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Thermotolerance of Feral and Managed Honey Bees (Apis mellifera) in Southern California.

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Thermotolerance of Feral and Managed Honey Bees (*Apis mellifera*) in Southern California.

A thesis submitted in partial satisfaction of the requirements for the degree Master of Science

in

Biology

by

Anngely Leeds

Committee in charge:

Professor James Nieh, Chair
Professor David Holway, Co-Chair
Professor Sara Jackrel

2022

The thesis of Anngely Leeds is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

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This thesis, in full, is currently being prepared for submission for publication. Anngely Leeds was the primary researcher and author of this paper.

ABSTRACT OF THE THESIS

Thermotolerance of Feral and Managed Honey Bees (*Apis mellifera*) in Southern California.

by

Anngely Leeds

Master of Science in Biology

University of California San Diego, 2022

Professor James Nieh, Chair
Professor David Holway, Co-Chair

Honey bees (*Apis mellifera*) provide pollination services for many crops globally, but extreme weather and changing temperatures due to climate change may threaten the health of this species. Although we know that honey bee survival can decrease due to thermal stress, we know less about how thermal stress affects feral honey bees given that they are potentially adapted to higher temperatures in certain climates. Our research investigated the effect of extreme heat and cold on feral and managed honey bees in San Diego County, California. In this

region, managed honey bees are pure European *Apis mellifera,* while the majority of feral honey bees are genetically admixed with *Apis mellifera scutellata*, from Africa, which are thought to be limited in their range by cold and to be more tolerant of heat. We measured individual survival between feral and managed honey bees after exposure to incubator heat shock or cold shock, critical thermal maxima and minima, and chill coma recovery time. Feral bees had higher survival compared to managed bees following heat shock or cold shock, but they did not differ with respect to CT_{max}, CT_{min}, or chill coma recovery time, suggesting that the effects of temperature upon survival require some time to manifest. Thus, the range of *scutellata* hybrids may not be constrained by individual survival following cold exposure but by colony-level effects or broader environmental effects linked with colder climates. These hybrids are, however, evidently better adapted to warmer conditions compared to pure European honey bees.

Introduction

Western honey bees (*Apis mellifera*) provide pollination services worldwide. More than half of the world's most important crop production relies on animal pollination (Klein *et al.*, 2007). Due to the importance of honey bees as pollinators, current research aims to understand factors that influence the health and survival of the species. The climate crisis may threaten honey bees, since extreme hot and cold temperatures can decrease honey bee survival (Koo *et al.*, 2015; Wang *et al.*, 2016). Furthermore, understanding how extreme temperatures affect honey bee survival is important for anticipating adverse effects of climate change on honey bees.

In Southern California, the majority of feral honey bees are genetic admixtures of *Apis mellifera* and the subspecies *Apis mellifera scutellata*, while the majority of managed colonies are pure *A. mellifera* (European honey bees, EHB, Kono and Kohn, 2015). Evidently, *scutellata* hybrids can tolerate higher temperatures compared to EHB due to their neotropical origins (De Jong, 1996). Such tolerance can be measured in multiple ways. Critical thermal maximum (CT_{max}) is the highest temperature an organism can withstand before losing muscle control, and critical thermal minimum (CT_{min}) is the lowest temperature an organism can withstand before losing muscle control (Sánchez-Echeverría *et al.*, 2019). For example, feral honey bees that are putative *scutellata* hybrids in Arizona have higher CT_{max} than do managed EHB (Atmowidjojo et al., 1997). Since the majority of feral bees in Southern California exhibit *scutellata* hybrid ancestry, feral honey bees in San Diego may tolerate heat stress better than managed honey bees.

Although *scutellata* hybrids tolerate heat better than EHB, there are inconsistent findings about cold tolerance. Cold climates are thought to limit the expansion of *scutellata* hybrids in North America. The northward expansion of *Apis mellifera scutellata* in the United States was estimated to be limited to areas with similar climatic conditions to their range in South America

(Taylor & Spivak, 1984). However, studies have found *scutellata* hybrids present in areas, to a limited degree, beyond this estimated range (Kono & Kohn, 2015; Lin *et al*., 2018). Some studies have shown that *scutellata* hybrids have decreased overwintering ability compared to EHB and greater mortality under cold conditions than EHB (Villa et al., 1991; Villa & Rinderer, 1993). However, other studies found that *scutellata* hybrids do not differ from EHB in their overwintering ability (Krell et al., 1985; Dietz & Vergara, 1995). Thus, additional research on the cold tolerance of *scutellata* hybrids is needed to determine their ability to tolerate cold temperatures similarly to EHB.

We also know relatively little about the thermotolerances of feral honey bees in Southern California, a region in which honey bees, mainly *scutellata* hybrids, are remarkably abundant (Kono & Kohn, 2015). I therefore investigated the thermotolerance of feral honey bees and managed honey bees through the following experiments: survival trials after exposure to heat shock or cold shock, measuring CT_{max} and CT_{min}, and chill coma recovery time. These experiments provide insights into the thermal ecology of FHB and MHB that will help us understand how each group might survive future extreme temperatures due to climate change.

Materials and Methods

Study site and colonies

Frames with capped brood nearing emergence and foragers were collected from the managed apiary at the Biology Field Station (BFS, University of California, San Diego) and the feral apiary at Elliot Chaparral Reserve (ECR, Kearny Mesa, CA). Across all survival trials, brood frames from 19 different colonies were used (seven feral colonies, 12 managed colonies). A recent genomic analysis of these colonies revealed that all of these feral colonies were admixed with *A. mellifera scutellata* (Zarate *et al.*, 2022). Foragers from nine different colonies kept at the managed apiary were used (three feral colonies, and six managed colonies). Feral and managed colonies generally do not coexist well within a common apiary, particularly in the fall and winter, because feral colonies tend to aggressively rob and thereby kill or cause the absconding of managed colonies. All of our survival experiments were therefore conducted with colonies at these two different sites. However, to measure CT_{max} and CT_{min}, and chill coma recovery time, we used three feral colonies brought from the ECR to BFS and kept them there for over 21 days (the time it takes for the queen to lay eggs and new adults to emerge) before making these measurements.

Maximum and minimum temperatures at field sites

Environmental temperatures at the feral apiary (Kearny Mesa, CA) were recorded with a HOBO data logger (model MX2201) to determine field realistic experimental temperatures. At the feral apiary, the minimum temperature recorded was 3 °C and the maximum temperature recorded was 50.2 °C. For the managed apiary (La Jolla, CA), we used public weather data (timeanddate.com) available for La Jolla, CA to determine field realistic temperatures. In La Jolla, CA, the minimum temperature recorded was 6.1 °C and the maximum temperature recorded was 33.9 °C. For heat shock trials, we chose 45 °C after a series of preliminary trials that proved 40 °C was not hot enough to decrease the survival of either feral or managed honey bees. For cold shock trials, we chose 7 °C as it was within the range of environmental temperatures at both apiaries, and preliminary trials proved it was cold enough to decrease survival of honey bees.

Caging bees for survival trials

Brood frames were left in nuc boxes in a warm room at 30 °C, 50% humidity for 24 h. Newly emerged workers (25 per cage) were then transferred to ventilated plastic cages (11.5 cm x 11.5 cm x 9.0 cm). Bees in each cage were provided with 30% sucrose solution via a 3 ml plastic syringe with its tip cut off to prevent the formation of an air bubble that could restrict bee access to the sucrose solution. Depending upon the trial (see **Table 1**) cages of heat treatments could also receive a separate syringe of tap water.

Effects of heat shock or cold shock on survival

Three to five cages were used per trial for each treatment: feral heat shock, managed heat shock, feral cold shock, managed cold shock, feral control, and managed control. The effects of heat and cold shock were tested for different temperatures and times of exposure. Heat shock treatment bees were placed in a VWR BOD refrigerated incubator (model LRI201WWW/0) at 40 °C (0.5 h, 1 h, 2 h, or 4 h) and 45 °C (1 h or 2 h). Cold shock treatment bees were placed in the same incubator at 7 °C (4 h or 24 h), 10 °C (4 h), and 12 °C (4 h). The incubators were kept at 50% humidity for the duration of the heat and cold shocks by placing plastic bins of water inside each incubator and adjusting the water surface area while measuring humidity. Temperatures and humidity were measured with a Hobo temp/RH datalogger (UX100-003) and temperature was calibrated with a T-Pro RTD PT100 platinum temperature sensor and a SYL-2352 Auber PID temperature controller. After exposure to heat and cold shock, bees were kept at 30 °C and 50% relative humidity (RH) for 14 days. The control bees were kept at 30 °C and 50% RH for the entire trial. The number of dead bees per cage was counted and recorded every other day. The sucrose and water syringes were weighed daily.

Capturing foragers

We captured foragers from managed colonies and feral colonies both kept at our managed apiary. We used a bee brush to sweep foragers at hive entrances into ventilated plastic cages. We immediately transferred the caged foragers to a heated room (30 °C, 50% RH) and provided 30% sucrose solution in 3 mL syringes (1 syringe per cage). We transferred each forager to a 20 mL snap cap vial with holes poked in the cap for ventilation. Each vial had half a cotton ball saturated with 1 mL of DI water to prevent desiccation.

CT_{max}, CT_{min} , and Chill Coma Recovery Time

We used a static approach to determine critical thermal maxima and minima in which foragers in vials were held at a constant temperature, and we recorded the time at which muscle control was lost and the bee could not return to an upright position (Lutterschmidt & Hutchison, 1997; Sánchez-Echeverría et al., 2019). We also measured the chill coma recovery time, defined as the time until a honey bee's first movement after being moved from a critically low-temperature exposure (7 °C for 1 h) to a warmer temperature (30 °C, Free & Spencer-Booth, 1960). We set the incubator to 50 °C to test critical thermal maxima, and, in separate trials, to 7 °C to test critical thermal minima (Atmowidjojo *et al*., 1997; Free & Spencer-Booth, 1960). Inside the incubator, we recorded the bees for 1 h per trial with two video cameras (5MP 4MP Dome Super Hybrid Security Camera, Vonnision) recording onto an 4K UltraHD 4 channel DVR system (Amcrest AMDV8M4-1TB). We watched the video recordings to determine the time at which each bee lost muscle control.

Statistics

We used JMP v12 software to conduct all statistical analyses. We used a Proportional Hazards fit model for the survival analyses. We looked at the effect of treatment (heat shock, cold shock, or no thermal stress). We also looked at the effect of origin (feral or managed). Additionally, we looked at the interaction effect between treatment and origin. We used Mixed Models (REML algorithm) to analyze CT_{max}, CT_{min}, and chill coma recovery time (all continuous variables). In these models, colony was a random effect and origin was a fixed effect.

Results

Testing the effect of heat shock or cold shock on survival

The proportion of surviving feral bees was greater than that of managed bees following heat shock exposure (**Fig. 1a, Table 2**). In heat shock trials, there was a significant effect of origin (Wald χ^2=15.44, 1 df, P<0.0001) and treatment (Wald χ^2=59.66, 1 df, P<0.0001). There was also a significant effect of origin x treatment (Wald χ^2=8.96, 1 df, P=0.0028) because feral heat-shocked bees had higher survival than managed heat-shocked bees. In these heat shock trials, heat-shocked feral bees survived better than heat-shocked managed heat bees (Risk ratio=0.74, P<0.0001), while there was no difference in survival between the feral bees and managed bees that were not heat shocked (Risk ratio=0.96, P=0.52).

Similarly, the proportion of surviving cold-shocked feral bees was greater than that of cold-shocked managed bees relative to the baseline survival seen in control groups (**Fig. 1b, Table 3**). In cold shock trials, there was a significant effect of origin (Wald χ2=12.58, 1 df, P=0.0004) and treatment (Wald χ^2=53.02, 1 df, P<0.0001). There was also a significant effect of origin x treatment (Wald χ^2=3.96, 1 df, P=0.047) because feral cold-shocked bees had higher survival than managed cold-shocked bees, when considering the baseline higher survival in the control managed bees. For the control treatment bees, feral bees survived worse than managed bees (Risk ratio=1.29, *P*=0.0001). Despite the greater baseline survival of control managed bees, there was no difference in survival between cold-shocked feral bees and cold-shocked managed bees (Risk ratio=1.08, *P*=0.26).

CT_{max}, CT_{min}, and Chill Coma Recovery Time

There was no difference in CT_{max} (R^2_{adj}=0.09, $F_{1,1}$=1.69, *P*=0.37, **Fig. 2a**) or CT_{min} (R^2_{adj}=0.13, $F_{1,5}$=0.01, *P*=0.93, colony accounted for 15% of model variance, **Fig. 2b**) between feral honey bees and managed honey bees. There was no difference in the time it took for feral honey bees and managed honey bees to recover from a chill coma (R^2_{adj}=0.27, $F_{1,4}$=0.04, *P*=0.85, **Fig. 2c**).

Discussion

We found that feral bees survived heat shock and cold shock better than managed bees, although there were no differences in CT_{max}, CT_{min}, or chill coma recovery time between feral and managed bees. The ecological relevance of our findings lies in the fact that honey bees in Southern California, especially those that are inland, can be exposed to similarly harsh temperatures while swarming, in which approximately half of the colony leaves to find a new nest site, exposing the outer layer of the swarm cluster to environmental temperatures (Nagy & Stallone, 1976). Feral honey bees for survival trials were sourced from the feral apiary (ECR) which is 13.8 km east of the managed apiary (BFS) and subject to more extreme temperatures than the managed apiary near the coast (see **Table 5**). A common garden design would be ideal to control for variation in study site temperatures, but was not initially used because of issues with managed bees surviving at the ECR apiary. Our conclusions, based upon the survival data, are therefore limited and we cannot disentangle the effects of rearing climate (i.e., acclimation) from bee type (feral vs. managed). However, to collect the CT_{max} and CT_{min}, and chill coma recovery time data, we used colonies placed at the same site (BFS) and found that it is possible to run such an experiment for a short period of time. We collected preliminary survival data from feral and managed colonies at the BFS, during this period, and found similar survival results.

Our results indicated that FHB survived better than MHB after exposure to heat shock or cold shock, although there were inconsistencies in control survival. Prior research showed that FHB can survive longer than MHB in laboratory survival trials (Ward *et. al*., 2022). For our heat shock trials, there was no difference in the survival of control FHB and MHB. For our cold shock trials, the control MHB survived better than the control FHB, and the reason for which is unclear. The interaction of origin x treatment was significant, suggesting that FHB survived cold shock better than MHB despite the higher survival of control MHB compared to control FHB. Regarding the inconsistencies in control survival, we suggest that the season at which the survival trials occurred had an effect on whether FHB or MHB survived better overall, as FHB survived better in winter and spring (see **Table 4**).

Previous research showed that Yemini honey bees, a honey bee race that tolerates the hot and dry climate of Saudi Arabia, had higher survival during a constant exposure to 40 °C compared to Carniolan honey bees, from more temperate climates (Abou-Shaara *et al*., 2011). Our research aligns with these results considering that feral honey bees in San Diego are admixed with *A. mellifera scutellata,* a tropical honey bee adapted to high temperatures. Together, these findings suggest that different honey bee races differ in their temperature tolerances and that *scutellata* hybrids retain the heat tolerance abilities of *A. mellifera scutellata*. Prior studies have shown that *scutellata* hybrid bees and EHB exhibit similar survival rates overall under experimental cold temperature conditions, including whole colonies exposed to 0 °C for 73 days, and worker bees exposed to 0 °C for 1, 2, or 3 h (Villa & Rinderer, 1993; Abramson *et al*., 1997). Surprisingly, we found that FHB with *scutellata* ancestry were able to survive cold temperatures better than EHB. The increased cold tolerance of FHB may be due to local acclimation, or the level of EHB hybridization in the FHB we used for the study, an average of 54% European ancestry (Zarate *et al.* 2022).

A potential thermotolerance mechanism that can be compared between FHB and MHB is the expression of heat shock proteins (HSPs) in response to thermal stress. HSPs are widely conserved proteins that are expressed under normal conditions, but HSP expression increases under stressful conditions such as extreme heat and cold exposure (Kregel 2002). HSPs act as molecular chaperones that prevent the denaturation of other proteins during cellular stress (Feder & Hoffman, 1999). In honey bees, the expression of HSPs (*hsp70*, *grp78*, and *hsp90*) increases after heat shock (1 h at 45 °C), and expression of certain HSPs (*grp78*) increases after cold shock (1 h at 4 °C, Koo *et al*., 2015). Foragers of *A. mellifera jemenitica*, a honey bee subspecies native to the Arabian Peninsula, which is adapted to high temperatures, shows less HSP expression following heat shock for 4 h (40 °C and 45 °C) in comparison to foragers of *A. mellifera ligustica* or *A. mellifera carnica*, EHB acclimated to temperate regions (Alqarni *et al*., 2019). In two leaf miner insect species (*Liriomyza huidobrensis* and *Liriomyza sativae*), HSP expression in response to cold temperatures is induced at lower temperatures in species from colder climates (Huang & Kang, 2007; King & MacRae, 2014). Increased expression of HSPs in response to thermal stress indicates that an organism is not adapted to tolerate that temperature without recruiting molecular chaperones to mitigate protein damage. Considering our FHB tolerated thermal stress better than MHB, we might expect less HSP expression in FHB compared to MHB following exposure to heat shock or cold shock.

We found that our feral honey bees and managed honey bees showed no significant difference in static CT_{max}. Prior research showed that FHB in Arizona had a higher dynamic CT_{max} (50.7 ± 1.9 °C) compared to managed honey bees (42.8 ± 2.8 °C), in which FHB could withstand a constant ramping rate up to a higher temperature (Atmowidjojo *et al*., 1997). The findings of Atmowidjojo *et al*. suggest that FHB can withstand a gradual increase up to a higher temperature compared to managed honey bees, but our results suggest there is no difference in the time that FHB and MHB can withstand a static, critically high temperature. It is possible that the static approach induced a relatively high heat injury accumulation rate at which the rate of injury imposed by a high temperature was much faster than the rate at which repairs can occur (Winther Bak *et al*., 2020).

We found no difference in the static CT_{min} or chill coma recovery time of feral honey bees and managed honey bees. This contradicts the assumption that FHB with *scutellata* ancestry are less cold tolerant than EHB from temperate regions. FHB in San Diego are genetically diverse, as they have an average genetic composition of 37. 5% ± 1.12% s*cutellata*, 19.1% ± 0.41% Western European, 34.8% ± 1.14% Eastern European, and 8.51% ± 0.24% Middle Eastern (Zárate, *et al*., 2022). Due to the higher percentage of EHB ancestry than *scutellata* ancestry, FHB in San Diego may have similar cold tolerance abilities to MHB, which are largely pure EHB. Additionally, San Diego is within the climatically favorable range for both *A. m. scutellata* and EHB (Taylor & Spivak, 1984). Prior studies suggest that hybrid zones form in areas that are climatically favorable to both *A. m. scutellata* and EHB, as seen in the established hybrid zone in temperate areas of Argentina in which cold temperatures limit the southern expansion of *A. m. scutellata* and *scutellata* hybrids (Villa & Rinderer, 1993; Sheppard *et al*., 1991). The favorable climatic conditions of San Diego may form a hybrid zone in which *scutellata* hybrids and EHB exhibit similar levels of cold tolerance, while the hybrid zone may be limited further north where cold tolerance may begin to differ.

Interestingly, differences in thermotolerance were only seen when FHB were sourced from the inland apiary which was subject to more extreme temperatures than the coastal apiary where MHB were sourced. Next year, we plan to collect additional data from common garden survival trials. The survival data presented in this thesis combined with data from common garden survival trials can be informative. If feral bees are better able to tolerate cold and heat shock at both the BFS and ECR, it is likely that they are genetically better adapted to these temperature extremes. However, if feral bees do not have better survival than managed bees following heat or cold shock when feral bees are moved to the BFS, we will have evidence that the differences are largely due to habitat temperatures. In addition to common garden survival trials, we will also investigate potential molecular mechanisms for cold and heat shock tolerance by quantifying heat shock protein expression after heat shock or cold shock (Koo *et al*., 2015). Understanding the thermotolerance of feral and managed honey bees is critical to understanding how climate change may affect honey bees overall, and furthering this knowledge can help guide future decisions on the management of these essential pollinators.

This thesis, in full, is currently being prepared for submission for publication. Anngely Leeds was the primary researcher and author of this paper.

Figures

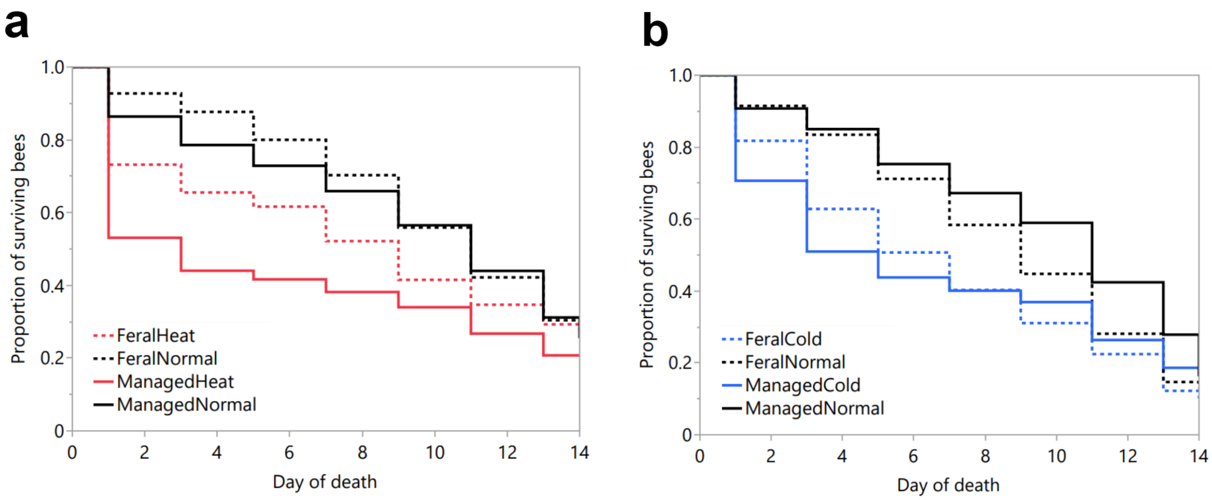

Figure 1. (a) Feral bees survived heat shock better than managed bees**.** Feral honey bees (n=1,348) from six colonies and managed honey bees (n=1,318) from 11 colonies were put in cages (25 bees per cage) and were either exposed to a 2 h heat shock at 45 °C on Day 0 (Feral Heat, Managed Heat) or kept at normal hive conditions (30 °C, 50% relative humidity) for the duration of the trial (Feral Normal, Managed Normal). After heat shock, the heat treatment cages were kept at hive conditions (30 °C and 50% RH) for the rest of the trial. Feral Heat survived better than Managed Heat (P<0.0001). There was no difference in survival between Feral Normal and Managed Normal (P=0.23). **(b)** Feral bees survived cold shock better than managed bees. Feral honey bees (n=1,132) from six colonies and managed honey bees (n=1,142) from eight colonies were put in cages (25 bees per cage) and were either exposed to a 24 h cold shock at 7 °C on Day 0 (Feral Cold, Managed Cold) or kept at normal hive conditions (30 °C, 50% relative humidity) for the duration of the trial (Feral Normal, Managed Normal). After cold shock, the cold treatment cages were kept at hive conditions (30 °C and 50% RH) for the rest of the trial. Feral Cold survived better than Managed Cold (P=0.04). Managed Normal survived better than Feral Normal (P<0.0001).

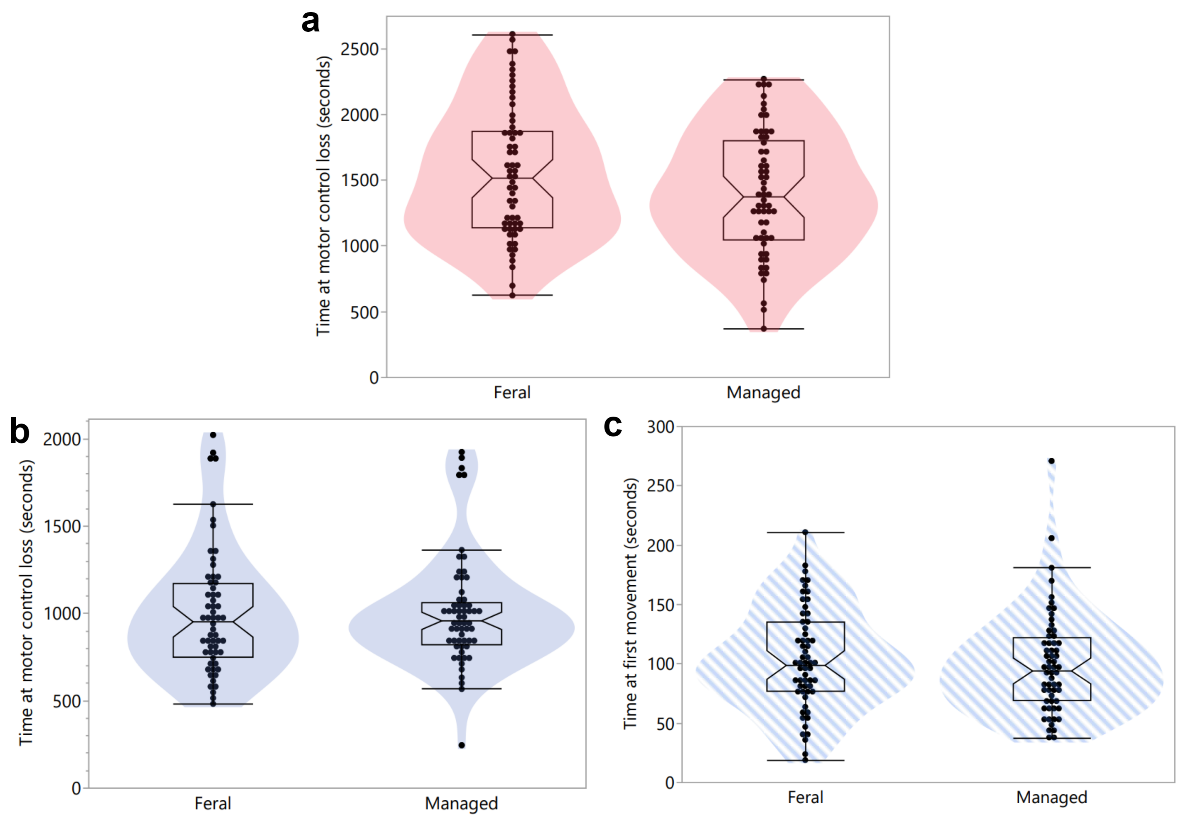

Figure 2. (a) Feral foragers (n=60) from three colonies and managed foragers (n=59) from five colonies were placed in vials and held in an incubator set to 50 °C until motor control was lost, at which the time was recorded in seconds. There was no significant difference between the time it took for feral honey bees and managed honey bees to knock down (*P*=0.37). **(b)** Feral foragers (n=59) from three colonies and managed foragers (n=60) from four colonies were placed in vials and held in an incubator at 7 °C until motor control was lost, at which the time was recorded in seconds. There was no difference in the time it took for feral bees and managed bees to knock down (*P*=0.93). **(c)** Feral foragers (n=59) from three colonies and managed foragers (n=60) from three colonies were placed in vials and held in a refrigerator set to 7 °C for 1 h to induce chill coma and then transferred to an incubator set to 30 °C. The chill coma recovery time was recorded in seconds as the time at which the first movement of each bee occurred. There was no difference in the chill coma recovery time between feral honey bees and managed honey bees (*P*=0.85).

Tables

Table 1. The conditions of the cold shock trials and heat shock trials are indicated.

No. of trials	Temperature (°C)	Duration (h)	Water syringes
5	7	24	No
7	45	2	Yes

Table 2. Risk ratios from heat shock trials indicate there was no difference in survival between control feral bees and control managed bees, while heat-shocked feral bees survived better than heat-shocked managed bees.

Level 1	Level 2	Risk Ratio	Prob>χ^2
Feral normal	Managed normal	0.96	0.52
Feral heat	Managed heat	0.74	<0.0001
Feral heat	Feral normal	1.23	0.0009
Managed heat	Managed normal	1.60	<0.0001

Table 3. Risk ratios from cold shock trials indicate that control managed bees survived better compared to control feral bees, but there was no difference in survival between cold-shocked managed bees and cold-shocked feral bees.

Level 1	Level 2	Risk Ratio	Prob>χ^2
Feral normal	Managed normal	1.29	0.0001
Feral cold	Managed cold	1.08	0.26
Feral cold	Feral normal	1.28	0.0001
Managed cold	Managed normal	1.54	<0.0001

Table 4. Risk ratios for control treatments by season indicate that feral bees survived better compared to managed bees during winter and spring, while there were no differences in survival during fall and summer.

Season	Level 1	Level 2	Risk Ratio	Prob>χ^2
Fall	Managed normal	Feral normal	3.21	0.21
Winter	Managed normal	Feral normal	17.5	<0.0001
Spring	Managed normal	Feral normal	2.52	0.002
Summer	Managed normal	Feral normal	0.64	0.2

Table 5. Absolute maximum and minimum temperatures recorded during November 2021 to September 2022 at each apiary field site location.

Field site	Location	Maximum (°C)	Minimum (°C)
ECR (Feral)	Kearny Mesa, CA	50.2	3
BFS (Managed)	La Jolla, CA	33.9	6.1

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