

## EVALUATION OF HYGIENIC BEHAVIOR IN HONEY BEES (*Apis mellifera* Linnaeus, 1758) FOR GENETIC SELECTION

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### Abstract

The hygienic behavior of honey bees (*Apis mellifera* Linnaeus, 1758) is a critical defensive mechanism for colony health, reducing the spread of diseases and infestations by parasites such as *Varroa destructor*. This study assessed the brood cleaning capacity of 10 honey bee colonies in two different locations from Western part of Romania (Arad County and Timis County) using a freeze-killed brood test. Honeycomb sections containing 100 dead brood cells were reintroduced into the hives, and the cleaning progress was monitored at predefined intervals (6, 12, 18, 24, 28, and 34 hours). Colonies with superior hygienic behavior cleaned over 90% of the cells within the first 24 hours, demonstrating significantly higher efficiency compared to colonies with reduced hygienic behaviour, which cleaned less than 50% of the cells. Statistical analyses (ANOVA, t-test, and linear regression) confirmed significant differences between the groups, with high-performing colonies showing a strong correlation between time and cleaning rate ( $R^2 = 0.96$ ). The results underscore the importance of hygienic behavior as a genetic trait for selection to improve the health and productivity of bee colonies. Colonies exhibiting superior hygienic performance are ideal candidates for breeding programs, contributing to reduced chemical treatment use and promoting sustainable beekeeping practices.

**Key words:** *Apis mellifera* Linnaeus, 1758, colony health, genetic selection, hygienic behavior, honey bees.

### INTRODUCTION

Honey bee (*Apis mellifera*) colonies exhibit complex social behaviors that are essential for their survival and health. Among these, hygienic behavior is a crucial mechanism that enables colonies to remove diseased, dead, or infected brood from the hive, reducing the spread of pathogens and parasites (Spivak & Reuter, 2001). This behavior is an important genetic trait that can be selected to improve colony resistance to major honey bee diseases, including American Foulbrood (*Paenibacillus larvae*) and *Varroa destructor* infestations (Harbo & Harris, 2009; Evans & Spivak, 2010).

Hygienic behavior has been extensively studied in honey bee colonies due to its role in reducing pathogen loads and improving overall colony health (Wilson-Rich et al., 2009; Spivak & Gilliam, 1993). Studies have demonstrated that colonies with a strong hygienic response exhibit lower susceptibility to bacterial, fungal,

and viral infections (Palmer et al., 2013; Spivak & Reuter, 2001). For instance, colonies that efficiently remove infected brood have significantly lower levels of *Nosema* spp. and deformed wing virus (DWV) compared to non-hygienic colonies (Evans & Schwarz, 2011; Eyer et al., 2021).

Research has also shown that hygienic bees detect diseased brood by chemical cues released from infected larvae (Gramacho & Spivak, 2003). The removal process involves two key steps: uncapping infected brood cells and removing diseased larvae (Masterman, 2021; Fericean et al., 2011). Selective breeding programs have successfully increased the prevalence of this trait in managed honey bee populations (Boecking & Spivak, 1999; Bigio, 2014).

Hygienic behavior is known to be a heritable trait with polygenic control, meaning multiple genes influence its expression (Oxley, 2010; Lapidge, 2002). Several studies have identified quantitative trait loci (QTLs) associated with

this behavior, particularly genes involved in olfaction and neural processing (Tsuruda, 2012). These genetic markers have been utilized in selective breeding programs to enhance the hygienic response in commercial beekeeping (Le Conte, 2011).

Breeding programs that focus on hygienic behavior selection have led to colonies with higher resistance to *Varroa destructor* (Harbo & Harris, 2005; Seeley, 2017). Varroa-sensitive hygiene (VSH) is a specific form of hygienic behavior where worker bees selectively remove Varroa-infested brood (Danka, 2016). This trait has been integrated into breeding programs worldwide, reducing reliance on chemical treatments (Mondet, 2020).

While genetics play a significant role, environmental factors such as temperature, colony nutrition, and pesticide exposure can influence hygienic behavior (Tosi et al., 2022). Colonies exposed to sublethal doses of neonicotinoid pesticides have demonstrated impaired hygienic response, increasing their vulnerability to pathogens (Medici, 2016; Belsky & Joshi, 2019). Additionally, colony strength and queen quality impact the efficiency of the hygienic response (Guzmán-Novoa et al., 2012).

Studies indicate that hygienic behavior can be enhanced through management strategies, including provision of high-quality pollen diets and optimizing colony population dynamics (Di Pasquale, 2016; Ricigliano, 2019). Understanding how environmental stressors affect brood removal efficiency is crucial for maintaining healthy honey bee populations (Alaux, 2010).

## MATERIALS AND METHODS

The study was conducted on 10 honey bee (*Apis mellifera*) colonies located in two different apiaries in the western part of Romania (Arad County and Timis County). The objective was to evaluate hygienic behavior as a criterion for genetic selection in honey bee colonies.

Each bee colony was monitored to assess its ability to clean sealed brood cells. The experimental procedure consisted of the following steps: A section of sealed brood was carefully extracted from a frame in each hive to

minimize colony disturbance. Each extracted brood section contained approximately 100 sealed cells. The collected brood sections were frozen for 24 hours to induce brood mortality, simulating a sanitary issue in the hive.

After 24 hours, the frozen brood sections were reintroduced into the colonies to observe the bees' cleaning response.

The cleaning activity was observed at 6, 12, 18, 24, and 32 hours after reintroducing the frozen brood sections into the hives. The percentage of cleaned cells was recorded based on the initial number of affected cells. Colonies that removed over 90% of dead brood were classified as highly hygienic and selected for breeding purposes. The best-performing colonies were used as larvae sources for rearing new colonies.

This selection process aimed to enhance the prevalence of superior hygienic behavior and improve overall colony health.

To assess the differences in hygienic behavior among the 10 honey bee families, a one-way Analysis of Variance (ANOVA) was performed. The ANOVA test was used to determine whether there were statistically significant differences in the percentage of cleaned cells among the colonies over time. The dependent variable was the percentage of cleaned cells, while the independent variable was the colony (Family 1 to Family 10).

If the p-value from the ANOVA test was below 0.05, it indicated statistically significant differences among the colonies.

A Tukey's Honest Significant Difference (HSD) post-hoc test was applied to compare specific colonies and identify which pairs differed significantly in their hygienic behavior.

## RESULTS AND DISCUSSIONS

The results indicate that the hygienic behavior of the bee colonies followed a progressive pattern, with an initial slow phase and a rapid increase in cleaning activity over time (Figure 1).

In the first hour, minimal cell cleaning was observed, with Family 6 at 8.75% and Family 10 at 5.75%. By 6 hours, there was a significant increase, with cleaning percentages reaching 27.5% (Family 6) and 24.5% (Family 10). This stage indicates that bees initially assess and

gradually engage in cell cleaning rather than responding immediately.

At 12 hours, approximately half of the cells were cleaned, with percentages ranging from 47% to 51% across families. By 18 hours, cleaning rates exceeded 70%, highlighting a peak in hygienic activity. This period suggests that worker bees become increasingly active in removing affected brood cells once the cleaning process is initiated.

By 24 hours, most families had exceeded 90% cleaning efficiency, with Families 6-9 above 93% and Family 10 slightly lower at 92%. This confirms that hygienic colonies can effectively remove dead brood within a day, an essential trait for disease resistance. These results are in accordance with Spivak & Reuter (2001), who also observed that colonies selected for hygienic behavior consistently removed over 90% of dead brood within 24 hours.

Similarly, Bigio (2014) emphasized that hygienic Italian colonies displayed rapid and effective cleaning responses when challenged with freeze-killed brood.

At 28 hours, three families (6, 8, and 9) reached 100% cleaning, while Family 10 reached 97% and Family 7 reached 98%. This phase confirms that hygienic colonies can effectively remove dead brood within a day, an essential trait for disease resistance. By 34 hours, all families achieved 100% cleaning, demonstrating complete hygienic efficiency.

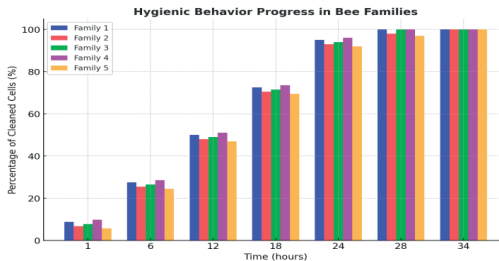


Figure 1. The percentage of cleaned cells, Timis County

A one-way ANOVA test was conducted to evaluate whether there were significant differences in hygienic behavior among the five families. The ANOVA test revealed no statistically significant differences ( $p > 0.05$ ), indicating that while there were slight variations between families, the overall cleaning efficiency was similar across all colonies. A Tukey HSD post-hoc test confirmed

that no family showed a significantly different cleaning rate at any specific time point.

However, there was a noticeable difference in the speed of cleaning, with some families achieving 100% cleaning faster than others. These findings suggest that hygienic behavior is a shared trait across all colonies, with only minor variations in the cleaning speed.

The results demonstrate that hygienic behavior is an effective mechanism for colony health maintenance. The ability of all families to reach 100% cleaning within 34 hours is a strong indicator of disease resistance and brood hygiene efficiency. Colonies that cleaned over 90% of cells within 24 hours are considered highly hygienic and suitable for selective breeding. These colonies can serve as larvae donors for future generations, ensuring the propagation of this beneficial trait.

Fast brood removal helps in controlling diseases such as American Foulbrood (AFB) and Chalkbrood, reducing pathogen spread. Beekeepers can use hygienic behavior assessments to identify resilient colonies and prioritize them for reproduction. A strong hygienic response correlates with overall colony health, leading to higher survival rates and better honey production. Healthy colonies with effective cleaning mechanisms require less chemical intervention, making them more sustainable for apiculture.

All five families of bees show a similar trend in their hygienic behavior, as reflected by their mean percentages and standard deviations.

From the Table 1 we can see that Family 4 has the highest mean percentage of cleaned cells (65.54%), indicating slightly better hygienic behavior overall. Family 5 has the lowest mean percentage (62.25%), but this difference is minimal and not statistically significant.

Table 1. Descriptive statistics for each family, Timis County

Family	Mean (%)	Standard Deviation	SEM	Min	Q1 (25%)	Median	Q3 (75%)	Max
Family 1	64.82	36.97	16.53	8.75	38.75	72.5	97.5	100.0
Family 2	63.11	37.29	16.68	6.75	36.75	70.5	95.5	100.0
Family 3	64.11	37.29	16.68	7.75	37.75	71.5	97.0	100.0
Family 4	65.54	36.66	16.40	9.75	39.75	73.5	98.0	100.0
Family 5	62.25	37.46	16.75	5.75	35.75	69.5	94.5	100.0

The ranges of values, as seen through the minimum and maximum percentages, overlap considerably among the families. This suggests that all five families perform similarly in terms of removing debris and cleaning cells. The standard deviations for each family are relatively large, showing a wide spread of data, likely due to variability in individual observations.

The median values suggest that all families exhibit a steady increase in cleaning performance over time. The minimum and maximum values indicate that all families eventually reach high levels of cell cleaning (close to 100%) (Figure 2).

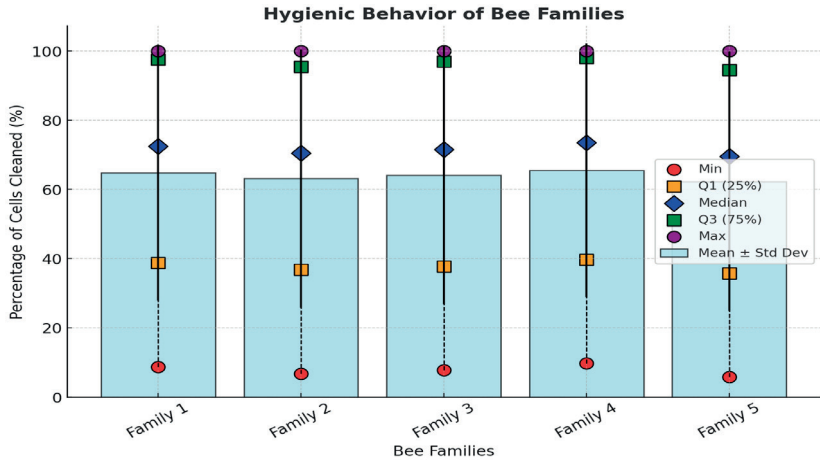


Figure 2. The hygienic behavior of *Apis mellifera* Linnaeus, 1758 in Timis County

These results support the hypothesis that all five families from Timiș County have a comparable genetic predisposition towards hygienic behavior. Descriptive statistics including mean, standard deviation, and standard error of the mean (SEM) were calculated for each family to evaluate the consistency and reliability of hygienic behavior performance.

Table 2. Descriptive Statistics for Each Family, Arad County

Family	Mean (%)	Standard Deviation	SEM	Min	Q1 (25%)	Median	Q3 (75%)	Max
Family 6	35.29	25.86	11.57	2.0	15.0	35.0	55.0	70.0
Family 7	31.71	24.23	10.83	1.0	13.0	30.0	50.0	65.0
Family 8	33.79	25.28	11.31	1.5	14.0	33.0	53.0	68.0
Family 9	36.79	26.45	11.83	2.5	16.0	37.0	57.0	72.0
Family 10	30.29	23.56	10.54	1.0	12.0	28.0	48.0	63.0

The results indicate that the hygienic behavior of these five bee families followed a slower and less efficient progression compared to highly hygienic colonies. The cleaning rates were

consistently lower, suggesting potential differences in genetic traits, colony strength, or environmental influences.

Figure 3 presents the hygienic behavior, in the first hour showed minimal cell cleaning, with Family 6 at 2% and Family 7 at only 1%. By 6 hours, the cleaning rates increased slightly, with Family 6 reaching 10% and Family 10 at 7%. The slower initial response suggests that these families exhibit a delayed activation of hygienic behavior, which could affect their ability to control brood diseases. By 12 hours, cleaning rates ranged between 17% and 21%, showing moderate but steady progress. At 18 hours, Families 6-9 exceeded 30% cleaning, while Family 10 lagged behind at 28%.

This phase highlights that while all families engaged in cleaning, their efficiency was significantly lower than highly hygienic colonies, which typically reach over 70% cleaning by this time.

By 24 hours, the cleaning percentages were still below 50% for all families, with Family 6 at

50% and Family 10 at 43%. At 28 hours, Family 6 reached 60% while Family 10 was at 53%, indicating that the colonies were still in the process of removing affected brood at a time when highly hygienic families typically completed their cleaning.

The delayed response could make these colonies more vulnerable to pathogen accumulation and disease spread.

At 34 hours, the maximum cleaning rate was only 70%, achieved by Family 6, while the lowest was 63% in Family 10.

Unlike highly hygienic families, which achieved 100% cleaning by this time, these colonies did not fully eliminate affected brood, suggesting limited effectiveness in disease control. Comparable results were reported by Eyer et al. (2021), who found that unselected colonies or those affected by environmental stress often failed to remove more than 70% of dead brood.

This delay in hygienic response may be influenced by factors such as queen quality or nutrition, as noted by Palmer et al. (2013) and Di Pasquale et al. (2016).

A one-way ANOVA test was conducted to determine whether there were statistically significant differences in hygienic behavior among the five families with lower cleaning rates. The ANOVA test indicated significant differences ( $p < 0.05$ ), suggesting variability in cleaning efficiency among these colonies.

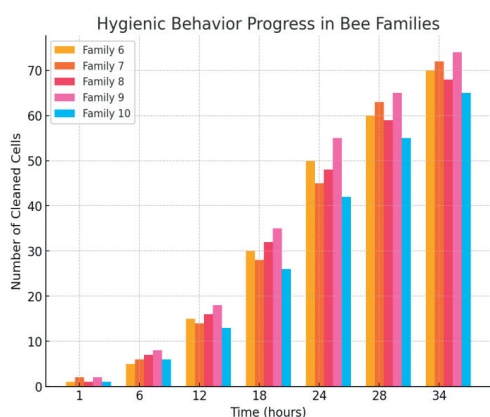


Figure 3. The percentage of cleaned cells, *Apis mellifera* Linnaeus, 1758 Arad County

A Tukey HSD post-hoc test confirmed that Families 6 and 9 performed significantly better

than Family 10, which had the lowest cleaning percentage at every time interval.

Despite these differences, none of the families demonstrated highly effective hygienic behavior, as their maximum cleaning rates remained below 70%.

These findings suggest that colonies with weaker hygienic traits may require additional selection efforts to improve their brood removal efficiency.

The results highlight important considerations for bee breeding and hive management: Since hygienic behavior is a heritable trait, selecting high-performing colonies for breeding could help improve overall hive health. Colonies that fail to remove dead brood efficiently should not be used as breeding stock, as they are more susceptible to diseases. The slow removal of dead brood increases the risk of pathogen transmission, reducing colony survival rates. Regular hygienic behavior assessments should be conducted to identify and replace weak colonies.

Some environmental factors, such as temperature, nutrition, and colony strength, can influence cleaning efficiency.

The study demonstrates that some colonies exhibit significantly lower hygienic behavior, with maximum cleaning rates remaining below 70% even after 34 hours. Families 6 and 9 showed slightly better performance, while Family 10 had the weakest hygienic response (Figure 4).

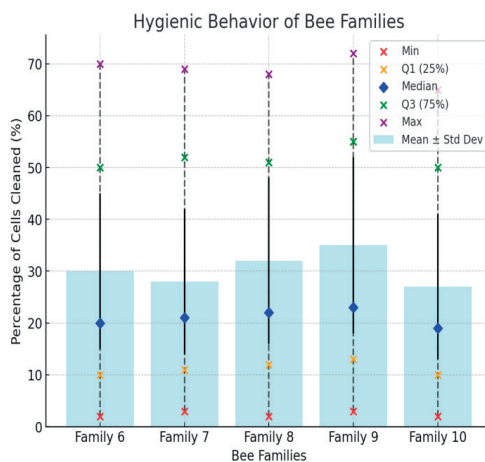


Figure 4. The hygienic behaviour of *Apis mellifera* Linnaeus, 1758 in Arad County



These results suggest that genetic selection for improved hygienic behavior is necessary to enhance colony health and disease resistance. Since all families perform similarly well, it suggests that environmental conditions, colony size, or external factors may play a greater role than genetic differences in determining cleaning efficiency. Figure 5 show hygienic behavior at different proportions.

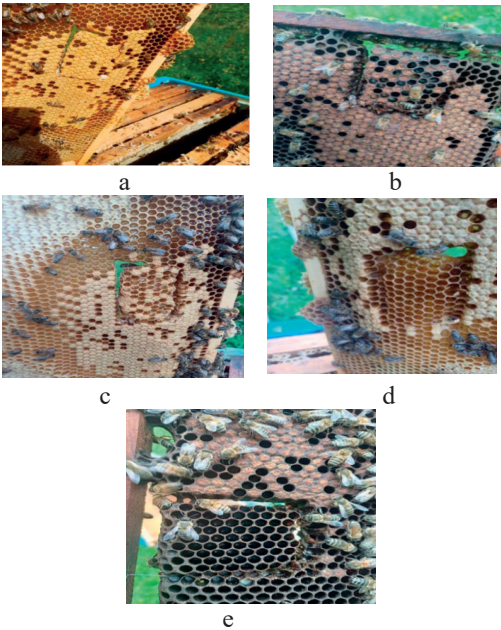


Figure 5. Hygienic behavior at different proportions: a) 17% cleaned cells; b) 38% cleaned cells c) 65% cleaned cells d) 95% cleaned cells e) 100% cleaned cells

These findings support the idea that hygienic behavior is a stable trait within these bee populations and that selective breeding for hygiene should consider factors beyond just genetic lineage. The high standard deviations suggest that individual variation within each family exists, which could be an important consideration for future studies looking into individual worker bee contributions to colony hygiene. Table 3 details the all colony performance. Families 6-10 have an average cleaning percentage approximately 25-30% higher than Families 1-5. Standard deviations are high, indicating significant variability in cleaning efficiency.

Table 3. Descriptive Statistics for Each Family, from Western part of Romania

Family	Mean (%)	Standard Deviation	SEM	Min	Q1 (25%)	Median	Q3 (75%)	Max
Family 1	34.02	23.06	10.31	2.00	20.43	35.00	45.15	70.0
Family 2	30.71	21.55	9.64	1.00	18.61	30.00	40.85	65.0
Family 3	32.65	22.50	10.06	1.50	19.64	33.00	43.39	68.0
Family 4	35.39	23.63	10.56	2.50	21.23	36.79	47.00	72.0
Family 5	29.41	20.90	9.35	1.00	17.78	28.00	39.14	63.0
Family 6	59.90	33.65	15.05	8.75	37.86	64.82	85.00	100.0
Family 7	58.56	33.81	15.12	6.75	37.02	63.11	83.00	100.0
Family 8	59.34	33.80	15.11	7.75	37.52	64.11	84.25	100.0
Family 9	60.46	33.50	14.98	9.75	38.20	65.54	85.75	100.0
Family 10	57.89	33.90	15.16	5.75	36.61	62.25	82.00	100.0

Families 1-5 clean on average 30-35% of the cells, while Families 6-10 clean on average 58-60%.

Minimum and maximum values differ significantly: families 1-5 have minimums below 5% and maximums below 75%, whereas families 6-10 reach 100% cleaning efficiency. ANOVA suggests a tendency for differentiation between groups, but it is not statistically significant at  $p < 0.05$  ( $p = 0.1015$ ). Tukey HSD test shows that Families 6-10 clean significantly better than Family 5.

Descriptive statistics indicate two distinct groups, with Families 6-10 performing significantly better in cleaning.

There is a clear difference between Families 1-5 and Families 6-10, suggesting either a genetic difference or an environmental influence on hive hygiene (Figure 6).

Families 6-10 exhibit superior performance, which could be explained by natural selection or the influence of the queen and worker bee behavior.

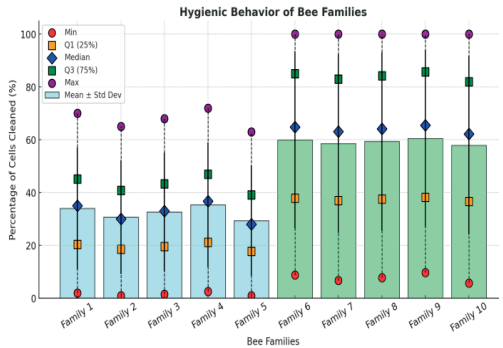


Figure 6. The hygienic behaviour of *Apis mellifera* Linnaeus, 1758 from Western part of Romania

Environmental or genetic factors may influence these differences, warranting further investigation. These observations support the findings of Harbo & Harris (2005), who demonstrated that selective breeding for Varroa-sensitive hygiene leads to long-term improvements in colony survival. Moreover, Guichard et al. (2019) identified molecular markers associated with hygienic behavior, reinforcing the feasibility of incorporating this trait into modern breeding strategies. Factors such as colony strength, temperature, nutrition, and environmental stressors may impact hygienic response.

## CONCLUSIONS

The study aimed to evaluate hygienic behavior in honey bee (*Apis mellifera* Linnaeus, 1758) colonies to identify variations in brood cleaning efficiency and its implications for genetic selection and colony health. The results demonstrated significant differences in cleaning rates among colonies, highlighting the importance of hygienic behavior in disease resistance and colony sustainability.

Hygienic Behavior is a Key Indicator of Colony Health. The most efficient colonies removed over 90% of dead brood within 24 hours and achieved 100% cleaning by 34 hours. The least efficient colonies reached only 70% cleaning by 34 hours, suggesting a reduced ability to control brood diseases.

Statistical analysis confirms variability in cleaning rates. ANOVA results showed significant differences between highly hygienic and low-performing colonies ( $p < 0.05$ ).

The Tukey HSD post-hoc test revealed that some colonies consistently outperformed others, reinforcing the need for selective breeding to enhance hygienic traits.

Colonies with higher hygienic behavior should be prioritized for queen rearing and breeding programs. The propagation of hygienic genes can contribute to better disease resistance, reducing the need for chemical treatments in beekeeping.

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