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Original article

Genetic Evaluation of Tunisian Honeybees for In-Hives and Broods' Temperature and Relative Humidity: Critical Traits for Tolerance to Climate Changes

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Abstract

Honeybee colonies are essential for global food security and genetic diversity due to their critical role in pollination. Within a colony, honeybees engage in activities like honey production and maintaining hive conditions, which are vital for the colony's health and productivity. This study aimed to deepen our understanding in three key areas: (1) The ability of honeybees in the southern Mediterranean to regulate hive and brood conditions, (2) Estimation of genetic parameters for hive Temperature and Relative Humidity, and (3) Prediction of Breeding Values (PBVs) for honeybee colonies. Data on Temperature and Relative Humidity were collected from sensors placed inside hives and at the brood level over a three-year period (2021-2023). The study analyzed 22,364 records of in-hive Temperature and Relative Humidity from 118 sensors, along with 1,664 brood-level humidity records from 14 sensors. PBVs were predicted using a BLUP Animal model. The results showed that monthly variations significantly influenced both Temperature and Relative Humidity within the hives. Heritability estimates were 0.21 for in-hive temperature, 0.33 for in-hive relative humidity, and 0.22 for brood relative humidity. A high genetic correlation (0.65) between Temperature and Relative Humidity within the hives suggests shared genetic and physiological mechanisms for these traits. The study also found that 77% of in-hive Temperature records and 48% of Relative Humidity records fell within optimal ranges, indicating that many Tunisian bees effectively regulate their hive environment. Based on PBVs, 24 colonies were selected for their superior adaptation to environmental conditions. This research underscores the importance of connected beehives and their impact on honeybee management and selection. Understanding genetic parameters and trait relationships aids in improving the long-term success and productivity of honeybee populations.

Keywords: Honeybees, Food security, Genetic, Temperature, Relative Humidity.

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INTRODUCTION

Numerous studies have demonstrated that temperature and relative humidity play crucial roles in regulating the activities of honeybee colonies. Both internal and external activities of worker bees are highly dependent on temperature. Specifically, for the development of brood, maintaining a suitable temperature range of 32 to 36 °C was reported to be essential (Stabentheiner et al., 2003; Petz et al., 2004). Deviations from this range can have adverse effects on various aspects, including the duration of immature honey bee development, emergence rate, coloration of emerged bees, adult brain function, learning ability, disease prevalence, and wing morphology (Tautz et al., 2003; DeGrandi-Hoffman et al., 1993; Groh et al., 2004; Ken et al., 2005). However, ambient temperature positively influences foraging activity (Blažytė-Čereškienė et al., 2010). High temperatures negatively impact bee foraging, while temperatures below 10 °C hinder flight activity (Joshi & Joshi, 2010).

Relative humidity also holds significant importance within the honey bee colony. The development of brood predominantly requires high humidity (Doull, 1976; Human et al., 2006), with a favorable range considered to be around 75% humidity inside beehives (Ellis et al., 2008). In the case of external activities, such as foraging, relative humidity does not have a direct impact on honey bees (Joshi & Joshi, 2010). However, when relative humidity levels drop inside the hives, honey bee workers employ various behaviors like nectar water evaporation and water collection to increase humidity (Human et al., 2006). Studies have indicated that caged bees exposed to high temperatures exhibit an increased uptake of water (Free & Spencer-Booth, 1960). Thus, the integration of relative humidity and temperature is crucial for honey bee activity monitoring.

It is important to note that the response to thermal stress and relative humidity levels may vary among different honey bee breeds and strains. The ability of honey bee breeds to thrive in particular regions is a result of their adapted responses to ecological stresses. The main purpose of the present study was to: 1) examine the ability of honeybees in the southern Mediterranean region to maintain optimal conditions within the hive and brood, 2) estimate genetic parameters for two important traits of honeybee colonies, namely Temperature and Relative Humidity, and 3) predict Breeding Values (PBVs) for honeybee colonies.

MATERIALS and METHODS

SmartBee device and generated data

The data used in the present study were collected using state-of-the-art monitoring and tracking technologies implemented in a cutting-edge device. Developed by a Tunisian company called "Beekeeper.Tech" and known as "Smartbee", this device incorporates advanced features including a GPS tracking subsystem, as well as temperature and relative humidity sensors. These sensors are strategically placed both inside the hive and at the brood level, allowing for comprehensive and accurate monitoring of temperature and humidity conditions throughout the beekeeping process.

These devices are based on the internet of things (IoT) which involves extending internet connectivity beyond standard devices, such as desktop, laptop and smart phones. Therefore, SmartBee devices can communicate and interact over the internet. Measured variables are sent to the cloud services of the company, every 6 hours, for storage and processing (https://en.smartbeekeeper.com/). If an abnormal measurement is detected for humidity or/and temperature, the data will be sent immediately to notify the beekeeper at an early stage to move fast and fix the problem with minimum effort.

Temperatures (Temp) within the beehives and Relative Humidity (RH) within the beehives and at the brood level were collected over 3-year periods (2021-2023). A total of 22’364 records of temperature and relative humidity within the hives, collected from 118 devices, were used. At the brood level, a total of 1’664 RH records generated by 14 devices were also used. The original data were edited using SAS software (SAS Inst. Inc., Cary, NC. 2002) and only records falling within the range of trait means ± 2 × standard deviation was kept. After the complete editing phase, 1.7%, 8.63% and 4.2% of the original data of temperature within hives, RH within hives and RH at the brood level, respectively, were discarded. After filtering the original data, means and standard deviations were computed for Temp and RH traits. Frequency of records falling within optimal ranges of temperature and humidity were reported.

Data analysis

Data were analyzed using linear model (1):

$$Y_{ijkl} = \mu + byd_i + m_j + bt_k + e_{ijkl} \quad (1)$$

where:

y_{ijkl}: The hives temperature, the hives RH and the brood RH;

μ: The population means;

byd_i: The effect of the beekeeper-year-day class which represents the beekeeper management on measured variables;

m_j: The effect of the month of the year;

t_k: Covariate time when the record is taken;

b: The linear regression coefficient;

e_{ijkl}: The random residual error.

Genetic evaluation

Heritability estimates, predicted breeding values (PBVs) and their accuracy (rTI: the correlation coefficient r between the true breeding value and the predicted index) were computed by MTDFREML software (Boldman et al., 1995). In matrix notation (Henderson, 1976), the single trait animal model (2) was used to estimate variance components and their standard errors and to predict Breeding Values (PBVs).

$$Y = Xb + Z_1 a + Z_2 pe + e \qquad (2)$$

where:

Y: The vector of observations of beehives temperature, beehives and brood relative humidity;

b: The vector of fixed and covariate effects (beekeeper-year-month class and day-time class);

a: The vector of colony random additive genetic values;

pe: The vector of random permanent environmental effects and non-additive genetic effects;

X, Z_1 and Z_2: known incidence matrices;

e: The error vector.

RESULTS and DISCUSSION

Means and variation of beehives temperature, beehives and brood relative humidity

The edited data showed that *Apis Mellifera Intermissa* bees, raised under southern Mediterranean conditions, are able to maintain an average (± s.d.) temperature of 18.75 ± 8.6 °C inside hives (Table 1). The humidity at the brood level was higher than that recorded inside hives. Average relative humidity was 70.66 % (± 26.46) and 77.10 % (± 15.03) inside hives and at the brood level, respectively. These results were the scale reported by Djemali et al. (2022).

Table 1. Means and variation of beehives temperature, beehives and brood relative humidity

Trait	Number of hives	Number of records	Mean	Standard deviation
Hive temperature	118	21 986	18.75	8.59
Hive relative humidity	118	22 302	70.66	26.46
Brood relative humidity	14	1593	77.10	15.03

Frequency of hives with an optimal environment

The optimal temperature range enabling bees to successfully feed and produce enough ventilation inside beehives should be between 20 and 25 °C in spring and summer, while in fall and winter it should be between 10 and 25 °C (Heidi De Los Reyes in management BootstrapBee.com; Joshi & Joshi 2010). Bees actively regulate the temperature within the hive to ensure the well being of the brood, the development of the colony, the proper storage and preservation of food. The recommended temperature range in the brood area is around 30°C to 36°C (Stabentheiner et al., 2003). When the in-hive temperature matches the desired brood temperature, the brood rearing process becomes more efficient. Worker bees, responsible for regulating the hive temperature, can focus their efforts on other tasks, such as foraging and caring for broods, rather than expending additional energy to adjust temperature within the hive (Hemeida et al., 2015; Jones & Oldroyd, 2006). Therefore, maintaining a suitable range of temperature from 10°C (or 20 °C) to 36 °C inside hives is crucial to ensure a normal growth and development of the brood and to avoid nest heating energetic cost. When ambient or in-hive temperature drops from 28 to 17 °C, the metabolic rate of a bee colony rises from 7 to 19 watt kg^{-1}. A comfortable temperature within hives will avoid colony losses and the interruption of production of brood cells especially during cold periods of the year (Borges & Blochtein, 2006). Bee workers began to cluster and generate their own heat by consuming carbohydrates (in the form of stored honey), as a result they starve and reduce honey production.

In addition to the effect of temperature on colony activities, relative humidity is an important microclimatic variable for bee worker longevity, brood health, development and egg hatching (Kühnholz & Seeley, 1997; Human et al., 2006; Ellis et al., 2008). The brood requires a slightly higher humidity level compared to the rest of the hive. The optimal brood humidity is typically around 50 % to 90-95% (Aupinel et al., 2005; Silva et al., 2009; Kaftanoglu et al., 2011). In fact, honeybee eggs require a relative humidity of above 50 % to hatch successfully (Abou-Shaara et al., 2017), with the highest survival between 90-95 % (Doull, 1976). Such high humidity allows decreasing the reproduction of Varroa parasitic mites (Kraus & Velthuis, 1997). However, it has been shown that the longevity of adult honeybee decrease with increasing humidity (Woodrow, 1935). Honey bee workers have been found survive better at relative humidity of 75 %, whereas at low relative humidity of 15 % to 50 % their survival was negatively impacted, especially at 15 % (Abou-Shaara et al., 2013). Hence, the overall humidity inside the beehive should ideally be maintained between 50 % and 75 %. This range helps prevent excessive moisture that can lead to mold or fungal growth, while also avoiding overly dry conditions that cause dehydration in the bees and their food stores. Honey bee workers would therefore need to regulate relative humidity to optimal levels in the brood nest using different behavior such as fanning behavior (Lensky, 1964) and carbon dioxide regulation (Seeley, 1974; Southwick and Moritz, 1987), nectar dehydration, water collection and spreading in the nest (Kühnholz & Seeley, 1997).

Beekeepers can also intervene to approach the temperature inside hives to the optimal one, especially during extreme weather conditions, by reducing the entrance size or by using an entrance reducer in case of high temperature and by adding an inner cover to the hive to help retain heat in case of low temperature. Therefore, it is essential to install specialized equipment such as hive thermometers, temperature and humidity sensors (SmartBee devices), and digital temperature control systems.

The capacity of Tunisian bees to maintain an appropriate environment inside hives, by month, was examined and presented in Figure 1. Results showed that the trend of optimal temperature frequency in beehives has been always higher than the one obtained for the optimal relative humidity. This can be explained by the presence of constraints on potential regulation mechanisms since optimal relative humidity could vary in the different areas of the nest. Relative humidity may also depend on numerous external factors, such as the availability of water, which significantly impair the regulation. Furthermore, there are trade-offs related to temperature regulation and respiratory gas exchange. These factors can prevent the establishment of optimal relative humidity levels. Human et al. (2006) argued that honeybee workers can only adjust humidity within sub-optimal limits.

Suitable in-hive temperature records represent 62.95 % and 90.37 % of the total in season 1 (Spring-Summer) and season 2 (Autumn-Winter), respectively. These results prove that Tunisian honeybees are able to effectively regulate hives' biophysical parameters since 76.66 % of the whole data belongs to the suitable range. In Tunisia, bees may be more capable of maintaining an optimal temperature inside hives during winter and autumn compared to summer and spring due to several factors. This can be explained by the fact that season 2 is characterized by a cooler external temperature unlike summer and spring. Honeybees in colder weather tend to exhibit better insulation practices within the hive (they may seal off cracks or gaps in the hive to minimize heat loss and maintain a more suitable internal temperature). During winter and autumn, honeybees reduce their foraging activity. Therefore, with fewer bees venturing outside the hive to collect nectar and pollen, the hive's internal temperature is better maintained as there are fewer openings for heat to escape.

However, optimal relative humidity records frequency is higher in season 1 (50.22 %) than the one obtained in season 2 (46.67 %). This could be explained by the increased foraging activity for nectar and pollen in spring and summer. Nectar has high water content, and bees collect and store it in hives. This source of water is used for evaporative cooling and aids in humidity regulation. Moreover, season 1 is a crucial time for brood rearing that requires a specific humidity range for proper development. Thus, honeybee workers actively regulate humidity levels around the brood area to maintain optimal conditions for the brood's growth and survival.

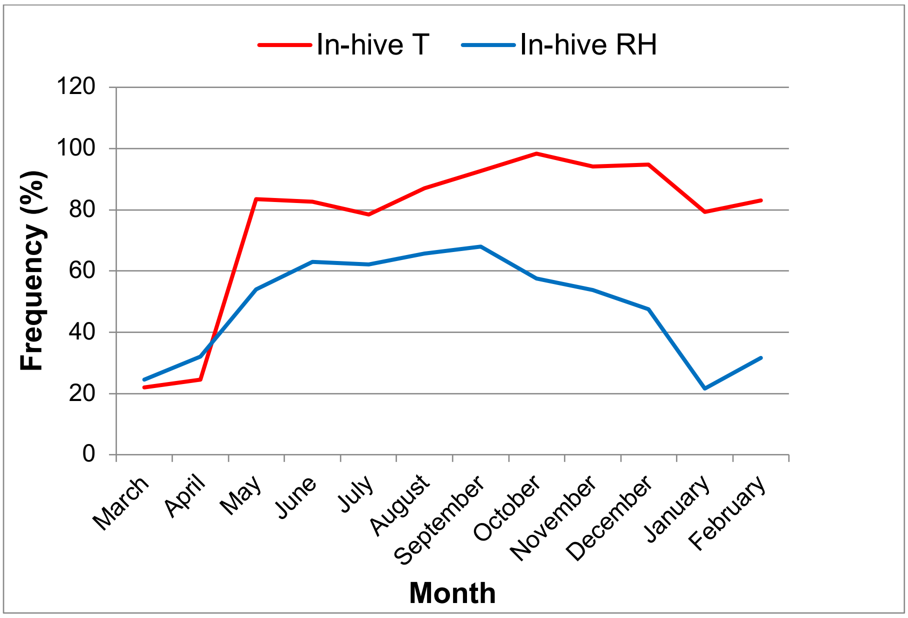

Figure 1. Trend of the frequency of optimal in-hive temperature (T) and relative humidity (RH) records.

Sources of variation

Preliminary analyses revealed that the effect of time when the record was taken was highly significant only for hive temperature and approached significance for hives and brood relative humidity trait (Table 2). Beekeeper-year-day contemporary group and the month were highly significant sources of variation ($P < 0.001$) for all the traits. These results indicate that each type of beekeeper appears to have specific management decisions.

Table 2. Sources of variation of temperature and relative humidity

Source of variation	Hive temperature	Hive relative humidity	Brood relative humidity
Beekeeper-year-day	**	**	**
Month	**	**	**
Time	**	0.086	0.012
Error df	20 411	20 727	1419
R2	0.62	0.40	0.61

Error df: The degrees of freedom associated with the error term or residuals; $\mathbf{R^2}$, The coefficient of determination.

Effect of the month

The effect of the month on temperature and relative humidity inside hives and at the brood level is shown in Figure 2. The Least Squares Solutions translated the effect of the month on both temperature and relative humidity (inside the hive and at the brood level). These results were of the scale reported by Djemali et al., (2022).From November to April, the recorded temperatures were relatively low. The coldest month was January with 14.28 °C ± 5.86. A difference of 3 °C approximately was found between January and April. Then, the temperature increases from April to attend its maximum in September

(30.28 °C ± 4.07) and starts decreasing again from September to December (17.24 °C ± 8.68). Between the coldest and the hottest month, there was a difference of 6 °C.

The highest value of relative humidity at the brood chamber (92.29 % ± 10.25) was recorded in April. Then, its values decrease until August (43.41 % ± 2.78) to be increased again continuously. The lowest relative humidity values at the brood level were obtained in July and August (43.26 % and 43.41 %, respectively). The high temperature characterizing this period may explain such humidity decrease. The relative humidity was always higher at the brood level where there is little nectar available as a water source and a tendency to evaporation due to the high temperature maintained, but where there is also high need moisture for optimal brood rearing and development (Doull, 1976). This suggests that humidity inside hives is regulated through bee workers that maintain: 1) High brood humidity by carefully managing evaporation and moisture distribution and 2) Lower humidity in the rest of the hive areas, such as the honey storage cells and the entrance, to dehydrate nectar to honey and prevent microbial growth (Human et al., 2006).

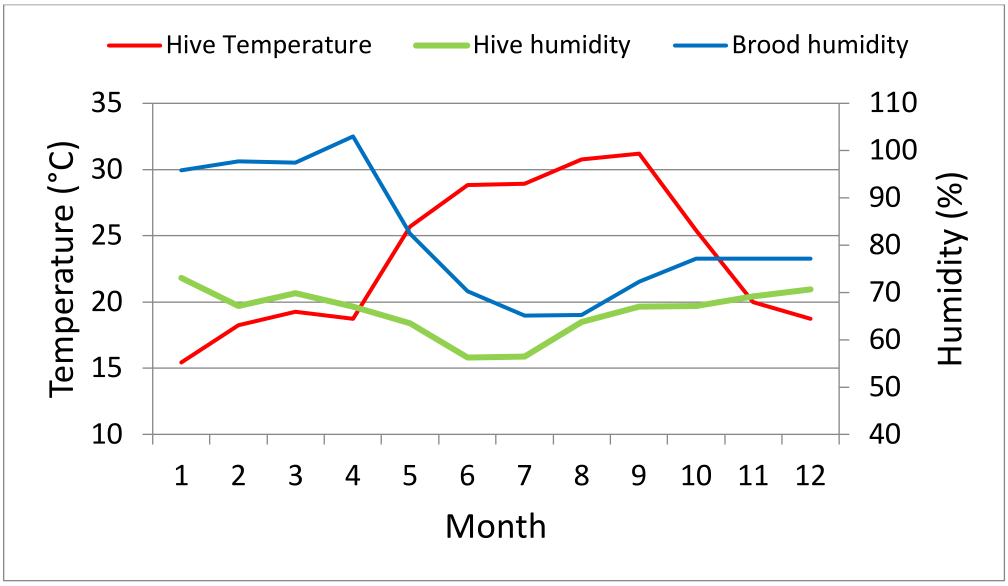

Figure 2. The effect of month on temperature and relative humidity inside beehives and at the brood level.

Heritability estimates

Heritability estimates, permanent environmental and residual effects of in-hives temperature, in-hives and brood relative humidity were reported in Table 3. Medium to high values of heritability obtained for the three studied variables reveals that these variables are heritable and, therefore, they could be improved through genetic selection methods. The lowest heritability was found for the temperature inside hives. In fact, even though this trait can be considered as a fascinating honey bee behavior since they are engaged in thermoregulation by funning their wings to cool the hive or clustering together to generate heat (Jarimi et al., 2020). The in-hives temperature can also be extremely influenced by external factors that are beyond the control of bee workers. The ambient temperature and the availability of heat sources or cooling mechanisms in the surrounding environment can all affect the ability of honey bees to regulate the temperature inside the hive. Higher heritability estimates were obtained for humidity, inside hives and at the brood level. Indeed, it has been reported that honey bees have the ability to regulate the humidity in its hive regardless of weather conditions throughout the year (Eouzan et al., 2019). In a research study conducted by (Sudarsan et al., 2012), honeybee workers maintain different humidity levels in the different parts of the hive mainly via an air ventilation mechanism. This is not only important to expel the heat build-up in the hive to avoid overheating but also to reduce the CO2 concentration and water vapor and replace them continuously with a fresh supply of oxygen. This temperature and relative humidity regulation technique is very similar to a heat recovery and mechanical ventilation unit in a building.

Table 3. Estimates of heritability, residual and animal effects of in-hives temperature, in-hives and brood humidity traits.

Trait	Heritability	Residual effect	Animal effect
In-hive temperature	0.21	0.51	0.28
In-hive humidity	0.33	0.34	0.33
Brood humidity	0.22	0.41	0.37

Genetic and phenotypic correlations

Genetic (upper diagonal) and phenotypic (lower diagonal) correlations of temperature and relative humidity inside honeybee hives and at the brood level are shown in Table 4. Observed genetic correlations ranged from 0.006 to 0.65, with the highest genetic correlation obtained between the temperature and the relative humidity inside hives, and the lowest one between the in-hive relative humidity and the relative humidity measured at the brood level. The positive and low genetic correlation between the two measured humidity indicates that these traits are genetically associated but the strength of this association is low. The high genetic correlation between the temperature and the relative humidity inside beehives suggests that the same or closely linked genes are involved in determining both traits. However, very small but positive phenotypic correlations were obtained between In-hive temperature and relative humidity (inside beehives and at the brood level). The in-hive relative humidity and brood relative humidity were negatively correlated (-0.2). The very small and negative correlation indicates that there is little to no systematic relationship between the two traits at the phenotypic level. Therefore, the variations in one trait do not consistently predict or explain the variations in the other trait.

Table 4. Genetic (above diagonal) and phenotypic (below diagonal) correlations (with standard deviation in parentheses) for in-hive temperature, in-hive and brood humidity.

	In-hive Temperature	In-hive humidity	Brood humidity
In-hive Temperature	1	**0.65 (< .0001)**	0.13 (0.69)
In-hive humidity	0.1 (< .0001)	1	0.006 (0.98)
Brood humidity	0.07 (0.74)	-0.2 (0.3)	1

Prediction of breeding values

Predicted breeding values (PBVs) were determined for the three studied biophysical traits. The computed PBVs were added to trait averages, the sums falling within the suitable range of each trait (10 °C ≤ In-hive T ≤ 36 °C; 50 % ≤ In-hive H ≤ 75 %; and 50 % ≤ Brood H ≤ 95 %) were identified. Colonies found in common between the three identified groups were selected as a result of this work (Table 5). Just three colonies were found for brood humidity variable because of the lack of data for this trait.

Table 5. Selected hives for the studied traits.

Device (hive code)	**PBV + average of In-hive Temperature (°C)**	**PBV + average of In-hive Humidity (%)**	**PBV + average of Brood Humidity (%)**
sb00211	19.347	70.963	70.064
sb00217	19.192	68.245	77.322
sb00220	19.241	65.681	80.63
sb00219	19.501	55.101	-
sb00081	18.194	57.471	-
sb00202	19.661	64.497	-
sb00204	19.694	65.451	-
sb00034	18.892	66.802	-
sb00205	18.118	67.429	-
sb00224	17.791	69.838	-
sb00107	19.281	70.427	-
sb00222	18.813	71.019	-
sb00031	17.065	71.329	-
sb10025	19.252	73.794	-
sb00085	18.242	73.869	-
sb10021	18.531	73.921	-
sb00183	18.767	74.467	-
sb00094	19.993	74.794	-
sb10072	18.383	75.397	-
sb10018	19.278	75.501	-
sb00309	18.492	75.646	-
sb00145	18.783	75.920	-
sb10019	19.262	75.922	-
sb00175	18.827	75.992	-

Conclusion

In conclusion, this study has demonstrated the significant impact of temperature and relative humidity on both adult and immature honeybees. The findings indicated that Tunisian colonies have the ability to maintain optimal biophysical conditions within their hives and brood under South Mediterranean climate conditions. Moreover, the study has revealed promising heritability estimates and positive genetic correlations, suggesting the potential for genetic selection to improve the three traits investigated.

The implementation of a recording system, such as the one developed by Beekeeper. Tech company, holds great potential not only for the genetic enhancement of bees but also for providing valuable guidance to beekeepers regarding hive management, nutrition, honey quality, and overall health. By utilizing this tool effectively, beekeepers can make informed decisions to optimize their practices and outcomes.

While this study involved the selection of 24 colonies based on predicted breeding values, it is important to acknowledge the possibility of achieving more accurate genetic evaluations. This can be achieved by increasing the amount of standardized and consistent phenotypic data through the integration of measuring devices in beehives. Additionally, improving pedigree information and implementing reliable breeding strategies for queens are crucial steps in enhancing the precision and relevance of genetic evaluations. To accomplish these requirements, a collaborative effort involving beekeepers, scientists, and industry stakeholders is essential. By working together, valuable insights can be gathered, ensuring that the most accurate and up-to-date information is incorporated into the genetic evaluation process. This collaborative approach will contribute to the continuous improvement of honeybees, benefiting both the beekeeping industry and the broader ecosystem.

The slightly higher optimal relative humidity records frequency in season (1), compared to the one obtained in season (2), could be explained by the increased foraging activity for nectar and pollen in spring and summer. Nectar has high water content, and bees collect and store it in hives. This source of water is used for evaporative cooling and aids in humidity regulation. Moreover, season (1) is a crucial time for brood rearing that requires a specific humidity range for proper development. Thus, honeybee workers actively regulate humidity levels around the brood area to maintain optimal conditions for the brood's growth and survival.

Disclosure statement: The authors report there are no competing interests to declare.

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