



Assessing Hybridisation in *Apis mellifera ruttneri*: Morphometric Insights from Gozo and Malta

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About Author: After completing his M.Sc. at the University of Malta, Dylan Farrugia co-founded the NGO-VO Foundation for the Conservation of the Maltese Honey Bee, where he currently serves as president. He has also worked as a Research Support Officer on an EU-funded project at the same university, focusing on the conservation of Mediterranean honey bee subspecies, with particular emphasis on the endemic Maltese honey bee (*Apis mellifera ruttneri*).

Abstract

This study assessed hybridisation in Malta's endemic honey bee, *Apis mellifera ruttneri* using traditional and geometric morphometry. Findings revealed hybrid traits in both Gozitan and Maltese samples, linked to foreign subspecies and/or other breeds introductions. Linked to foreign subspecies introduction. Results highlight urgent conservation needs to preserve the native subspecies' genetic integrity and adaptive value amid ongoing genetic introgression.

Introduction

Long-term geographic barriers drive reduced gene flow in honey bees, leading to allopatric evolution and formation of distinct subspecies (Ruttner, 1988). The Maltese Islands found 93 km south of Sicily and 288 km east of Tunisia in the centre of the Mediterranean Sea host the endemic honey bee *Apis mellifera ruttneri*, commonly referred to as the Maltese Honey Bee (Sheppard et al., 1997). It is well-suited for Malta's dry conditions and displays distinct traits and adaptive strategies (Farrugia et al., 2022). However, extensive importation of foreign honey bee subspecies and breeds, particularly *A. m. ligustica* and *A. m. carnica* due to their perceived beekeeping superiority, has raised concerns regarding genetic hybridisation and loss of adaptive alleles. Controversially in 2015, a commercial queen rearing enterprise using foreign subspecies on Gozo was authorised to operate despite local beekeepers' genetic integrity concerns for the native *A. m. ruttneri*.

Aim

This study aimed to assess the morphological integrity of *A. m. ruttneri* in Gozo and Malta through separate discriminant analyses using traditional and geometric morphometric data to give indications on the extent of hybridisation.

Methodology

Sampling

Between August and November 2021, a total of 55 colony samples were collected from 14 apiaries in Gozo (n=34) and 7 apiaries in Malta (n=21), with multiple colonies being sampled from each apiary (Fig. 1).

Traditional morphometry

Assessment using traditional morphometry was based on 11 angles (A4, B4, D7, E9, G18, J10, J16, K19, L13, N23 and O26), four distances (right forewing length, width, lengths of segment a and b of cubital cell) and one index ratio (Cubital Index) measured with Swift Imaging 3.0 software. Character measurements were averaged per colony sample.

Geometric morphometry

Geometric morphometry utilised 19 landmark points at vein intersections plotted with Identify software version 1.6.2, which were then aligned using Procrustes fit method and averaged per colony with Morpho version 1.04 software.

Discriminant analysis

SPSS Version 25.0 was used to construct separate discriminant analysis models for each approach to classify sampled Gozitan and Maltese colonies relative to reference subspecies. The reference subspecies data for the traditional model was based on *A. m. intermissa*, *A. m. ligustica*, *A. m. ruttneri* and *A. m. sahariensis*, and the geometric model was based on *A. m. carnica*, *A. m. intermissa*, *A. m. ligustica*, *A. m. ruttneri* and *A. m. sahariensis*. Each model generated discrimination scores, probabilities, scatterplots and predicted classified subspecies.



Figure 1: Sampling locations across Gozo (blue) and Malta (red).

Results

Discriminant analyses indicated clear hybridisation trends. Traditional morphometry classified 82.35% of Gozitan colonies and 76.19% of Maltese colonies as *A. m. ruttneri*, with others showing affinity to *A. m. ligustica*. The scatterplot generated from the traditional data discriminant analysis showed a continuous distribution from Gozitan and Maltese *A. m. ruttneri* sampled colonies towards *A. m. ligustica* reference colonies (Fig. 2), with some also clustering near A-lineage reference subspecies.

Geometric morphometry revealed a stronger hybridisation trend, with only 41.18% of Gozitan and 23.81% of Maltese colonies classified as *A. m. ruttneri*. The scatterplot generated from the geometric data discriminant analysis showed that most Gozitan and Maltese *A. m. ruttneri* sampled colonies were distributed continuously between the A and C-lineage reference subspecies (Fig. 3). The wireframe analyses showed subtle but consistent wing venation differences between hybrid and pure colonies.

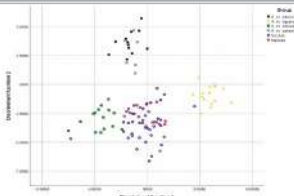


Figure 2: The scatterplot generated from the traditional data discriminant analysis.

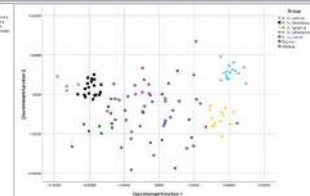


Figure 3: The scatterplot generated from the geometric data discriminant analysis.

Discussion & Conclusion

Hybrids of two different honey bee breeds/subspecies exhibit intermediate wing venation patterns, reflecting continuous morphometric measurements and reduced subspecies resolution. The presence of hybrid traits in both Gozitan and Maltese populations highlights ongoing genetic introgression, likely exacerbated by the operation of a commercial queen-rearing enterprise in Gozo since 2015. As hybridisation can occur between colonies from 15 km away (Jensen et al., 2005), and Gozo's coastlines are only 14 km apart, hybridisation potential is markedly higher given the island's small area of 67 km². Furthermore, this is worsened by a relatively high mean hive density on Gozo, with around 20 colonies per km² from 2021 data (Gemma, P. & Francesco Luca, A., unpubl. data, 2023).

Hybridisation threatens the adaptive genetic traits of *A. m. ruttneri*, which are crucial for local apiculture sustainability. Studies on performance and *Varroa destructor* tolerance of *A. m. ligustica* versus *A. m. ruttneri* on the Maltese Islands has categorically shown the native subspecies' superiority in hygienic behaviours and performance (Uzunov et al., 2023).

Urgent conservation measures are necessary to preserve this unique subspecies. A study by Chetcuti Dimech et al. (2023) outlining legal measures to holistically safeguard the Maltese honey bee, and thereafter action to declare *A. m. ruttneri* as Malta's national insect, is a positive step towards conservation. However more decisive actions needs to be taken, including restricting foreign honey bee imports and establishing nation-wide breeding programs for *A. m. ruttneri*. Without intervention, *A. m. ruttneri* faces irreversible decline due to continued hybridisation.

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Assessing Hybridisation in *Apis mellifera ruttneri*: Morphometric Insights from Gozo and Malta

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INTRODUCTION: The Maltese Islands host the endemic honey bee *Apis mellifera ruttneri*, which has evolved distinct traits suited to local environmental conditions. However, extensive importation of foreign honey bee subspecies, particularly *A. m. ligustica* and *A. m. carnica*, has raised concerns regarding genetic hybridisation and loss of adaptive alleles.

Aim

This study aimed to assess the morphological integrity of *A. m. ruttneri* in Gozo and Malta through traditional and geometric morphometric analyses to determine the extent of hybridisation. Sampled colonies from Gozo and Malta were compared to reference honey bee subspecies using traditional and geometric morphometry methods. The findings aim to provide baseline insights into conservation strategies for protecting the genetic purity of this endemic subspecies.

Methodology

Between August and November 2021, 34 *A. m. ruttneri* colonies were sampled from Gozo and 21 from Malta. Traditional morphometry was conducted by measuring 11 wing angles, four distances, and the cubital index, while geometric morphometry involved 19 landmark-based wing shape analysis. Separate discriminant analysis models were applied to each approach to classify sampled colonies relative to reference subspecies (*A. m. ruttneri*, *A. m. ligustica*, *A. m. intermissa*, *A. m. carnica*, and *A. m. sahariensis*).

RESULTS: Discriminant analyses indicated clear hybridisation trends. Traditional morphometry classified 82.35% of Gozitan colonies and 76.19% of Maltese colonies as *A. m. ruttneri*, with others showing affinity to *A. m. ligustica*. Geometric morphometry revealed a stronger hybridisation trend, with only 41.18% of Gozitan and 23.81% of Maltese colonies classified as *A. m. ruttneri*. The wireframe analyses showed subtle but consistent wing venation differences between hybrid and pure colonies.

Discussion & CONCLUSION: The presence of hybrid traits in both Gozitan and Maltese populations highlights ongoing genetic introgression, likely exacerbated by the operation of a commercial queen-rearing enterprise in Gozo since 2015. Hybridisation threatens the adaptive traits of *A. m. ruttneri*, which are crucial for local apiculture sustainability. Urgent conservation measures, including restricting foreign honey bee imports and establishing nation-wide breeding programs for *A. m. ruttneri*, are necessary to preserve this unique subspecies. Without intervention, the genetic integrity of *A. m. ruttneri* faces irreversible decline due to continued hybridisation.