©, version 16.1, SAS Institute Inc, Cary, NC). We log transformed the stinging data prior to running the analysis which improved normality of data. We then calculated adjusted univariate tests. To account for treating ordinal data as continuous, we used the Huynh-Feldt adjusted p-values. This method has been shown to perform well for repeated ordinal data

with small sample sizes, resulting in lower type II error and greater power than alternative methods (Stiger *et. al.,* 1998).

We also conducted analyses for each month using a multivariate analysis of variance (MANOVA) in JMP where we included all defensive measures as response variables. As our analysis of variance showed no significant differences in colony size between sites for August through November, we did not include size in the model (Table 4). If the MANOVA reported a significant F test ($p < 0.025$), we then ran an individual analysis of variance (ANOVA) per defense measure including site as a model effect.

Genomic admixture analysis

Raw reads generated from sequencing, and those downloaded from NCBI, were trimmed and filtered for quality and length, aligned to the reference genome assembled by Wallberg *et al., (*2019) and then used to estimate ancestry proportions from either the A, M, C, or O clades following a bioinformatic pipeline previously described in Zarate *et. al.,* (2022). We used a previously assembled honey bee reference panel of 159 whole honey bee genomes representing the 4 honey bee clades and 7 different honey bee subspecies (29 genomes from Harpur *et. al.,* 2014; 70 genomes from Wallberg *et. al.,* 2014). The program ANGSD v0.930 (Korneliussen, Albrechtson, & Nielson, 2014) was used to call variant sites and estimate genotype likelihoods across all 159 honey bee genomes. We estimated honey bee clade ancestry proportions using NGSadmix and a panel of 201,975 SNPs (Skotte et al., 2013) using the admixture pipeline described in Zarate *et. al.,* (2022) with the number of assumed genetic clusters ranging from 2-6. We included only SNPs that were reported in at least 94% of all individuals and had a minimum minor allele frequency of 5%. We used R (R Core Team 2014) to graph admixture estimates.

Assessing relationship between amount of African genomic ancestry and nest defense

To assess variation of African ancestry within vs. between colonies and sites we conducted an analysis of variance (ANOVA) of percent African ancestry across all colonies from both sites with colony nested within site as a random effect. We then assessed the extent to which African ancestry predicted the various defense measures examined. For the following analyses we used only the dataset collected in November as that was the time period the honey bees were sampled for genetic analysis. To assess the extent to which amount of African ancestry predicted the various defensive measures, we ran a MANOVA with all defense metrics as response variables and African ancestry as a model effect. We analyzed BFS and ECR separately as there was little variation in percent African ancestry in ECR. All analyses were conducted using JMP©, version 16.1.

RESULTS

Honey bee colony size across time.

Honey bee colony size differed between site for May ($p = 0.0412$), but did not differ between site for the months of August through November (Figure S1, Table 2.4). For the month of July, we did not have size data so this analysis was not conducted for that month.

Nest defense behavior across time.

For four of the seven defensive measures (fly off comb, hit the operator's veil, pursuit to 50 meters, and the stings on gloves), there were significant effects of site, time and the interaction of time and site (Figure 2.1, Table 2.3). For the other three metrics, two of the effects were

significant although it varied by measure. For stings on flag, both time and site were significant, but the interaction was not. In contrast, pursuit to 25 meters lacked a significant time effect and running on comb did not show a significant effect for site.

Nest defense behavior within each month

In May, honey bees from BFS exhibited higher defensiveness for three measures, whereas ECR showed greater defensives in just one (the number of stings on the flag) (Figure 2.1, Table 2.2). In July, there were no significant differences in measured behaviors between sites. In August, the sites only differed in two defensive behaviors (stings on flag and pursuit to 50 meters) with ECR showing more defensiveness. In the final two months studied (October and November) four and six of the seven traits showed significantly higher levels of defensive behavior for ECR bees, respectively (Figure 2.1, Table 2.2).

Genomic admixture and extent of Africanization in honey bees

ECR and BFS differed significantly in the amount of African ancestry present across colonies ($p < 0.001$). BFS colonies were on average 57% Eastern European (C Clade), 16% Western European (M Clade), 19% African (A Clade) and 7% Middle Eastern (O Clade) (Figure 2.2, Table 2.6). In contrast, ECR colonies exhibited higher African ancestry on average (38%), followed by Eastern European (37%), Western European (19%) and Middle Eastern (6%) (Table 2.6). There existed considerable variation in African ancestry between colonies in the BFS apiary, with some possessing as much as 39% and one colony exhibiting none at all. In contrast, the honey bees at ECR exhibited little between colony variation for African ancestry, ranging from 33-46%.

There was relatively little genomic variation among bees sampled from the same colony. Colony identity explained ~75% of variation in African ancestry at both ECR and BFS apiaries ($p < 0.01$).

For the month of November, for both ECR and BFS, amount of African ancestry was not a significant predictor of the amount of times a colony stung either the leather flag or the operator's gloves ($p > 0.05$) (Table 2.5). Neither was defensiveness as measured by either of the five other ranked measures (tendencies of honey bees to run on the comb, fly off the comb, hit the operator's veil, or pursue the operator to 25 or 50 meters) significantly predicted by amount of African ancestry at either site.

DISCUSSION

From our assessment of nest defense in honey bee colonies sourced from managed, European and feral, Africanized stock in San Diego, CA, we find that defensive behavior is modulated by both season and honey bee type. AHB colonies from the ECR site showed an increase in defensiveness across most measured behaviors as the year progressed and were more defensive than managed bees for nearly all measured behavioral traits in October and November (Table 2). EHB colonies from the BFS site showed somewhat elevated defensiveness for some measures in the initial month of our study (May) but did not show sharply increased defensive behavior in later months (Figure 2.1; Table 2.1 & 2.2).

There was significant effect of site, time, and the interaction of site and time for four of the seven defense measures assayed (Table 2.3). However, for some measures, only two of the three were significant. For the measure of stings deposited on the flag, there was a significant effect of site and time, but not of the interaction of site and time. For other measures, such as the tendency

for honey bees to run on the comb or pursue to 25 meters, the interaction and one of the fixed effects was significant, but not both fixed effects were significant.

While the AHB colonies assessed here exhibit higher defensiveness than the EHB colonies when assessed in the fall, they do not appear to be as defensive as AHB colonies studied in Mexico (Guzman-Novoa & Page, 1994; Guzman-Novoa *et. al.,* 2002, 2003; 2005), Colombia (Villa, 1988), or Brazil (Collins *et. al.,* 1982; Harpur *et. al.,* 2020) using similar field tests. The most comparable data come from data on the rate at which stings accumulate on a flag over a given amount of time (ranging from 15 seconds to 1 minute). While we used the traditional ratings test as detailed by Guzman-Novoa *et. al.,* (2003) which included the measurement of various ranked factors, many studies of defensiveness only assessed the amount of stings deposited on a target (sometimes a leather ball instead of a flag). Sting rates by AHB colonies reported in various studies differed widely, although on average AHB are reported to sting ~100 stings per minute and deposit 5.7 X more stings than EHB. The AHB assessed here report lower numbers of stings as assessed by the flag test (approximately ~2.5X higher than BFS colonies or ~66 stings per minute) in October, the month where AHB on general exhibited highest defensiveness. In May, the month where AHB were the least defensive, this number dropped to ~23 stings per minute, a rate much more comparable to that measured in EHB in the aforementioned studies.

Surprisingly, our AHB honey bees reported less stinging than the gentle AHB of Puerto Rico (on average stinging 220 times per minute), although their study differed from ours in that they initiated the assay by causing a strong disturbance in the form of dropping a brick from a proscribed height onto the hive before presenting the flag (Rivera-Marchand *et. al.*, 2012) The EHB colonies they measured with this same assay reported relatively high stinging (197 stings / minute) as well, resulting in gAHB being only 10 percent greater than EHB in stinging propensity.

In the same study, Rivera-Marchand *et. al.*, (2012) found various other aspects of honey bee behavior (tendency to fly off the comb, run on the comb, hanging off the comb), that did not differ between gAHB and EHB and values for gAHB were lower than those reported from AHB in Mexico with the same test procedures (Giray *et. al.,* 2000).

The lower amount of African ancestry (~40%) reported in the AHB of southern California might explain some of the reduced defensiveness seen here. The AHB of Mexico or Brazil, where many AHB defensiveness studies have occurred, have a greater percentage (70-84%) of their genome from the African lineage (Clarke *et. al.,* 2002; Wallberg *et. al.,* 2014; Nelson *et al.,* 2017; Zarate *et. al.,* 2020). However, the crossing of AHB and EHB colonies in Mexico resulted in F1 colonies that showed levels of defensive behavior similar to that seen in the parental AHB strain. Thus, even AHB with intermediate levels of African genomic content can continue to exhibit high defensiveness (Guzman-Novoa & Page, 1994). Therefore, lower defensive behavior seen both in our study and in the gAHB may involve selection for reduced defensiveness and not be entirely explained by reduced African genomic content.

We found a substantial amount of African ancestry (average 23%) in bees from our managed BFS apiary. In addition, African ancestry at this site varied a great deal among colonies (range 4% to 41%). The amount of African ancestry that had introgressed into our managed apiary was not completely unexpected given that this stock is consistently requeened with queens bred in commercial bee-breeding operations located in southern California, where European queens (largely derived from the Italian subspecies *A. m. ligustica*) are allowed to mate openly with any drone they encounter. Thus, there remains the very real possibility of gene flow from feral Africanized colonies introgressing into the breeding population, at least where breeding is done within the range of the AHB.

BFS colonies originate as nucleus colonies which are bred and reared outside the range of the AHB. However, if the original queen dies, these colonies are requeened with commercially produced queens from San Diego county that originate from Italian (*A. m. ligustica*) stock, come from hives that are carefully screened for gentle behavior, but queens produced from these hives are allowed to mate freely with drones that may come from the producer's hives but also could come from the feral AHB population. Therefore, it is possible that African ancestry is of BFS bees is at least partially due to the manner in which queens are mated. Nevertheless, BFS bees showed markedly lower levels of African genomic content, on average, and were also markedly more gentle during October and November measurements when ECR exhibited elevated levels defensive behaviors. Lastly, it is possible that the EHB colony at the BFS site that was most highly Africanized resulted from usurpation of the hive by an AHB swarm, a behavior that is much more common in AHB than EHB (Danka *et. al.,* 1992).

We showed that defensive behaviors of AHB colonies in southern California were highly affected by the time period in which they were measured. In spring and summer months, feral AHB colonies were not generally more defensive than the managed colonies they were compared to which had much lower levels of African genetic content. In the late fall months of October and November, however, AHB colonies were markedly more defensive than managed colonies, though perhaps still less so than AHB colonies measured in Central and South America which have approximately twice the level of African genomic content. The seasonality of these behaviors is a reminder that judging a colony or swarm's ancestry based on its behavior at any given time may be unreliable. At the same time, the seemingly lower level of defensive behavior observed in AHB from southern California provides some hope that potentially beneficial traits possessed by this feral population, such as their ability to thrive in the face of pathogens for which managed hives

require treatments (Geffre *et al.,* 2021), can be bred into commercial honey bees without raising defensiveness to levels that adversely affect beekeeping practice.

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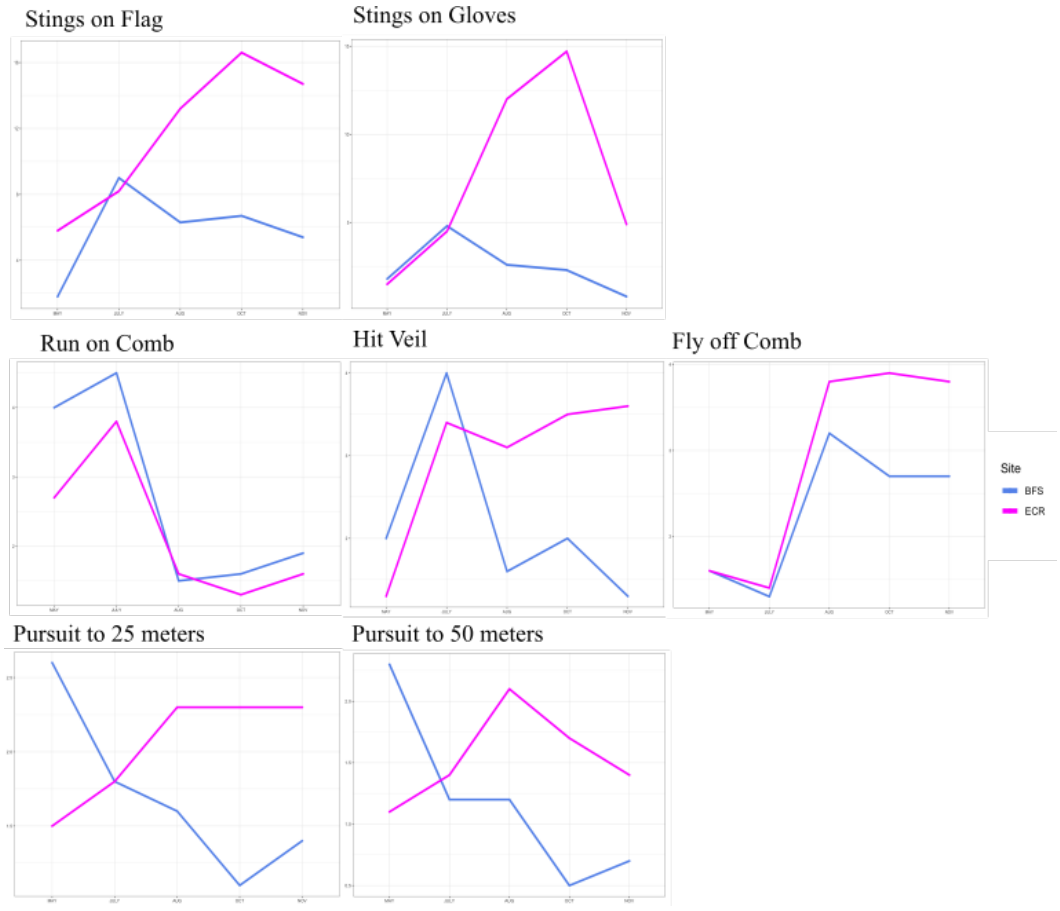

Figure 2.1: Honey bee colony defense measure averages between managed EHB (BFS) and feral AHB (ECR) honey bee colonies from May to November 2021. The stinging measures are continuous variables while all others are ordinal variables, ranked on a scale of 1 to 5 with 1 being the least defensive and 5 the most. Defensive assays adapted from Guzman-Novoa *et. al.,* (2003).

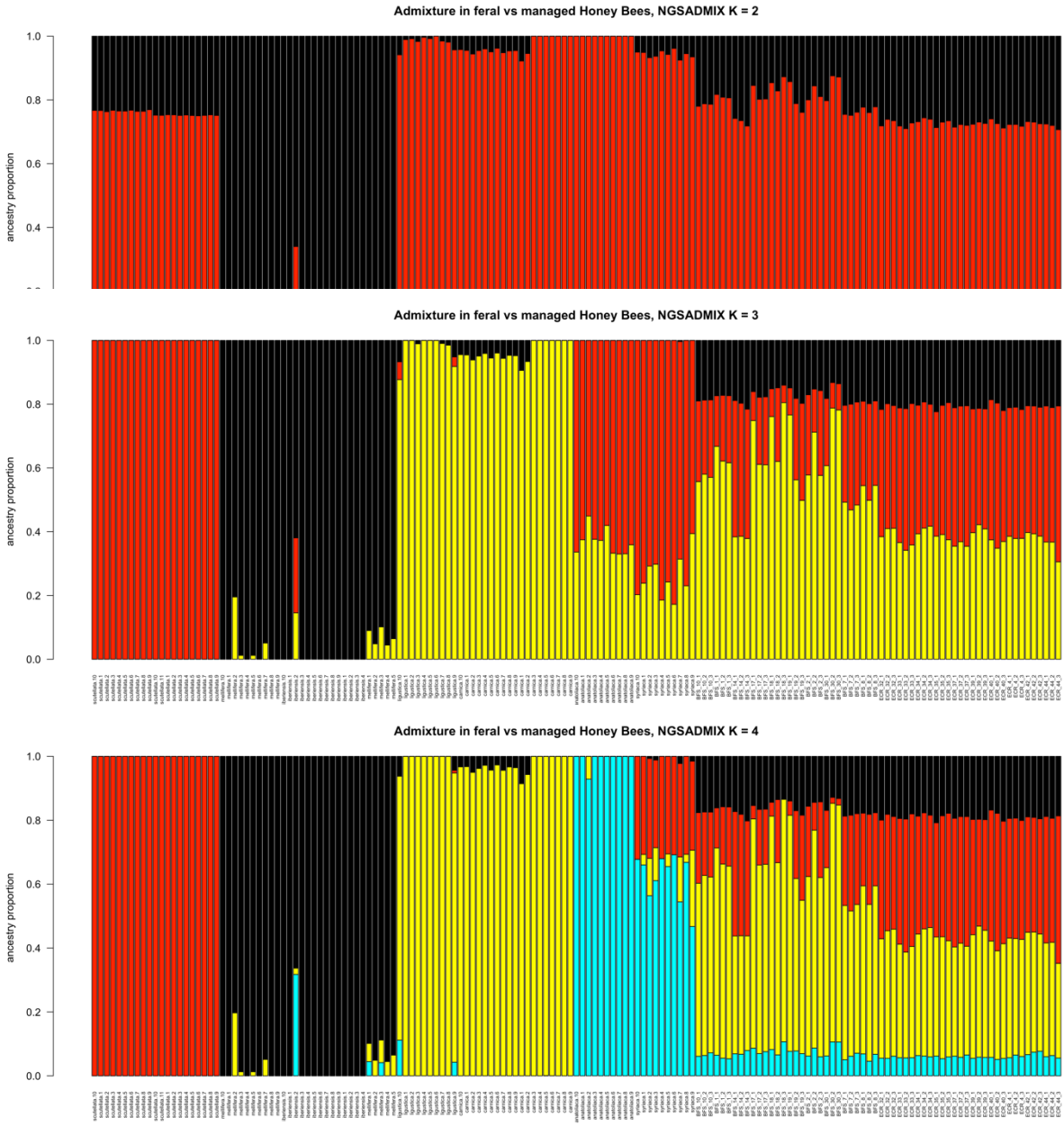

Figure 2.2: NGSadmix barplot of ancestry (K=2-6) between feral and managed honey bees. Each vertical bar represents one honey bee genome and colors represent the estimated proportion of ancestry derived from each assumed ancestral genetic cluster. Starting from the left of the figure and beginning with the African clade, we list reference genomes belonging to the four major evolutionary lineages of *Apis mellifera* (A, M, C, O). Following these, we list 60 admixed honey bee genomes, half collected from feral hives and half collected from managed hives present in San Diego, CA.

Table 2.1: Honey bee defensiveness measured between site per month using a multivariate analysis of variance (MANOVA) with site as a fixed effect. All ordinal variables were treated as continuous variables.

Month	Test	Value	Exact F	NumDF	DenDF	Prob>F P < 0.05
May	F Test	4.004	6.8645	7	12	**0.0020***
July	F Test	0.7763	1.3308	7	12	0.3164
August	F Test	1.0444	1.7905	7	12	0.1791
October	F Test	1.7550	3.0086	7	12	**0.0453***
November	F Test	3.2091	5.5014	7	12	**0.0051***

Table 2.2: Summary of nest defense measures assessed between managed European honey bee colonies (EHB) and feral, unmanaged AHB honey bee colonies (ECR) assessed between May and November 2021. Trials were repeated three times for each colony per month and the averages reported. Differences between continuous measures (Stings on flag and gloves) and ranked ordinal variables (Tendency for honey bees to fly off comb, run on comb, hit veil, pursuit at 25 and 50 m) both assessed using one-way ANOVAs.

Month	Measure	BFS Average ±SEM	ECR Average ±SEM	Effect Test for Site Prob > F	Direction of increased defensiveness
May	Stings on flag	1.8 ± 0.68	5.8 ± 1.36	**0.0028***	ECR
	Stings on gloves	1.8 ± 0.38	1.5 ± 0.67	0.3715	-
	Fly off comb	1.6 ± 0.10	1.6 ± 0.12	1.0000	-
	Run on comb	4.0 ± 0.21	2.7 ± 0.26	**0.0217***	BFS
	Hit Veil	2.0 ± 0.37	1.3 ± 0.39	0.3870	-
	Pursuit 25m	2.6 ± 0.15	1.5 ± 0.20	**0.0094***	BFS
	Pursuit 50m	2.3 ± 0.15	1.1 ± 0.20	**0.0088***	BFS
July	Stings on flag	9.0 ± 1.42	8.2 ± 1.22	0.5953	-
	Stings on gloves	4.8 ± 1.00	4.5 ± 0.78	0.5494	-
	Fly off comb	1.30 ± 0.09	1.4 ± 0.09	0.6601	-
	Run on comb	4.5 ± 0.18	3.8 ± 0.23	0.1360	-
	Hit Veil	4.0 ± 0.28	3.4 ± 0.33	0.3447	-
	Pursuit 25m	1.2 ± 0.13	1.8 ± 0.13	1.000	-
	Pursuit 50m	0.9 ± 0.10	1.4 ± 0.11	0.3553	-
August	Stings on flag	6.3 ± 1.14	13.2 ± 2.50	0.1607	-
	Stings on gloves	2.6 ± 0.60	12.0 ± 2.30	**0.0233***	ECR
	Fly off comb	3.2 ± 0.23	3.8 ± 0.27	0.3217	-
	Run on comb	1.5 ± 0.09	1.6 ± 0.09	0.6733	-
	Hit Veil	1.6 ± 0.31	3.1 ± 0.38	0.0858	-
	Pursuit 25m	1.6 ± 0.16	2.3 ± 0.16	0.0982	-
	Pursuit 50m	1.2 ± 0.11	2.1 ± 0.16	**0.0168***	ECR
October	Stings on flag	6.7 ± 1.50	16.6 ± 2.44	**0.0385***	ECR
	Stings on gloves	2.3 ± 0.58	14.7 ± 2.61	**0.0050***	ECR
	Fly off comb	2.7 ± 0.25	3.9 ± 0.24	0.0558	-
	Run on comb	1.6 ± 0.15	1.3 ± 0.09	0.2790	-
	Hit Veil	2.0 ± 0.37	3.5 ± 0.34	0.0698	-
	Pursuit 25m	1.1 ± 0.14	2.3 ± 0.14	**0.0013***	ECR
	Pursuit 50m	0.5 ± 0.13	2.6 ± 0.18	**0.0120***	ECR
November	Stings on flag	5.4 ± 0.97	14.7 ± 2.2	**0.0218***	ECR
	Stings on gloves	0.8 ± 0.20	4.9 ± 0.75	**0.0008***	ECR
	Fly off comb	2.7 ± 0.20	3.8 ± 0.23	**0.0303***	ECR
	Run on comb	1.9 ± 0.13	1.6 ± 0.09	0.2323	-
	Hit Veil	1.3 ± 0.29	3.6 ± 0.31	**0.0016***	ECR
	Pursuit 25m	1.4 ± 0.11	2.3 ± 0.14	**0.0310**	ECR
	Pursuit 50m	0.7 ± 0.12	1.4 ± 0.10	**0.0179***	ECR

Table 2.3: Repeated Measures Analysis of Variance for various measures of defensiveness for honey bee colonies between ECR and BFS across the time period of May through November. We report the effects of site, colony size, and the interaction of site and colony size. In order to account for treating ordinal data as continuous, we report the Hunyh-Feldt (Univar H-F) adjusted value. To account for multiple testing, we used the Bonferroni correction with k = 2, alpha = 0.025 and designate significant tests as BF.

Defense Metric	Effect	Value	Exact F	NumDF	DenDF	Prob > F **< 0.025**
Fly Off Comb	Site	1.4184	15.6032	1	11	**0.0023*BF**
	Time	0.9198	5.9377	3.6792	40.471	**<0.0001*BF**
	Time*Site	0.9198	5.9377	3.6792	40.471	**0.0010*BF**
Hit Veil	Site	1.3432	14.7759	1	11	**0.0027*BF**
	Time	0.7521	4.7656	2.1921	24.113	**0.0158*BF**
	Time*Site	0.7521	5.6074	3.0084	33.092	**0.0032*BF**
Run on Comb	Site	0.1564	1.7211	1	11	0.2163
	Time	1	85.635	4	44	**<0.0001*BF**
	Time*Site	1	2.5363	4	44	**0.0533**
Pursuit at 25 m	Site	1.1844	13.0292	1	11	**0.0041*BF**
	Time	0.9570	13.9273	3.8283	42.112	0.0660
	Time*Site	0.9570	13.9273	3.8283	42.112	**<0.001*BF**
Pursuit at 50 m	Site	0.7403	8.1433	1	11	**0.0157*BF**
	Time	0.8631	8.2936	3.4526	37.979	**0.0001*BF**
	Time*Site	0.8631	9.8762	3.4526	37.979	**0.0001*BF**
Stings on Flag	Site	2.2637	24.900	1	11	**0.0004*BF**
	Time	1	5.1648	4	44	**0.0017*BF**
	Time*Site	1	2.5074	4	44	0.0555
Stings on Gloves	Site	1.2739	14.0139	1	11	**0.0032*BF**
	Time	1	11.5202	4	44	**<0.0001*BF**
	Time*Site	1	9.4605	4	44	**<0.0001*BF**

Table 2.4: Analysis of variance (ANOVA) of honey bee colony size between ECR and BFS between May and November.

Month	Nparm	DF	Sum of Squares	F Ratio	Prob > F
May	1	1	78166626	4.8350	**0.0412***
July	-	-	-	-	-
August	1	1	21819605	2.5333	0.1289
October	1	1	15617690	1.4331	0.2498
November	1	1	7745145.8	0.8570	0.3668

Table 2.5: Multivariate analysis of variance (MANOVA) of various honey bee defensiveness measures with percent African ancestry as a fixed effect for each site.

Site	Value	Exact F	NumDF	DenDF	Prob > F
BFS	5.6589	1.6168	7	2	0.4342
ECR	5.0278	1.4365	7	2	0.4700

Table 2.6: Genomic composition of honey bee workers sampled from each colony by site.

	BFS	ECR
African (A)	0.192 ± 0.019	0.382 ± 0.004
Western European (M)	0.165 ± 0.004	0.190 ± 0.001
Eastern European (C)	0.572 ± 0.022	0.369 ± 0.004
Middle Eastern (O)	0.0718 ± 0.003	0.060 ± 0.001

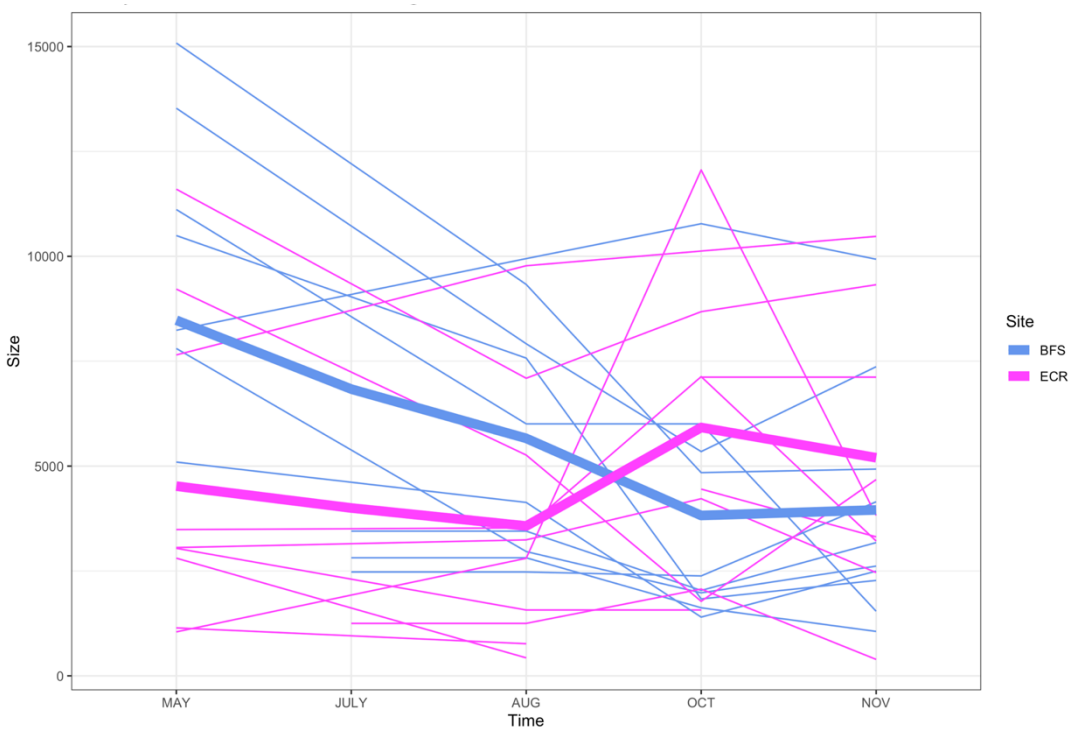

Figure S1: Average honey bee colony size from May through November. Averages represented by thick lines, individual colonies by thin lines. ECR colonies shown in magenta, BFS colonies in blue.

Chapter 3: Three Decades of Africanized honey bees (AHB) in California

ABSTRACT

Africanized honey bees (AHB) have been part of California's agricultural and natural landscapes for nearly three decades. Prior to their arrival in 1994, leading honey bee experts expressed concern over the potentially disastrous impact of AHB on California agriculture and public safety. AHB were almost universally seen as unsuitable for agriculture and beekeeping due to undesirable behavioral traits inherited from the African subspecies *Apis mellifera scutellata* (e.g. fierce nest defense). Despite these dire predictions, the state's agricultural production has not been significantly impacted by AHB. However, some evidence suggests that the abundance of AHB in natural habitats can have negative consequences for native pollinators. Here, we review the status of AHB in California and assess their impact on agriculture, natural resources, as well as their potential as a genetic resource for improving managed honey bee health. Finally, we provide recommendations for updating the term "AHB" to reflect their biology more accurately and to avoid unnecessary connotations.

Admixture and the origins of AHB

Now a common feature of California ecosystems and commercial agriculture, honey bees are not native to the American continents having been first introduced in the early 1500s. European lineages such as the German, Italian, and Carniolan races were mainly used. These temperate-adapted subspecies did well in the northern latitudes, but in some cases fared poorly in the tropical regions of the Americas. To fortify managed honey bee populations, Brazilian geneticists interbred African and European honey bee (EHB) lineages. They hoped to create an improved hybrid

combining the tropical hardiness of the African honey bee with the honey production capabilities and gentle nature of the popular European subspecies (reviewed in Schneider et al., 2004). To this end, scientists imported 47 queens of *Apis mellifera scutellata* from South Africa and Tanzania to Sao Paulo, Brazil in 1956 for experimental breeding.

These African honey bee queens and their admixed "Africanized" offspring were inadvertently released from research apiaries and quickly established themselves in the surrounding regions where they interbred with pre-existing European lineages. AHB rapidly replaced pre-existing European honey bees with admixed bees in which most genes came from *A. mellifera scutellata*. From their Brazilian origin, AHB expanded their range at a rapid rate (160-500 km/year), extending south into parts of Argentina and north throughout the rest of South and Central America and Mexico (Schneider *et al.,* 2004).

Predictions about the impact of AHB on agriculture

As African hybrid honey bees spread north, their impending arrival into California caused great concern. Page (1992), writing in this journal two years before the arrival of AHB, declared that the "imminent arrival of Africanized honey bees in California … threatens the foundation of the honey bee pollination service industry and those agricultural commodities that depend on bees. Once feral Africanized honey bees arrive in California, it will be extremely difficult to maintain hives with pure European honey bees — and Africanized bees are not amenable to commercial methods of transportation." AHB appeared to pose a looming threat for California agriculture and public safety. Scientists and beekeepers alike feared that African genes would pervade the domesticated, largely European, commercial stock and "Africanize'' the managed honey bees, causing substantial economic impacts because AHB have heightened defensive behavior and

would not be amenable to large-scale husbandry and to truck transport of hives for agricultural pollination. Page (1992) warned that, with AHB present in California, apiarists from states outside the range of the AHB could be reluctant to send their hives to California for fear of genetic mixing with AHB. Page (1992) also expressed some concern for public safety given AHB defensiveness and multiple reported AHB-caused fatalities in Central and South America.

Fortunately, nearly three decades after AHB entered California, the impact on agriculture has not been as disastrous as Page (1992) predicted. In what follows, we review current knowledge concerning AHB in California, and its impact on beekeeping and honey bee pollination services. We also assess topics Page (1992) did not consider, such as the potential impacts of feral AHB on California's diverse bee fauna as well as the possible benefits that California's AHB may provide for future honey bee breeding given their ability to thrive without human assistance in the face of diseases and other stressors that currently threaten commercial and hobbyist beekeeping in the state.

Range and Genetics of AHB in California

The first AHB in the U.S. were identified in Texas in 1990 and reached California in 1994. AHB are thought to require warmer winter temperatures than European honey bee races (Schneider *et al*., 2004), and their northern range limit is of considerable interest. All feral honey bees sampled in southern California now have approximately ~40% African (*Apis mellifera scutellata*) genomic content, with their remaining ancestry coming from several different European and Middle Eastern lineages (Cridland et al., 2017, Calfee *et al.,* 2020; Zarate *et al.,* in press). The frequency of feral bees with African ancestry, as well as their amount of African genomic content, declines with increasing latitude reaching its California limit in Napa and Sacramento counties (Calfee *et al.*

2020; Kono & Kohn, 2015; Lin *et al*., 2017). Rapid range expansion has ceased, although further, slow northern expansion may be expected under warming climate conditions. Interestingly, AHB in southern California have only about half as much African genomic content as those from Mexico and Central America or AHB from U.S. states such as Texas and Arizona (Calfee *et al*., 2020; Cridland *et al*., 2017; Pinto *et al*., 2005; Whitfield *et al*., 2006; Zarate *et al*., in press).

Effect on Apiculture, Agriculture and Public Health

California beekeepers anticipated that the arrival of AHB would impair honey production, as occurred in several South and Central American countries when AHB became the dominant managed honey bee (Guzman-Novoa *et al*., 2020). While California honey production slightly decreased the first year AHB were discovered in the state, a subsequent quick rebound of production suggests that other factors such as disease, weather, and reduced honey demand caused the downturn (Livanis & Moss, 2010). Additionally, in the years following AHB arrival, California beekeepers did not purchase more European colonies, suggesting that AHB had a negligible effect on the maintenance of managed colonies (Livanis & Moss, 2010). The presence of AHB also may not increase requeening costs. Beekeepers in areas with AHB regularly requeen their colonies (Schneider et al., 2004) to maintain their European ancestry, but this occurs even in areas without AHB because of declining honey bee queen longevity (Amiri *et al.*, 2017).

Pollination services provided by managed honey bees also appear to have been relatively unaffected by AHB. Annual yields of nuts, fruits, vegetables, and seeds that require commercial bee pollination have steadily increased from 1994 to the present, despite the presence of feral AHB in the southern central valley where many of these crops are grown (California Department of Food and Agriculture, 2020). Almonds, one of California's most profitable crops, require a

majority (>60%) of all U.S. commercial honey bee colonies to produce expected yields (Sáez *et al.,* 2020). In the last 25 years, the state's almond production has increased more than 8-fold, with perturbations in annual production primarily attributed to poor weather during the plant's short flowering period (USDA, 2021). The success of almond production given the presence of California AHB suggests that importation of commercial hives from states outside the current range of AHB has not been seriously affected.

The main impact of California AHB on apiculture has been on hive management in southern California, where AHB dominate the feral bee population. For both hobbyist and commercial beekeepers, jurisdictions in southern California enacted policies aimed at preventing the spread of genes from feral AHB into managed bee populations. In general, colonies are expected to be requeened frequently with queens that are produced and mated in regions outside the range of AHB (Schiff & Sheppard, 1996). How effective these measures have been in keeping the gene pools of managed and feral bees separated has received little study, though Kono and Kohn (2015) reported that mitochondrial DNA from the African lineage, found in most feral bees in San Diego County, was rare in hobbyist beekeeper hives. However, beekeepers in southern California often report their hives becoming increasingly defensive as time passes from the last requeening. Presumably this is due the death of the original queen and the mating of the next queen to drones from feral AHB colonies or, less commonly, from nest usurpation by feral AHB swarms (Schneider *et al*., 2004).

With respect to public safety, following the arrival of AHB there have been a considerable number of reported honey bee attacks on humans, pets, or livestock. A small number of these resulted in fatalities (Kunthara & Vara, 2015), and thus these press reports have served to keep the

term “killer bees” in the popular lexicon even if attacks by bees are relatively rare—though no doubt serious to those affected. Nevertheless, this threat is minor in comparison to other daily risks.

Potential impacts on native pollinators

While agricultural production and commercial apiculture have been largely unaffected by the arrival of AHB, both European and Africanized honey bees are non-native and their prevalence in California’s habitats may have negative consequences for native species. Much of the state is in the California Floristic Province, a biodiversity hotspot that extends from central Oregon to northern Baja California, Mexico. California is home to about 6,500 species of vascular plants and over 1,600 species of bees, many of which are endemic. Multiple pollinators are in decline due to a variety of threats including pollution, habitat destruction, climate change, and, potentially, resource competition from exotic species, particularly honey bees.

Today, AHB dominate the feral bee population in southern California (Kono & Kohn, 2015; Lin et al., 2017; Zarate *et al.,* in press). Unlike their primarily arboreal nesting European relatives, AHB colonies often nest in cavities found in rocks or in the ground, as well as in anthropogenic structures (e.g. irrigation boxes, attics, cinder block walls, etc.). Their nesting habits may be among the traits facilitating their current high level of abundance. In San Diego County, feral AHB are the dominant floral visitor to native vegetation, accounting for 75% of all flower visitors even though there are >600 species of native bees in the county. This degree of dominance of the pollinator community by honey bees is among the highest reported anywhere in the world (Hung *et al.* 2018). In addition, feral AHB are even more dominant, accounting for >90% of all visitors, on the most abundantly blooming plant species in wildlands (Hung *et. al*., 2019). Thus,

the great majority of pollen and nectar resources gathered by insect pollinators in this region likely go to honey bees.

It is difficult to directly assess the effect that resource competition with honey bees may have on native bee populations. During one summer season, a strong managed EHB hive in wildlands can collect 10 kg of pollen, enough to feed 110,000 progeny of an average native solitary bee species (Cane & Tepedino, 2016). Impressively, AHB remove even more pollen from the environment than their EHB counterparts, because they allocate more foragers to collect pollen rather than nectar (Fewell & Bertram, 2002).

While there is debate as to whether native pollinator populations are limited by floral resources, evidence suggests that, when honey bees are present at high densities, they compete with other insects for pollen and nectar (Goulson *et al*., 2015; Potts *et al.,* 2010). Research conducted across a variety of environments has shown that where honey bees are present in wildlands, wild bee diversity and abundance decreases (Mallinger *et al*., 2017; Torné-Noguera *et al*., 2016; Valido *et al*., 2019). Wild bees and other pollinators are often displaced from their preferred floral resources when honey bees are present, reshuffling their diets to presumably lower quality resources and potentially decreasing the number or fitness of their offspring (Portman *et al., 2018;* Magrach *et al*., 2017; Roubik & Villanueva-Gutierrez, 2009). Large, social pollinators such as bumble bees may be particularly susceptible to competition with honey bees because of significant niche overlap and their higher energy requirements compared to smaller, solitary bees (Thomson, 2006). This is of concern in California where native bumble bees are important pollinators of both agricultural and native plants. In a California study, placing honey bee colonies near bumble bee nests resulted in bumble bees collecting less pollen and producing smaller and fewer offspring, indicating significant resource competition (Thomson, 2004).

In addition to resource competition with native bees, honey bees may serve as disease reservoirs, spreading pathogens among managed and feral populations as well as to or from native bees, mediated by the flowers they all visit (Alger *et al*., 2019; Burnham *et al*., 2021; Graystock *et al*., 2015). While generally of good health, feral honey bees harbor several viral diseases, such as deformed wing virus, that infect multiple pollinator species (Alger *et al*., 2019; Geffre *et al*., 2021; Graystock *et al*., 2015). The degree to which native bees and both feral and managed honey bees transmit pathogens among each other, and the effects of these pathogens on native bee species, deserves further study.

AHB have potential to improve managed honey bee health

Currently, managing the health of European honey bee colonies is a major challenge for beekeepers and adds to the time and expense of maintaining colonies (Brodschneider *et al.,* 2018). Because pathogens vectored by mites are a serious threat to honey bees, beekeepers often use a variety of anti-mite treatments. However, feral honey bees, such as the AHB of southern California achieve high densities without such human intervention, even though they carry several viral diseases at levels similar to those found among managed honey bees (Geffre *et al*. 2021). Several traits of AHB may account for their ability to thrive in the face of exposure to diseases that currently plague the honey bee industry.

Due to their hybrid origin, AHB harbor higher levels of genetic diversity than the European honey bee strains currently used by beekeepers (Harpur *et al*., 2012; Themudo *et al*., 2020; Wallberg *et al*., 2014). Genetic diversity in any population allows more evolutionary flexibility in response to environmental challenges but has been decreasing in managed honey bees (Themudo

et al., 2020). Thus, the input of genetic variation from feral populations could be beneficial, particularly for combating diseases.

In comparison with European honey bees, AHB can exhibit higher levels of hygienic behavior (Aumeier *et al.,* 2000; Guzman-Novoa *et al.,* 1999), including successful grooming to remove *Varroa* mites (Invernizzi *et al.,* 2015), which vector multiple viruses. AHB also exhibit other behaviors that, while perhaps not beneficial to commercial beekeeping, may reduce the impact of diseases and parasitism. Such traits include higher swarming rates, smaller colony sizes, and enhanced defensive behavior (Carr *et al*., 2020; Herb *et al*., 2018; Loftus *et al.*, 2016; Schneider *et al*., 2004). The higher swarming rates are particularly intriguing because swarming induces a broodless period that decreases the population of brood parasites such as *Varroa* mites. In fact, broodlessness induced by colony cold storage is being studied as way to control *Varroa* (Kulhanek, 2017).

Feral honey bees generally may harbor useful genetic variation because they have been subject to natural selection. Like AHB, non-AHB feral honey bees elsewhere in the U.S. are also more robust to environmental and disease stressors than their managed counterparts (Locke, 2016; Loftus *et al*., 2016; Seeley *et al*., 2015; Seeley, 2017). For example, feral bees of European descent have adapted to resist the negative effects of *Varroa* mites and now thrive unaided in areas where commercial beekeepers use a variety of preventative measures but still suffer considerable hive mortality from *Varroa* (Seeley *et al.,* 2015). Further research on the traits associated with robust health in feral honey bee populations, including AHB, may elucidate how these insects mitigate the impact of pests and pathogens. Such knowledge can inform honey bee breeding programs, possibly allowing for the development of new varieties that combine the genetic diversity and

health associated with AHB with desirable behavioral qualities associated with European varieties (e.g. gentle nature, low absconding rates, and higher honey production).

However, AHB defensive behaviors raise concerns about breeding them with European varieties. So far there have been no quantitative studies comparing the defensive behaviors of California AHB and European honey bees. The relatively low African genomic content of California AHB in comparison with other AHB populations could correspond to reduced defensive behavior. As an example, non-defensive "Africanized" bees are known to occur in Puerto Rico (Acevedo-Gonzalez *et al*., 2018). The discovery of AHB with desirable traits and low defensiveness in southern California could strengthen the argument for breeding some AHB with European varieties.

Final note: Are Africanized honey bees due for a name change?

While the term "Africanized" honey bee has become commonplace, this epithet is perhaps due for revision. Africanized as a descriptor is frustratingly broad and fails to accurately reflect the diversity of geographic lineages that an admixed honey bee of the American continents can encompass. In addition, there exist more than a dozen African honey bee subspecies exhibiting a diverse range of behavioral and life history traits distinct from those of the subspecies *A. m. scutellata* from which the AHB originated. In stark contrast to the elevated defensiveness of *A. m. scutellata* and the AHB, some African subspecies are known for their gentle characters (e.g. *Apis mellifera monticola*, the Ethiopian highlands honey bee) and so the term Africanized can lead to problematic generalizations regarding the larger African honey bee clade (Avalos *et al*., 2017; Ruttner, 1988). In fact, it can be argued that the use of the term Africanized reflects a larger Western cultural consciousness that perceives the African continent as a monolithic entity and

associates negative characteristics (e.g. aggression, violence, otherness) with African identity (Schwalbe *et al.,* 2000; Welch, 2007). Thus, the term "Africanized" is offensive to many people and we should move away from the use of the term "Africanized" because it resonates with racist human tropes. Considering this, a few researchers have begun to move away from the Africanized label to one of greater phylogenetic specificity: "*scutellata*-hybrid" (Calfee *et al*., 2020). This discussion raises valuable questions about the use of language in scientific discourse and how our language should evolve to reflect growth in scientific and cultural spheres. As we continue to learn more about these hybrids, a new name could counter the stereotypes evoked by the term "Africanized" and increase acceptance for using the beneficial traits of these bees to strengthen California beekeeping and agriculture.

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