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Integrative Phenotypic and Genomic Characterization of Genetic Diversity in *Apis mellifera intermissa*

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Abstract: The western honey bee "*Apis mellifera*" exhibits extensive phenotypic and genetic diversity shaped by long-term evolutionary processes and local environmental pressures. This diversity underpins the adaptability, ecological success, and resilience of honey bee populations across contrasting habitats. In North Africa, *A. m. intermissa*, represents a distinct subspecies characterized by unique morphometric traits, behavioral adaptations, and molecular features that reflect its adaptation to arid and semi-arid environments. This review synthesizes current knowledge on the integrative phenotypic and genomic characterization of *A. m. intermissa*, emphasizing morphometric variability, geographic differentiation, behavioral ecology, and molecular diversity. Landmark-based geometric morphometric and traditional linear measurements reveal pronounced clinal variation associated with climatic gradients, supporting ecological adaptation. Behavioral traits such as foraging strategies, defensiveness, and reproductive plasticity further illustrate subspecies specialization. At the molecular level, genomic and proteomic studies highlight elevated genetic diversity, enhanced immune responsiveness, thermal tolerance, and detoxification capacity. Together, integrative phenotypic and genomic approaches provide robust tools for subspecies identification, conservation planning, and the preservation of locally adapted honey bee populations. This synthesis underscores the importance of safeguarding *A. m. intermissa* as a valuable genetic resource for sustainable apiculture under increasing environmental pressures.

Keywords: *Apis mellifera intermissa*; phenotypic diversity; morphometrics; genetic diversity; landscape genomics; Tunisia; North Africa.

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1 Introduction

The western honey bee (*Apis mellifera*) is a globally significant pollinator, supporting approximately 35 % of crop production and contributing up to USD 577 billion annually to agriculture, while sustaining vital ecological functions in both natural and managed ecosystems (FAO, 2025). The success of this species is largely attributed to its remarkable phenotypic plasticity and genetic diversity, which have enabled adaptation to a wide range of ecological conditions.

Approximately 30 subspecies of *A. m.* have been described, traditionally grouped into major evolutionary lineages A (African), M (Western and Northern European), C (Southeastern European), and O (Middle Eastern) with a fifth lineage (Y) later identified in the southern Middle East (Franck et al., 2001; Cridland et al., 2017). These subspecies exhibit distinct morphological, behavioral, and genetic traits shaped by regional climatic and ecological pressures. In North Africa, the principal native honey bee subspecies are *A. m. intermissa* and *A. m. sahariensis*, with *A. m. intermissa* distributed throughout the Mediterranean regions of Tunisia, Algeria, and Morocco, and *A. m. sahariensis* occurring primarily in the Saharan zones of Algeria and Morocco (Bakhchou et al., 2025; Bouzeraa et al., 2020).

A. m. intermissa is particularly notable for its adaptability to high temperatures, seasonal drought, and fluctuating floral resources (Adjlane et al., 2012). Understanding the integrative phenotypic and genomic basis of this adaptation is essential for conservation, sustainable apiculture, and the prevention of genetic erosion through introgression from non-native stocks.

Therefore, in this review, we discuss the main available scientific literature regarding the integrative phenotypic and genomic characterization of genetic diversity in *A. m. intermissa*.

2 Morphometric characterization of *Apis mellifera intermissa*

2.1 Taxonomic diagnosis and external pigmentation

In North Africa, *A. m. intermissa* is generally dark, ranging from black to dark brown, and can be distinguished from the Saharan subspecies *A. m. sahariensis*, which exhibits lighter, yellowish coloration (Haider et al., 2025; Hali, 2023). Northern populations, particularly in Algeria and Tunisia, display relatively uniform dark pigmentation with minimal yellow abdominal banding, whereas populations near the Saharan regions show slightly lighter coloration and more prominent abdominal bands, reflecting a north–south morphological gradient. Coloration, including variation in scutellum pigmentation and abdominal tergite bands, has been identified as one of the most discriminating morphological traits among these populations (Bakhchou et al., 2025).

For instance, pigmentation of the scutellum varied from an average of 1.0 µm in northern Algerian populations (Tipaza, Blida) to 4.0 µm in Biskra, suggesting geographic differentiation (Hallati et al., 2023). Additionally, *A. m. intermissa* is visually distinct from *A. m. sahariensis*, which exhibits more pronounced yellow reddish abdominal bands (Bouslama et al., 2022; Boucheffa et al., 2022).

2.2 Wing morphometrics — landmark-based geometric approaches

Geometric morphometrics focuses on the analysis of shape by using homologous anatomical landmarks. In honeybees, these landmarks are placed on the intersections of wing veins (for example, the junctions between the radial, medial, and cubital veins). Once digitized, these coordinates are aligned through generalized Procrustes superimposition, a procedure that removes differences due to position, scale, and orientation, so that only true shape variation remains (Barour and Baylac, 2016) (Figure 1).

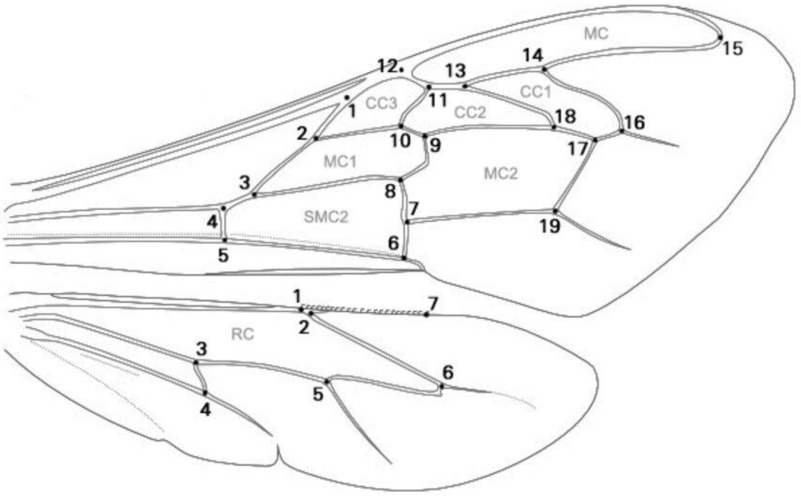

Figure 1. Landmarks on *A. m.* wings used for geometric morphometric analyses (Barour and Baylac, 2016).

The resulting data consist of two main components. The first is centroid size (CS), which provides a measure of overall wing size and is calculated as the square root of the summed squared distances of each landmark from the centroid. The second component is the Procrustes coordinates, representing shape variables obtained after removing the effects of size, position, and orientation through Generalized Procrustes Analysis, thereby capturing pure shape information (Barour and Baylac, 2016).

Population differences are often investigated using multivariate statistical methods. Principal components analysis (PCA) summarizes major axes of wing shape variation (Figure 2; Khedim et al., 2024), whereas canonical variate analysis (CVA) maximizes separation between predefined groups, with classification accuracy evaluated via cross-validation (Figure 3; Barour & Baylac, 2016).

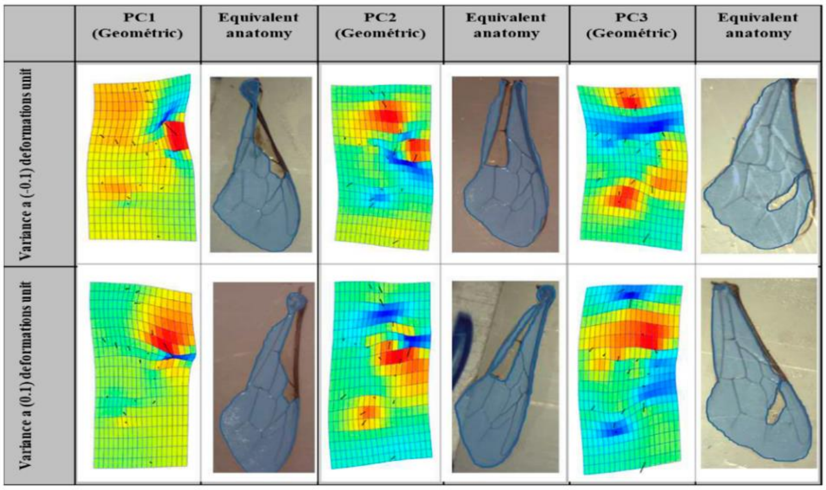

Figure 2. Principal Components Analysis (PCA) plot showing major axes of wing venation shape variation distinguishing geographic populations of *A. m. intermissa* in northern Algeria (Khedim et al., 2024).

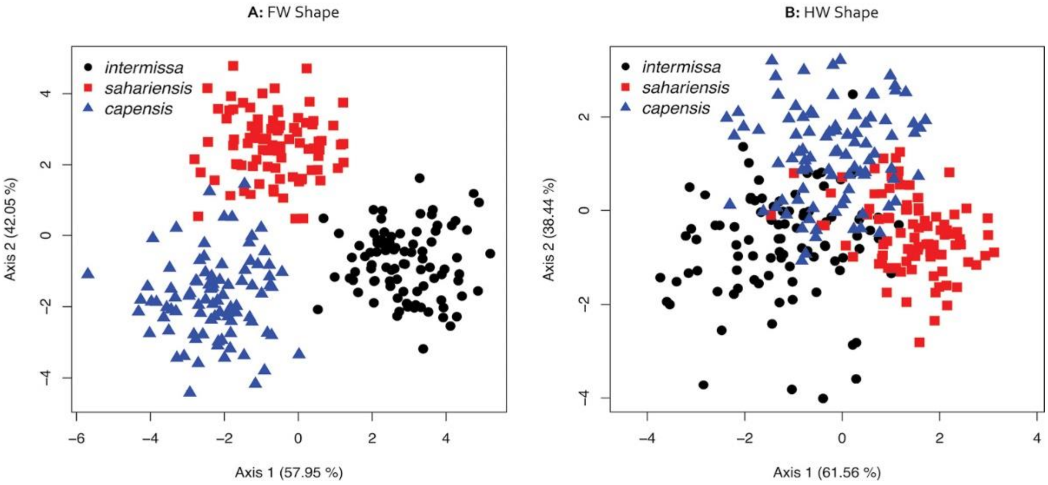

Figure 3. Canonical variate analysis (CVA) of wing shape variation among *A. m. subspecies*: A fore wing shape B hind wing shape (Barour and Baylac, 2016).

In a comparative study of three African subspecies (*A. m. intermissa, A. m. sahariensis, A. m. capensis*), Barour and Baylac (2016) analyzed 540 wings (270 workers). Their results showed that forewing shape alone allowed correct classification of *A. m. intermissa* individuals with an accuracy of ~96.7%. When both shape and size (form) were considered together, the accuracy exceeded 99%. This demonstrates that wing venation provides a powerful and objective tool for distinguishing *A. m. intermissa* from other closely related African subspecies.

2.3 Linear traditional body morphometrics

Complementary to geometric analyses of wing shape, traditional linear measurements remain informative for ecological and functional interpretation.

According to Barour and Baylac (2016), forewing length in *A. m. intermissa* ranges from 9.5 to 10.3 mm, with widths between 3.0 and 3.5 mm, while hindwing length varies from 7.2 to 7.9 mm.

In a broader survey across 22 Algerian apiaries, Sandra and Aicha (2022) reported that proboscis lengths varies between 6.5 and 7.2 mm, femur lengths of 3.6 to 4.0 mm, and thorax widths between 4.8 and 5.5 mm. These measurements, together with abdominal tergite widths around 3.1 to 3.7 mm, indicate notable phenotypic plasticity.

These linear morphometric traits provide valuable insights into the biology of *A. m. intermissa*. Wing dimensions serve as proxies for overall body size and flight performance, while proboscis length reflects potential adaptation to floral resource exploitation. Femur, thorax, and tergite measurements contribute to assessing structural robustness and population-level variability. Analyses of hindwings further confirmed significant shape and size variability within the subspecies, with patterns strongly correlated to climatic gradients (Khedim et al., 2024).

From the reviewed studies, it is clear that morphometric data underscore the rich phenotypic diversity of *A. m. intermissa*. The combination of geometric morphometric and traditional linear measurements offers precise tools for subspecies identification, detecting introgression from exotic bees, and guiding sustainable apiculture practices in North Africa. Taken together, these metrics also allow for the evaluation of allometric patterns, the detection of geographic (clinal) differentiation among populations, and the identification of potential adaptive responses to local environmental conditions (Khedim et al., 2024).

2.4 Geographic clinal variation and adaptive interpretation

Morphometric variability in *A. m. intermissa* displays clear geographic structure: individuals from cooler, higher-altitude or more temperate sites tend to have larger body and wing dimensions, while populations in hotter, lower-altitude environments are generally smaller.

This pattern is consistent with ecogeographical rules such as Bergmann's rule and can be interpreted in terms of thermal regulation and flight biomechanics: larger size and larger wing area improve heat retention and flight efficiency in cooler climates, whereas smaller size may be advantageous under thermal stress and for resource economy in arid landscapes (Khedim et al., 2023; Hallati et al., 2023).

2.5 Morphometric Approaches for Subspecies Detection, Conservation, and Introgression Monitoring

The combined use of geometric morphometrics (wing shape analysis) and traditional linear measurements provides a robust framework for honey bee research and management. This integrated approach allows for reliable subspecies identification and the monitoring of populations, as well as the detection of introgression from exotic or managed stocks, which is critical for preserving the genetic integrity of local honeybee populations. Additionally, it supports conservation planning and the delineation of local ecotypes, facilitating sustainable apiculture by identifying distinct populations that merit protection.

Recent advances in automated analysis, including tools such as ***DeepWings*** and related machine-learning algorithms, accelerate landmark detection and classification, enabling efficient large-scale monitoring (García et al., 2022).

For breeding and conservation programs, reporting both shape and form (shape combined with centroid size) is recommended, as incorporating size often increases classification accuracy and provides a more complete understanding of morphological variation (Kandemir et al., 2022; Barour & Baylac, 2016).

3 Behavior traits of A. m. intermissa

A. m. intermissa, the North African honey bee subspecies, exhibits distinct behavioral adaptations reflecting its ecological and evolutionary specialization.

Foraging activity closely tracks floral resource availability, with peak activity corresponding to maximal nectar secretion periods, demonstrating efficient exploitation of local floral phenology (Omar & Mohamed, 2020).

Hygienic behavior, involving detection and removal of diseased or dead brood, is well-developed, with reported brood removal rates exceeding 80–90% across seasonal cycles in Algerian and Tunisian populations, indicating strong colony-level disease resistance (Khelifi et al., 2021).

Defensive behavior is relatively heightened, likely as an adaptive response to predation and environmental pressures, and is coupled with a strong swarming tendency that supports rapid colony reproduction (Haider et al., 2025).

Reproductive resilience is further evidenced by the capacity of workers to initiate egg-laying in queenless conditions, ensuring colony continuity under queen loss or failure (Jean-Prost, 2005).

Collectively, these behaviors illustrate the integration of ecological adaptation and genetic specialization, emphasizing the significance of behavioral phenotypes in the evolutionary ecology and conservation management of *A. m. intermissa*.

4 Developmental Cycle

These behavioral adaptations are supported by the species' holometabolous developmental cycle, which comprises four stages: egg, larva, pupa, and adult. Eggs develop within brood cells and hatch into larvae after three days. The queen's developmental cycle averages 16 days, workers 21 days, and drones 24 days (Jean-Prost, 2005). Pupae undergo imaginal molt to emerge as adults, completing the brood cycle (Ayme, 2014).

Understanding the developmental timing in conjunction with behavioral traits provides insight into colony growth dynamics, resilience, and the capacity of *A. m. intermissa* to adapt to environmental pressures.

5 Molecular and Genomic Diversity of *Apis mellifera intermissa*

5.1 Genetic Diversity and Population Structure

Molecular studies using microsatellites, mitochondrial DNA, and single nucleotide polymorphisms consistently indicate high genetic diversity in *A. m. intermissa* compared with many European subspecies. Tunisian populations predominantly belong to the African (A) mitochondrial lineage, with limited introgression from European lineages in some managed apiaries (Shaibi et al., 2010).

Genome-wide analyses reveal population structure aligned with north–south climatic gradients, suggesting that environmental selection shapes genomic diversity across Tunisia.

5.2 Thermal Tolerance and Stress Response

Adaptation to high temperatures is a defining feature of *A. m. intermissa*. Heat shock proteins (HSPs), particularly HSP70, play a central role in cellular protection under thermal stress.

Benhassaini and Boudjema (2024) demonstrated that *A. m. intermissa* exhibits increased hemolymph protein concentrations under elevated temperatures compared with *A. m. sahariensis*, indicating enhanced physiological resilience.

These molecular responses are consistent with the subspecies' distribution in regions where summer temperatures frequently exceed 40°C (Alghamdi & Alattal, 2023).

5.3 Functional Genomics and Immune Response

Transcriptomic studies reveal upregulation of genes associated with immune defense, antioxidant activity, and stress response in *A. m. intermissa*. Key pathways include Toll and IMD signaling, as well as genes encoding antimicrobial peptides such as defensins and abaecins (Alaux et al., 2010).

These molecular features likely contribute to enhanced resistance against pathogens and parasites prevalent in North African environments.

5.4 Proteomic Insights into Detoxification and Adaptation

Proteomic analyses reveal adaptive molecular mechanisms in *A. m. intermissa*. Detoxification enzymes, including glutathione S-transferases (GSTs) and cytochrome P450 monooxygenases, show elevated activity in populations exposed to agricultural pesticides (Nabti et al., 2011; Goulson et al., 2015). Venom proteomes have identified bioactive peptides such as melittin, apamin, and phospholipase A2, which contribute to defense and immune regulation and may hold biomedical relevance (El Alaoui et al., 2022; El Hamdani et al., 2021).

5.5 Landscape Genomics and Environmental Adaptation

Genetic and ecophysiological studies on *A. m. intermissa* populations in Tunisia show that local climatic conditions are associated with functional differences among mitochondrial haplotypes. For example, Chouchene et al. (2010) documented that four *A. m. intermissa* haplotypes (A1, A4, A8, A9) exhibited different brood-nest temperature regulation and oviposition patterns across humid, sub-humid, semi-arid, and arid bioclimatic zones in Tunisia.

The occurrence of certain haplotypes exclusively in particular ecological zones suggests micro-evolutionary adaptation to local environments, providing evidence that environmental factors such as temperature and humidity influence physiological and reproductive traits in Tunisian honey bees.

6 Conclusion

This review highlights the integrative phenotypic and genomic diversity of *A. m. intermissa*, emphasizing its pronounced morphological variability, behavioral specialization, and molecular adaptations to North African environments. The convergence of morphometric, behavioral, and genomic evidence underscores the evolutionary distinctiveness and ecological resilience of this subspecies. Integrative approaches that combine phenotypic and genomic data are essential for accurate subspecies identification, monitoring, and conservation, as well as for preventing genetic erosion from introgression of non-native stocks.

Preserving *A. m. intermissa* as a locally adapted genetic resource is critical for the sustainability of apiculture and the resilience of pollination services under ongoing environmental and climatic changes.

Future studies integrating landscape genomics, epigenetics, and functional ecology will further enhance our understanding of adaptive mechanisms and inform targeted conservation strategies.

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