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1) A 7-year research project:

Exploring the life history traits of wild, unmanaged honey bee (*Apis mellifera*) colonies at the Galbreath Wildlands Preserve in Mendocino County, CA

2) Priority area foci:

Our priority area foci are the population dynamics, the level of health, and survival rates of wild Apis m. in the remote landscape of the Galbreath Wildlands Preserve, in Mendocino County, CA

3) Overview

Today, arguably the most harmful parasite of honey bee (*Apis mellifera*) colonies is the ectoparasitic mite *Varroa destructor*, which is found worldwide. *Varroa* primarily feeds on the fat body of developing and adult honey bees, whereby they can vector several honey bee-associated viruses (Ramsey et al. 2019). There is consensus among researchers and beekeepers that colonies not treated against the mite will usually die within a few years due to both direct damage and associated virus infections (Guzman-Novoa et al. 2010, Kulhanek et al. 2018, Brodschneider et al. 2018,). After the collapse of vast portions of wild, unmanaged honey bee populations in the late 20th century, some studies indicate that they may have stabilized within the last two decades (Seeley, 2017). Wild honey bee colonies seem to be abundant in large temperate woodlands in North America and the Southern Ural (Seeley, 2007; Ilyasov et al., 2015).

Wild honey bees exhibit different life history traits than managed colonies. For example, the density of naturally nesting colonies in remote woodlands is low and colonies nest individually in widely separated tree cavities (Visscher & Seeley, 1982; Seeley et al., 2015; Seeley, 2017). This life-history strategy of wild colonies makes them less vulnerable to parasites than beekeepers' colonies. Additionally, feral colonies stay smaller, rear less brood, and swarm frequently, all of which reduce the reproductive potential of *Varroa* mites. Both empirical and

theoretical studies suggest that these factors alone—the spacing of colonies, and their life history can reduce the infection levels of *V. destructor* considerably (Loftus, Smith & Seeley, 2016; DeGrandi-Hoffman, Ahumada & Graham, 2017; Seeley, 2017).

Furthermore, it is predicted that under certain conditions, populations of honey bee colonies not treated against parasites will evolve resistance mechanisms against these pests through natural selection (Neumann & Blacquière, 2017). A population of honey bees living in the deciduous forests of New York State was found to be stable over decades (Seeley, 2007; Mikheyev et al., 2015; Seeley et al., 2015; Seeley, 2017). This population has been shown to exhibit genomic signatures of selection after the arrival of *Varroa* (Mikheyev et al., 2015).

Indeed, obtaining precise information about the presence of wild honey bee colonies is important and should be considered when estimating overall wild pollinator abundances or when assessing the role of forest areas in providing pollination services to the surrounding landscape (Tscharntke et al., 2005; Jaffé et al., 2010; Mitchell, Bennett & Gonzalez, 2014; Decocq et al., 2016). The occurrence of wild, unmanaged honey bee colonies in woodlands also has special implications for the species itself, including its legal protection status and its perception among bee researchers, forest ecologists, and conservationists. In addition, wild, unmanaged honey bee colonies can be a rich source for studying the natural interaction of honey bees with the forest environment (Seeley, 1985) and they can represent an important reservoir of genetic diversity (Oleksa, Gawroński & Tofilski, 2013).

The primary goal of our project is to collect data about the life expectancy and genetic signature of wild, unmanaged honey bees living in remote landscapes of California in the absence of managed beekeeping of any kind. We will achieve this goal through two main objectives:

Objective 1: To determine the genetic diversity of wild honey bees in remote landscapes of Northern California.

Objective 2: To examine whether wild honey bees have developed a natural tolerance to *Varroa* mites in remote wildlands by monitoring the colonies' monthly survivorship status for up to 5 years.

Objective 3: To create baselines and evaluation of diverse pollinator communities to better understand the role of wild Apis m. as a possible benevolent co-constituent.

This study will provide us with the unique opportunity to contribute to a global field of research of wild honey bees that are persisting alongside *V. destructor*, and will increase our understanding of how unmanaged colonies can thrive in wild settings without any human intervention.

Methods

This is a 7-year-long research project at the Galbreath Wildlands Preserve (GWP). Sonoma State University is a (non-financial) supporter of this project and also the owner of GWP. 2023 will be the third year of our research project. In many regards, the preserve is an ideal location, as it is a large area (>3,600 acres), remote, surrounded by privately owned wilderness, and sheltered from most beekeeping activities. Each stage of this project has different funding requirements. Overhead and logistics are funded through the operational fund of Apis Arborea.

The first stage of the project has focused mainly on beelining, a time-intensive method and technique to locate wild honey bee nesting sites. As of January of 2023, we have identified 22 trees with wild *A. mellifera* and 21 additional areas that each contain a bee tree (not yet located bee trees), established monitoring protocols and data collection.

In 2023, Apis Arborea is collaborating with Dr. Juliana Rangel (see letter of collaboration) such that, upon collection of bee samples from tree cavities harboring active colonies, we will haplotype one worker per colony to determine its maternal genetic lineage, as done previously (Rangel et al. 2016). For cases in which colonies can collect a minimum of ten workers via netting at the nest entrance, we will also perform Nosema spp. spore counts, as done previously (Rangel et al. 2013). This sampling will allow us to explore the genetic makeup of wild stock in relation to *Varroa* tolerance, and establish baseline data for monitoring and evaluation of population dynamics. We will continue with beelining to locate nests of wild colonies in 2023, and will follow extensive monthly monitoring protocols to collect data about the health and survival rates of the colonies.

Apis Arborea is a nonprofit organization of citizen scientists and conservationists in Sonoma County, CA. The wild, unmanaged Apis m. research project dovetails with Apis Arborea's mission to promote innovative ways of conservation, engage community/citizen scientists and academia in the process, develop educational and conservation programs, and apply our research to sustainable beekeeping practices and breeding programs.

The project is designed to collaborate with a diverse field of researchers. Currently, we are collaborating with Juliana Rangel, Ph. D., Associate Professor of Apiculture in the Department of Entomology at Texas A&M University (TAMU) in College Station, TX. Apis Arborea is a member of COLOSS (https://coloss.org/tag/honey-bee/) and a research partner with Honey Bee Watch (https://www.honeybeewatch.com/team)

Project Location:

APIS ARBOREA

Wild / Naturalized Apis mellifera Research Project
2021-2028

Galbreath Wildlands Preserve
(Owned by Sonoma State University)
3,670 Acres

Near Yorkville,
Mendocino County, CA

b) Current data about nest density, 2023:

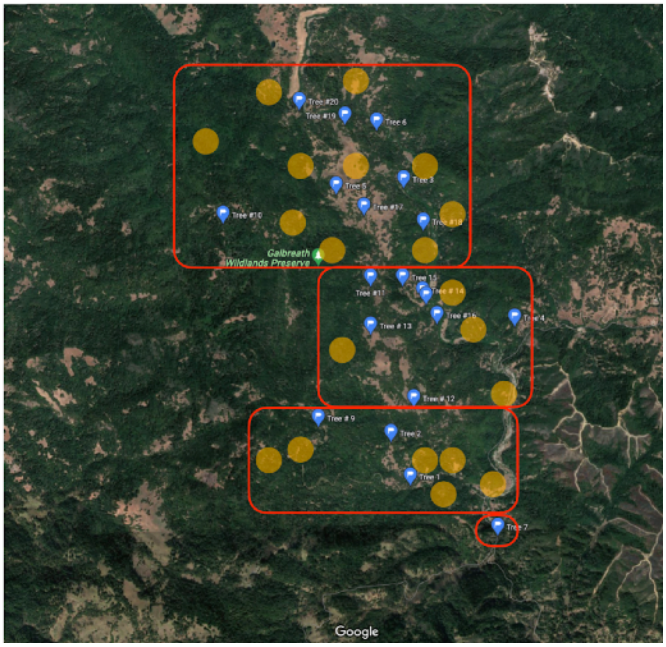
	Nests per mile ^{2}	Nests per kilometer ^{2}
42 BeeTrees: (22 located & 20 areas with BeeTrees)	9.03	3.5

As of 01/2023

= 4.65 miles ^{2} / 12 kilometers ^{2}

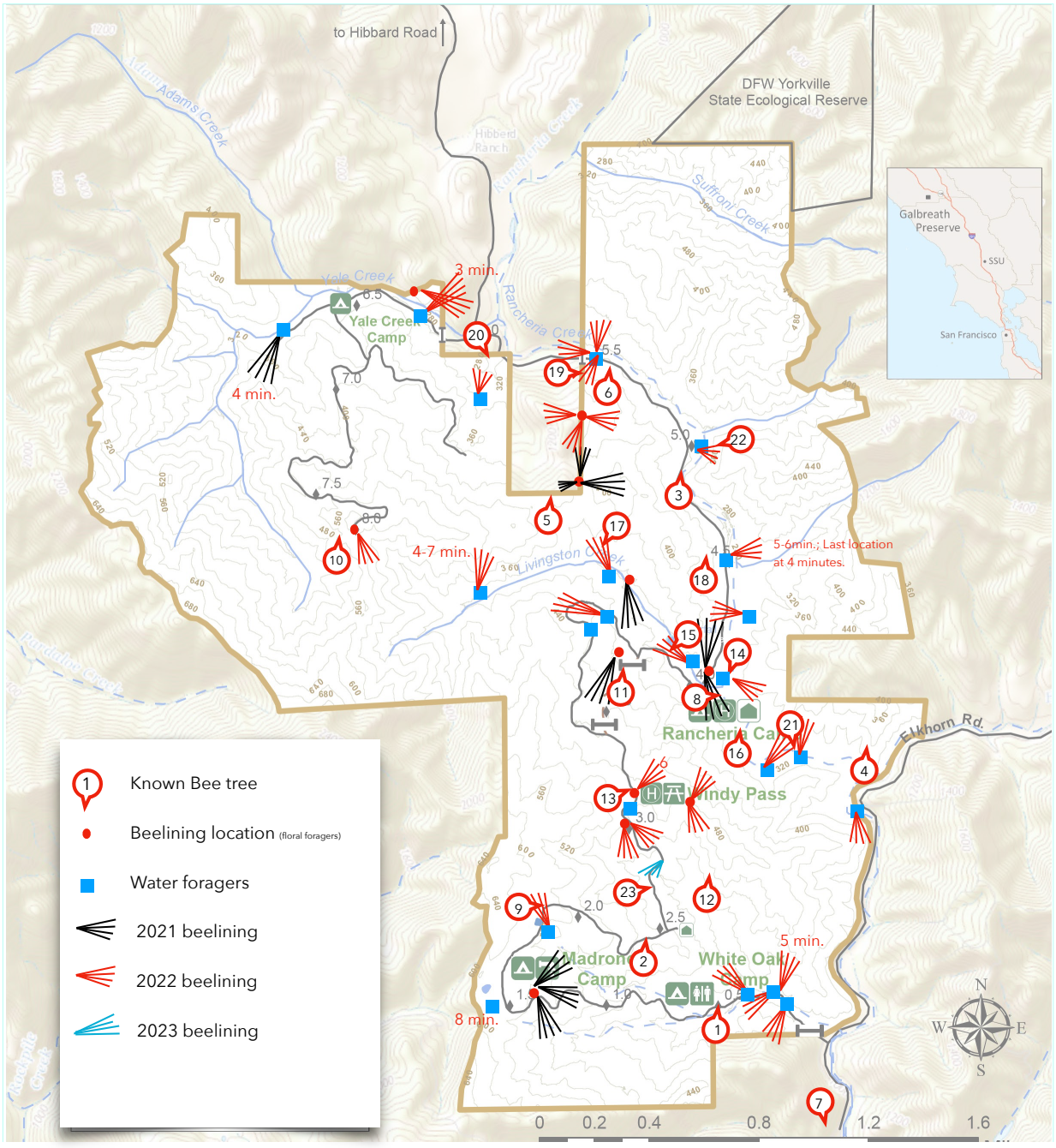
= identified BeeTree

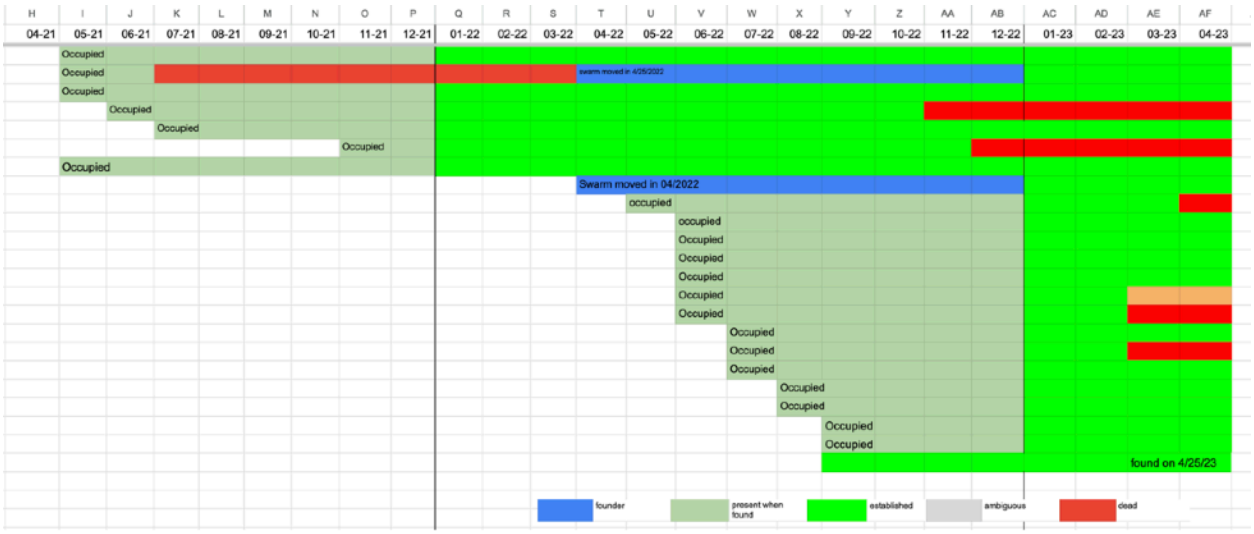
= area of an unidentified BeeTree, (indicated by beelining)



b) Details from Beelining; 04/2021-04/2023:

d) Nest monitoring from 04/2021 to 01/2023:

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04-21 05-21 06-21 07-21 08-21 09-21 10-21 11-21 12-21 01-22 02-22 03-22 04-22 05-22 06-22 07-22 08-22 09-22 10-22 11-22 12-22 01-23 02-23 03-23 04-23
Occupied
Occupied
Occupied
Occupied
Occupied
Occupied
Occupied
swarm moved in 4/25/2022
Swarm moved in 04/2022
occupied
occupied
Occupied
Occupied
Occupied
Occupied
Occupied
Occupied
Occupied
Occupied
Occupied
Occupied
Occupied
found on 4/25/23
founder
present when found
established
ambiguous
dead