

Evaluation of Hygienic Behaviour in Honey Bees *Apis mellifera intermissa* Through Pin Testing: Implications for Sustainable Beekeeping and Environmental Resilience

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Abstract

Honey bee health is increasingly challenged by parasites and environmental stressors. Hygienic behavior where bees detect and remove unhealthy brood is a key adaptive trait enhancing colony resilience. This study evaluated the hygienic behavior of *Apis mellifera intermissa* colonies in northern Algeria using the pin-killed brood (PKB) test across different seasons. Out of 40 colonies tested, 35% demonstrated a high hygienic response (>95% brood removal in 24 hours), while 40% showed moderate responses (50–95%). Colonies with strong hygienic behavior were also less affected by Varroa destructor and visible brood diseases. Seasonal variation was observed, with better performance during spring and early summer. These findings highlight significant intra-population variability in hygienic behavior within *A. m. intermissa*, suggesting potential for selective breeding to enhance disease resistance and colony survival under local environmental conditions.

Key words: Hygienic behaviour; *Apis mellifera intermissa*; pin test; disease resistance; colony health; selective breeding.

Introduction

Honey bees (*Apis mellifera*) are essential pollinators and key contributors to food security and ecosystem stability. However, their health is increasingly threatened by Varroa destructor, viruses, and climate-related stressors. Hygienic behavior—where worker bees detect and remove diseased or dead brood—is a natural defense that reduces parasite loads and limits disease spread. In Algeria, the local subspecies *Apis mellifera intermissa* plays a vital role in agriculture, yet its hygienic behavior is poorly documented. This study aims to evaluate the hygienic behavior of *A. m. intermissa* colonies using the pin-killed brood (PKB) test, in order to identify resilient colonies and support future selection programs.

Results & Discussion

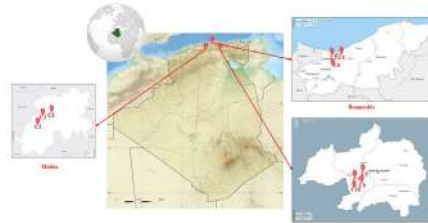


Figure 1. Distribution of colony locations.

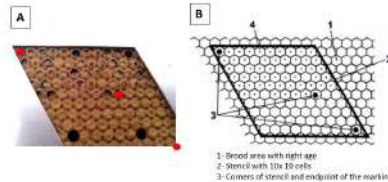


Figure 2. Pin Test Application for Assessing Hygienic Behaviour in *A. m. intermissa* Colonies. (A) The targeted brood area is punctured to simulate dead brood. (B) A pattern (10×10 cells) is placed over the comb to measure brood removal rates post-pin test.

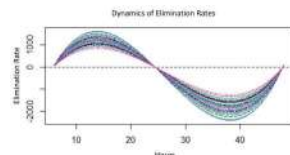


Figure 5. Dynamic elimination rate trends showing sinusoidal fluctuations influenced by environmental rhythms and colony activity.

Table 1. Analysis of variance (ANOVA) of the hygienic behavior of bee colonies by region and colony

source of variation	F-value	p-value	interpretation
Region	4.18	0.030	Significant: hygienic behavior varies significantly between regions.
Colony	1.72	0.150	Not significant: differences between colonies (in the same region) are not statistically significant.

Results & Discussion

The hygienic behavior of *Apis mellifera intermissa* colonies showed significant regional variation, with colonies from Médéa displaying the highest removal rates (83.6 ± 4.2%) compared to those from Boumerdes and Bordj Bou Arreridj. This suggests a potential influence of environmental conditions such as altitude and microclimate on the expression of hygienic traits. A seasonal decline in hygienic performance was also observed, with mean rates decreasing from 79.8% in March to 73.4% in September, possibly due to changes in colony strength, brood availability, or foraging pressure. Although inter-colony differences were not statistically significant, several colonies consistently exhibited high hygienic rates across all months, indicating potential candidates for selective breeding. A strong negative correlation was observed between hygienic behavior and Varroa infestation levels; colonies with higher hygiene rates had significantly lower Varroa loads. These findings reinforce the importance of hygienic behavior as a natural resistance mechanism and support its inclusion as a selection criterion in breeding programs for *A. m. intermissa* under Algerian conditions.

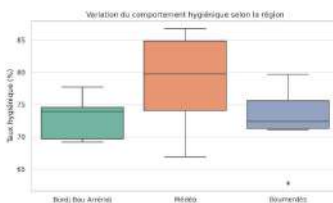


Figure 3. Box plot of hygiene rates by region.

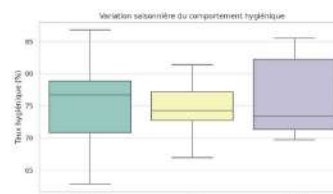


Figure 4. Seasonal boxplot of hygienic behavior.

Materials & Methods

Study Location and Period

The study was conducted from March to September 2024 in three Algerian regions with contrasting climates: Médéa, Tipaza, and Boumerdes. Each region hosted one apiary with 3 to 4 colonies, totaling 10 colonies of *Apis mellifera intermissa*.

Hygienic Behaviour Assessment

The pin-killed brood (PKB) test was used to evaluate hygienic behaviour. A 10×10 cm metal pattern (100 cells) was placed on brood combs to ensure standardized selection. Fifty sealed brood cells were pierced with an insect pin (No. 2), simulating diseased brood. The frame was reintroduced into the hive, and brood removal was observed at 6h, 24h, and 48h. The removal rate (%) was calculated based on the number of cleaned cells.

Varroa Infestation Monitoring

The sugar dusting method was applied to assess Varroa destructor infestation. 300 bees from the brood area were dusted with powdered sugar, dislodging mites for counting. Infestation rates were recorded monthly.

Data Analysis

Results were analyzed using R software. Seasonal and regional effects were tested via ANOVA. Correlations between hygienic behavior and Varroa infestation were examined using Pearson's correlation coefficient.

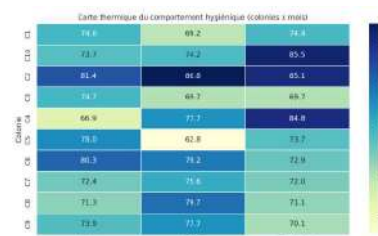


Figure 6. Carte thermique des performances hygiéniques.

Conclusion

This study confirms regional variability in hygienic behavior among *Apis mellifera intermissa* and highlights the effectiveness of the pin test as a practical, reproducible tool for behavioral selection in Algerian beekeeping. Among the ten colonies tested across three ecologically distinct regions, those in Médéa demonstrated significantly higher hygienic performance—suggesting that environmental factors (altitude, temperature, flowering) influence this adaptive trait. Although intra-apiary variation was not statistically significant, observable trends indicate exploitable genetic potential for local selection. Seasonal monitoring revealed a progressive decline in hygienic behavior by late summer, emphasizing the need for increased vigilance during critical colony health periods. In parallel, detergent-based assessment of Varroa destructor infestation rates revealed a clear negative correlation with hygienic behavior: colonies scoring >80% exhibited the lowest infestation levels. This supports the role of hygienic behavior as a natural defense mechanism and underscores the value of including the pin test in sustainable breeding programs aimed at improving colony resilience while reducing chemical treatments. Overall, these results demonstrate the strategic importance of hygienic behavior for improving colony health and preserving the local diversity of *A. m. intermissa*. Future research should explore associations between behavioral traits and additional vitality indicators such as varroa tolerance, viral load, longevity, and productivity to develop integrated selection profiles tailored to Algeria's ecological and socio-economic contexts.

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Evaluation of Hygienic Behaviour in Honey Bees "*Apis mellifera intermissa*" Through Pin Testing: Implications for Sustainable Beekeeping and Environmental Resilience

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This study evaluates the hygienic behavior of "*Apis mellifera intermissa*" of 8 colonies in Boumerdes, Algeria, from March to September 2024, using the pin test method. Hygienic behavior, crucial for colony health, involves the removal of dead or diseased brood and is a key defense against pathogens like "*Varroa destructor*". The removal rates were measured at 6-hour, 24-hour, and 48-hour intervals across different months. The results revealed seasonal variations, with higher removal rates during the spring and summer months (March-July), reaching up to 98% within 48 hours. A decline in removal rates was noted in late summer, likely due to environmental stress. Statistical analyses (for a single 5% significance) confirmed significant differences in removal rates across months and colonies ($p < 0.05$). Some colonies exhibited consistently strong hygienic behavior, highlighting the potential for selective breeding programs. These findings offer valuable insights for improving colony health and disease resistance, with implications for beekeeping practices in Algeria.