Original Article

INSIGHTS FROM TRADITIONAL AND GEOMETRIC MORPHOMETRIC ANALYSES OF SELECTED *APIS MELLIFERA RUTTNERI* COLONIES IN GOZO AND MALTA

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Abstract

The Maltese Islands host an endemic honey bee subspecies, *Apis mellifera ruttneri*. However, apiculture based on the imported foreign subspecies and strains greatly threatens the native *A. m. ruttneri*'s status through hybridisation. This study examined the morphometric attributes of selected *A. m. ruttneri* colonies (n=34) on Gozo, following several years of operation of a commercial queen rearing enterprise using foreign subspecies and strains. Additionally, colonies of *A. m. ruttneri* (n=21) from neighbouring Malta were also examined, where the importation of foreign honey bee colonies has been ongoing for a significantly longer time. Sampling was carried out between August and November 2021. Traditional and geometric morphometry were used for wing analysis, with separate discriminant analysis classification models applied to each approach. Results revealed clear hybridisation trends in both Gozitan and Maltese sub-populations of *A. m. ruttneri*. These findings align with and echo concerns regarding local honey bee decline. This study underscores the urgent need for more decisive and concrete actions to holistically protect the Maltese honey bee and its vital role in local apiculture.

Keywords: *Apis mellifera ruttneri*, conservation, geometric morphometry, hybridisation, Maltese Islands, traditional morphometry

INTRODUCTION

Long-term geographic barriers drive reduced gene flow in honey bees, leading to allopatric evolution and formation of distinct subspecies (Ruttner, 1988). Friedrich Ruttner's seminal work (1978; 1988) classified honey bees into twenty-five subspecies using traditional morphometry. Limited wing characters have also been used successfully to differentiate some subspecies (Nazzi, 1992; Tofilski, 2008; Uzunov et al., 2009). A newer approach to wing studies is geometric morphometry, through which shape differences in wings are measured (Klingenberg & Monteiro, 2005). Ilyasov et al.'s taxonomic revision (2020), considering various research conducted through diverse methods identified thirty-three subspecies categorised within evolutionary lineages (Ruttner et al., 1978; Ruttner, 1988; Franck et al., 2001).

Particularly high subspecies diversity in and along the Mediterranean basin can be noted, including the Maltese honey bee, *Apis mellifera ruttneri* which is endemic to the Maltese archipelago. The archipelago consists of three main islands; Malta, Gozo, and Comino, covering an area of 316 km^2 and found 93 km south of Sicily and 288 km east of Tunisia. Morphologically the Maltese honey bee is similar to the Sicilian honey bee *A. m. siciliana* and North African honey bee *A. m. intermissa* (Sheppard et al., 1997). Genetically its mitochondrial genome links it to *A. m. sahariensis*, *A. m. intermissa* and *A. m. iberiensis* (Henriques et al., 2022). The Maltese honey bee is well-suited for Malta's dry conditions and displays distinct traits and adaptive strategies (Farrugia et al., 2022; Uzunov et al., 2023). Although it defends against local predacious wasps (Sheppard et al., 1997), the growing *Vespa orientalis* population is increasingly posing an existential threat to the entire apicultural activity on the Maltese Islands (various unnamed beekeepers, unpubl. data, 2022).

Foreign colonies, notably *A. m. ligustica* and *A. m. carnica*, have been extensively imported due to their perceived beekeeping superior

traits for example better honey production and temperament, but compromise local subspecies genetic integrity. Since no reproductive barriers are present, mating between inter-fertile subspecies leads to genetic pollution, which creates uniformity in honey bee populations and erodes adaptive biodiversity for short-term financially driven goals. Indeed, *A. m. ruttneri* was believed extinct due to pathogens and hybridisation from introduced colonies and was followed by misguided but intensive replacements with foreign stock (Zammit-Mangion et al., 2017a). Despite this, genetic (Momeni et al., 2021) and morphometric (Zammit-Mangion et al., 2017b) studies indicated the presence of *A. m. ruttneri*, albeit with notable hybridisation (Janczyk et al., 2021). Controversially in 2015, a commercial queen rearing enterprise using foreign subspecies on Gozo was authorised to operate (Zammit-Mangion et al., 2017b), despite local beekeepers' genetic integrity concerns for the native *A. m. ruttneri*. Astonishingly, local authorities downplayed these worries and concerns (Maltatoday, 2015), and since then, socio-economic tensions have persisted in the Gozitan apicultural sector (Maltatoday, 2021).

This study used traditional and geometric morphometric methods with separate discriminant analyses conducted for each approach to provide insights about hybridisation status from selected colonies of *A. m. ruttneri* following introduction events in Gozo and Malta.

MATERIAL AND METHODS

Sampling

Between August and November 2021, a total of fifty-five colony samples were collected from fourteen apiaries in Gozo (n=34) and seven apiaries in Malta (n=21), with multiple colonies being sampled from each apiary (Fig. 1). Sampling was performed from apiaries owned by local beekeepers who had not imported foreign honey bees and confirmed that to their knowledge the colonies were of the native *A. m. ruttneri*. Around twenty adult worker bees per hive were collected from frames, immediately placed in 95% ethanol, refreshed after 24 hours to ensure optimal preservation, and stored at 4°C until morphological and genetic analyses.

Furthermore, an additional twelve colony samples of *A. m. intermissa* (n=1), *A. m. ligustica* (n=3) sampled in 2022 and *A. m. siciliana* (n=8) sampled in 2022 were obtained from international partners, hereafter referred to as 'additional reference colonies'. These samples were collected to expand reference subspecies data and improve the reliability of discriminant analyses. Preliminary statistical evaluation was conducted to determine whether the additional reference colonies should be included in the subspecies reference colony data, as outlined herafter.

Fig. 1. Map showing distribution of sampled apiaries in Gozo (1-14) and Malta (15-21).

Specimen preparation

Ten worker bees from each sampled colony were subjected to morphometric analysis. Abdominal colour patterns were noted before the preparation of wing slides. Right forewings were cut at the base and mounted on glass slides with Dibutylphthalate Polystyrene Xylene (DPX). After being cured for two days, the forewings were viewed under a stereomicroscope, and wing slides with misfolding or significant air bubble accumulation were removed. A total of 514 wing slides were included in the analysis. Digital images of the wings were taken with the use of a SWIFT Optical 10mp microscope camera.

Traditional morphometry

Assessment with the use of traditional morphometry was based on eleven 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 (Swift, 2020). Character measurements were averaged per colony sample.

Subspecies reference colony data for *A. m. intermissa*, *A. m. ligustica*, *A. m ruttneri*, *A. m. sahariensis* and *A. m. siciliana* were obtained from the Institut für Bienenkunde, Oberursel, Germany. Colony average data was uploaded to SPSS version 25.0 (IBM Corp., 2017). Multivariate Analysis of Variance tests (MANOVAs) compared additional reference colonies with Oberursel reference colonies, per subspecies. If no statistically significant difference was found, the additional reference colonies were incorporated into the subspecies reference colony data.

PAST Version 3.21 (Hammer et al., 2013) was used for traditional morphometry to construct a principal component analysis (PCA) with the use of subspecies reference colony data to identify general trends. The PCA plot positioned *A. m. siciliana* between *A. m. ligustica* and *A. m. ruttneri*, mirroring the placement expected for potential hybrid colonies (Kandemir et al., 2011). To prevent confounding results, *A. m. siciliana* was excluded from further analysis. This pattern was also observed separately with the use of geometric data, leading to its similar exclusion. The final subspecies reference colony data for the traditional morphometric analysis included: *A. m. intermissa* (n=12), *A. m. ligustica* (n=15), *A. m. ruttneri* (n=15) and *A. m. sahariensis* (n=6).

Geometric morphometry

Geometric morphometry utilised nineteen landmark points at wing vein intersections plotted with Identifly software version 1.6.2 (Tofilski, 2017). The landmarks were then aligned with the use of Procrustes fit method and averaged per colony with MorphoJ version 1.04 software (Klingenberg, 2011).

Subspecies reference colony data for *A. m. carnica*, *A. m. intermissa*, *A. m. ligustica*, *A. m ruttneri*, *A. m. sahariensis* and *A. m. siciliana* were received from Prof. Adam Tofilski (Krakow University of Agriculture, Poland) and Dr. Stefan Fuchs (Institut für Bienenkunde, Oberursel, Germany). Similarly to the traditional morphometry approach, colony-averaged landmark coordinates were uploaded to SPSS version 25.0 (IBM Corp., 2017). MANOVAs compared additional reference colonies with obtained (from Prof. Tofilski & Dr. Fuchs) reference colonies, per subspecies. If no statistically significant difference was found, the additional reference colonies were incorporated into the subspecies reference colony data.

MorphoJ version 1.04 software (Klingenberg, 2011) was used for geometric morphometry to construct a principal component analysis (PCA) with the use of subspecies reference colony data to identify general trends. The final subspecies reference colony data for the geometric morphometric analysis included: *A. m. carnica* (n=15), *A. m. intermissa* (n=19), *A. m. ligustica* (n=14), *A. m. ruttneri* (n=5) and *A. m. sahariensis* (n=4). Additionally, with the use of the same software, wireframes illustrating mean wing shapes based on reference subspecies or sampling location (Gozo or Malta) were generated. The differences in wing shapes (wireframes) were magnified twofold for clearer visualisation.

Discriminant analysis

Sampled Gozitan and Maltese colonies were classified to a subspecies with the use of separate discriminant analyses based on traditional and geometric morphometric data with SPSS Version 25.0 (IBM Corp., 2017). Each model compared the Gozitan and Maltese collected colony samples to subspecies reference colony data, generating discrimination scores, probabilities, scatterplots and predicted classified subspecies. Leave-one-out cross-validation was used to assess the accuracy of each classification model. Mahalanobis distances between reference subspecies used were also recorded from multivariate plots.

Abdominal colour pattern analysis

A Mann-Whitney U test was conducted to assess statistically significant difference between the predicted classified lineages (lineage A for *A. m. intermissa*, *A. m. ruttneri*, and *A. m. sahariensis*; lineage C for *A. m. carnica* and *A. m. ligustica*) of collected samples (Gozitan and Maltese) based on the geometric data discriminant analysis and on abdominal colour patterns). The classification of abdominal colour pattern was based on yellow markings, with samples grouped according to the pigmentation ratio: '100% black', '70-90% black', '40-60% black/yellow', '70-90% yellow' and '100% yellow'.

RESULTS

The results are based on the Gozitan and Maltese colonies collected during this study, which were then compared to reference colonies compiled from various established sources, as indicated in the methodology.

Traditional morphometry

Using traditional morphometry data, pairwise Mahalanobis distances based on group centroid of subspecies reference colony data revealed the largest difference between *A. m. ligustica* and *A. m. ruttneri* and the smallest difference between *A. m. intermissa* and *A. m. sahariensis* (Tab. 1). The leave-one-out cross-validation test returned a 91.7% accuracy rate.

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. Gozitan samples were classified as *A. m. ruttneri* (82.35%), *A. m. ligustica* (11.76%), and *A. m. sahariensis* (5.88%). Maltese samples were classified as *A. m. ruttneri* (76.19%) and *A. m. ligustica* (23.81%) (Tab. 2).

Geometric morphometry

Using geometric morphometry data, pairwise Mahalanobis distances based on group centroid of subspecies reference colony data revealed the largest difference between *A. m. carnica* and *A. m. sahariensis* and the smallest difference between *A. m. carnica* and *A. m. ligustica* (Tab. 1). The leave-one-out cross-validation test returned an 80.7% accuracy rate.

Similar to the scatterplot generated from the traditional data discriminant analysis, but in a more pronounced fashion, 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). Gozitan samples were classified as *A. m. ligustica* (44.12%), *A. m. ruttneri* (41.18%), *A. m. intermissa* (11.76%)

Table 1.

Mahalanobis distances based on separate traditional and geometric subspecies reference colony data in the discriminant plot

		Geometric				
		A. m. carnica	***A. m. intermissa***	***A. m. ligustica***	**A. m. ruttneri**	***A. m. sahariensis***
	A. m. carnica	-	24.10*	8.11*	23.17*	28.88*
Traditional	***A. m. intermissa***	-	-	22.31*	10.15*	9.87*
	A. m. ligustica	-	8.98*	-	21.06*	27.22*
	A. m. ruttneri	-	7.14*	10.15*	-	13.76*
	A. m. sahariensis	-	5.2*	9.01*	7.34*	-

*all pairwise differences were significant ($p<0.05$)

and *A. m. carnica* (2.94%). Maltese samples were classified as *A. m. intermissa* (42.86%), *A. m. ligustica* (28.57%), *A. m ruttneri* (23.81%) and *A. m. carnica* (4.76%) (Tab. 2).

The wireframes constructed using geometric morphometry analysis revealed slight differences in venation patterns between Gozitan and Maltese honey bee colony samples, with both populations exhibiting nearly identical shifts when compared to *A. m. ruttneri* and *A. m. ligustica* subspecies reference colony data. In these comparisons, landmarks 5 and 18

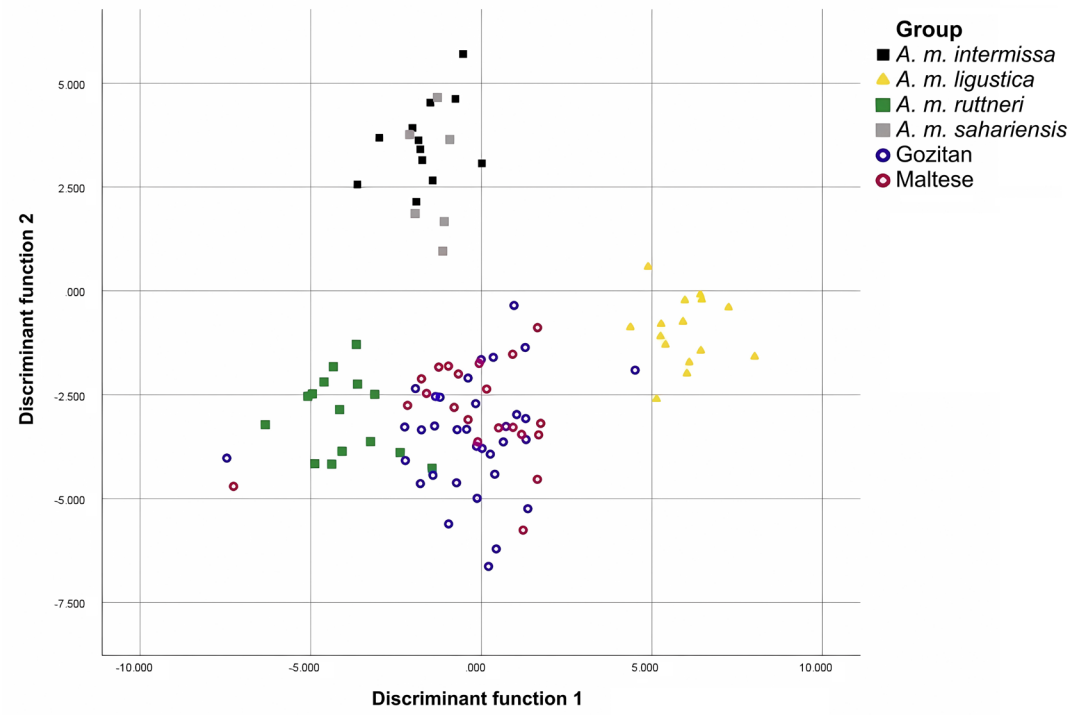

Fig. 2. Discrimination scatterplot using traditional morphometry showing Maltese and Gozitan colony samples projected into plot-space based on discrimination function 1 (65.7% of variance) vs. discrimination function 2 (27.9% of variance).

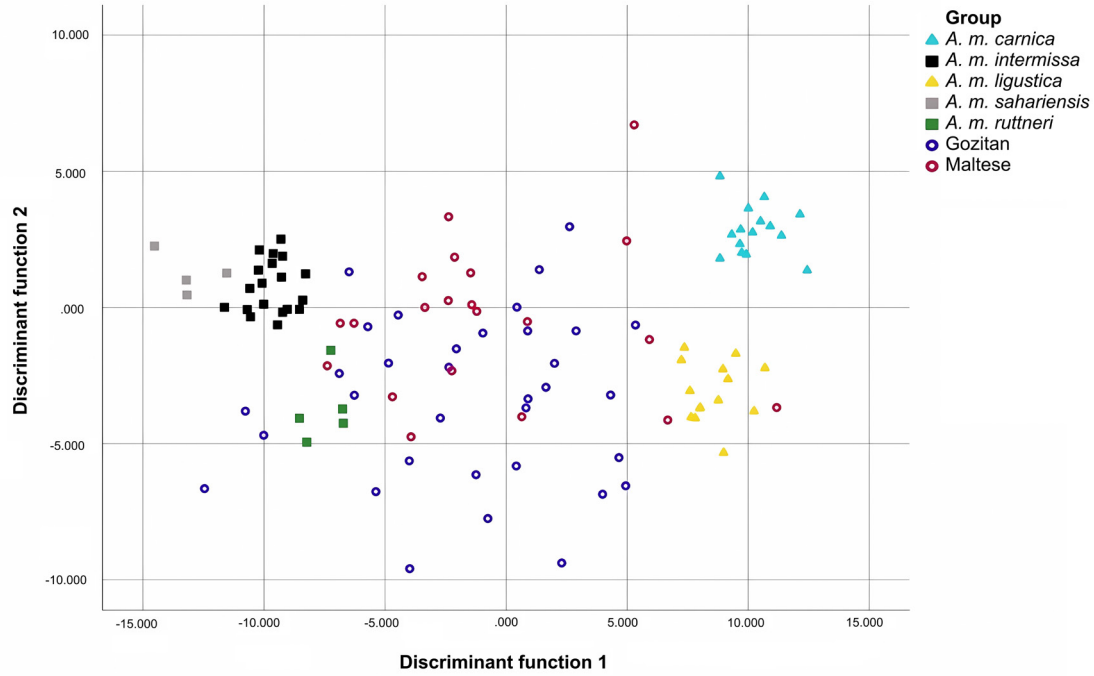

Fig. 3. Discrimination scatterplot using geometric morphometry showing Maltese and Gozitan colony samples projected into plot-space based on discrimination function 1 (87.3% of variance) vs. discrimination function 3 (5.5% of variance).

showed notable differences for the former, while landmarks 13 and 19 exhibited significant differences for the latter. The wireframe illustrating historical differences between *A. m. ruttneri* (A-lineage) and *A. m. ligustica* (C-lineage) highlighted distinctive differences most prominently in landmark 13.

Abdominal colour pattern

No statistically significant difference was found (Mann-Whitney U, p=0.28) between predicted classified lineages (A or C) of collected samples (Gozitan and Maltese) based on the geometric data discriminant analysis and abdominal colour pattern.

DISCUSSION

Hybridisation trends

Hybrids of two honey bee subspecies exhibit intermediate wing venation patterns, which reflect continuous morphometric measurements and reduced subspecies resolution (Nazzi, 1992; Kekeçoğlu, 2018; Węgrzynowicz et al., 2019). The results from this study show similar intermediate hybridisation patterns in Gozitan and Maltese samples of *A. m. ruttneri* (Fig. 2, 3) and minimal differences in wireframes. These observations underscore introgression, even among beekeepers rearing the native honey bee. Previous research on *A. m. ruttneri* reached differing conclusions on hybridisation status. Traditional morphometry studies indicated limited hybridisation (Zammit-Mangion et al., 2017b), while the geometric morphometry results from Janczyk et al. (2021) and the present study's traditional and geometric morphometry results showed hybrid traits in the collected samples. The temporal gaps between sampling efforts of the mentioned studies in 2014, 2018, and 2021 could be a significant factor contributing to the observed increase in hybridisation trends, particularly in Gozo, following the establishment of the commercial queen rearing enterprise in 2015.

Some samples were occasionally assigned to other A-lineage subspecies. The geometric data discriminant analysis model classified some Maltese (43%) and Gozitan (12%) samples as *A. m. intermissa*, while the traditional data discriminant analysis classified some Gozitan (6%) samples as being *A. m. sahariensis*. The importation of colonies from Northern Africa is unlikely. However, hybridisation has also been identified as a factor contributing to the occurrence of random wing venation patterns (Węgrzynowicz et al., 2019). Additionally, the low resolution among A-lineage subspecies is attributed to phylogenetic proximity (Henriques et al., 2022), also confirmed in this study through morphometry (Tab. 1). The smallest pairwise Mahalanobis distances were observed within the A-lineage subspecies reference colonies and within the C-lineage subspecies reference colonies respectively.

The genetic structure of drone congregation areas directly determines honey bee genetic dynamics (Jaffé et al., 2009). Some evidence points to partial reproductive isolation between

Table 2.

Summary of predicted classified subspecies for Gozitan and Maltese collected colony samples based on separate traditional and geometric data discriminant analyses

		Gozo		Malta	
		Count	%	Count	%
Traditional	*A. m. intermissa*	0	0.0	0	0.0
	A. m. ligustica	4	11.8	5	23.8
	A. m. ruttneri	28	82.4	16	76.2
	A. m. sahariensis	2	5.9	0	0.0
Geometric	*A. m. carnica*	1	2.9	1	4.8
	A. m. intermissa	4	11.8	9	42.9
	A. m. ligustica	15	44.1	6	28.6
	A. m. ruttneri	14	41.2	5	23.8
	A. m. sahariensis	0	0.0	0	0.0

drones of different subspecies (Koeniger et al., 1989; Szabo & Lefkovitch, 1992). The exhibiting of annual bimodal drone production by *A. m. ruttneri* suggests some temporal isolation from other subspecies or strains (Farrugia et al., 2022). Nevertheless, the introduction of foreign honey bees into habitats can lead to immediate hybridisation (De la Rúa et al., 2009). Particularly in Gozo, since the establishment of the commercial queen rearing enterprise using foreign subspecies and strains, an intensified influx of foreign drones has posed an immediate risk of introgression. Despite the potential of reproductive isolation in *A. m. ruttneri*, this study's hybrids' prevalence suggests that these processes are only limitedly effective. As hybridisation can occur between colonies from 15 km away (Jensen et al., 2005a), and Gozo's coastlines are only 14 km apart, hybridisation potential is markedly higher given the island's small area of 67 km^2. Furthermore, this is worsened by a relatively high mean hive density on Gozo, with around 20 colonies per km^2 from 2021 data (Gemma, P. & Francesco Luca, A., unpubl. data, 2023). These events are jeopardising the endemic *A. m. ruttneri* via hybridisation at a loss of desirable adaptive alleles that have evolved in it, including increased honey production, and disease resistance (Uzunov et al., 2023). Despite this, *A. m. ruttneri* phenotypes still emerged, aligning with Janczyk et al.'s (2021) findings.

Hybrid performance and implications

Research on honey bee hybridisation has shown that genetic admixture erodes adaptive genetic diversity by disrupting gene complexes, leading to reduced fitness and negative heterosis (Harrison & Hall, 1993; Schneider & Hall, 1997; Schneider et al., 2003; Büchler et al., 2014). This process also contributes to reduced resistance against biotic and abiotic stressors (Le Conte & Navajas, 2008). As gene flow between subspecies is asymmetrical and severity of the rate of displacement is dependent on various factors, outcomes remain unpredictable which have both ecological and commercial implications. The use of maladapted honey bees leads to poorer production and higher colony losses compared to local, more resilient subspecies (Büchler et al., 2014; Hatjina et al., 2014; Uzunov et al., 2023). 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). Research has also shown that subspecies maintain their annual colony developmental trajectories according to native habitat even when relocated, translating to imported colonies being less productive in introduced environments (Hatjina et al., 2014; Uzunov et al., 2023).

Imported foreign queens in the Maltese Islands have reportedly exhibited local traits or experienced colony decline over time, observations echoed by the owner of the commercial queen rearing enterprise set up in Gozo to water down concerns of hybridisation (Maltatoday, 2015). These observations align with the selective advantage of African matrilines, including *A. m. ruttneri* in their native warm climates (Schneider & Hall, 1997). European subspecies and hybrids appear to be less competitive unless actively managed (Schneider et al., 2003), and foreign queen preference for native drones likely due to higher fitness and adaptation has been documented (Muñoz et al., 2014a; Miguel et al., 2015). Nevertheless, these arguments are insufficient to justify risking the loss of a unique honey bee subspecies endemic to the Maltese Islands. The precautionary principle should always be applied to prevent irreversible consequences resulting in biodiversity loss (Chetcuti Dimech et al., 2023).

Morphometric approaches used

The accuracy of any predictive model depends critically on the reference samples used for classification. In this study, direct comparisons between the morphometric methods used to predict subspecies classification of the collected samples are of limited significance. This is because although the reference subspecies data for constructing the separate discriminant analyses based on traditional and geometric morphometry

respectively were officially sourced, they may have come from different colonies. Additionally, the separate models are not based on the same reference subspecies, and the common reference subspecies differ in the number of colonies used.

Expanding the reference colonies, particularly by including different reference subspecies from the O and M lineages, and number of reference colonies samples would significantly enhance the resolution of any discriminant analysis model. Future work could involve using the same reference colonies to enable direct comparisons between traditional and geometric morphometry, which would help determine which approach more accurately assesses the hybridisation status of *A. m. ruttneri* colonies. Complementing these methods with genetic studies could provide insights derived with a multifaceted perspective.

Both discriminant analysis classification models, independently applied to each morphometric approach, achieved high leave-one-out cross-validation accuracy rates, which reaffirm the effectiveness of morphometric tools. Traditional morphometry remains a simple and cost-effective technique applicable to ongoing research, while also serving as a valuable method for maintaining records of defining subspecies or ecotype characteristics. In comparison, geometric morphometry significantly reduces data collection time through software-assisted analysis, enhancing efficiency without compromising accuracy.

Abdominal colour pattern

Honey bee abdominal colour pattern is a recognisable feature often used by beekeepers to quickly establish purity (Henriques et al., 2020). Yellow bands in characteristically black coloured honey bees are interpreted as a sign of hybridisation events (Koeniger et al., 1989). Severe hybridisation notably on Gozo was reported by beekeepers, with colonies displaying more yellow abdominal bands in recent years since the establishment of the commercial queen rearing enterprise. Various intermediate forms showing distinctive patterns were observed during data collection. In this study, abdominal colour pattern alone was shown to be an unreliable feature to predict classified lineage (A or C) of collected samples based on the geometric data discriminant analysis. Case in point, some samples with high yellow pigmentation ratio were classified as being ≈100% *A. m. ruttneri* by both traditional and geometric discriminant analyses (samples *ZBG05* and *ZBG08*). These results echo similar findings for *A. m. iberiensis* (Henriques et al., 2020). Nevertheless, abdominal colouration in conjunction with other morphometric parameters may still be important in honey bee identification. Possibly abdominal colouration may be used as a preliminary tool, followed by genetic analysis to aid colony hybrid status identification.

Conservation initiatives

In various countries, sanctuaries have been established to protect or restore local honey bee diversity, with varying degrees of success (Kryger, 2009; Miguel et al., 2015). However, continuous monitoring is essential since instances where conservation efforts were implemented and foreign alleles persist are unfortunately common (Jensen et al., 2005b; Muñoz & De la Rúa, 2012; Muñoz et al., 2014b; Pinto et al., 2014). A nation or region wide action to mitigate introgressive genetic loss caused by human-mediated introductions would be more effective. Islands and island-nations such as Malta, with their natural geographic separation, which has also directly contributed to the evolution of unique honey bee subspecies, theoretically provide a more feasible environment for such measures. However, the successful conservation of honey bee biodiversity relies on comprehensive legal protection, supported by policies and effective enforcement to restrict the introduction of foreign honey bees.

A study by Chetcuti Dimech et al. (2023) outlined feasible legal measures to holistically safeguard the Maltese honey bee. Building on the findings of this latter study, Malta has taken a positive step forward by declaring *A. m. ruttneri* as its national insect. However, the article in the legislation intended to prevent the introduction of alien species (including subspecies) that

could directly threaten declared Maltese flora and fauna, was amended to not apply to the Maltese honey bee (Republic of Malta, 2024). Thus, this declaration does little to ensure the holistic protection of this endemic subspecies, primarily preventing genetic contamination. While sporadic local conservation efforts have taken place to protect the Maltese honey bee (Uzunov et al., 2018), the extent of participation and long -term effectiveness remain uncertain. Involving the local beekeeping community in conservation projects is crucial to avoid controversy and promote cooperation, as showcased prominently by the Læsø case (Kryger, 2009). Biodiversity protection ensures economic sustainability. Local adaptations of native honey bees are increasingly being recognised as one of the main key factors which will significantly impact long-term sustainability of apiculture. A notable global challenge is hybridisation of native subspecies, since this process dilutes adaptive alleles and thus subspecies' resilience. As an important receptacle of environmental adaptations, *A. m. ruttneri* and its genetic component should be protected as best as possible. Any beekeeping activities on the Maltese Islands should be centred around it. Foreign honey bee introductions jeopardise the genetic and morphological traits of *A. m. ruttneri*. Despite remaining *A. m. ruttneri*-like colonies, strong hybridisation trends surfaced in both Gozitan and Maltese colonies. The recent commercial queen rearing enterprise set up in Gozo could be likely driving rapid and severe introgression patterns in the Gozitan sub-population, despite dual insularity. Moreover, foreign colony introductions can also facilitate the establishment of new pests and pathogens, as observed historically, endangering also other native pollinators.

Conserving native subspecies and their adaptive potential hinges on recognising their value and implementing effective policies involving all stakeholders to prevent a 'tragedy of the commons'. With current regulations failing to holistically protect the Maltese honey bee allowing continuous introductions, the purging of foreign genetic alleles is unlikely. Robust regulatory policies and enforcement are crucial to prevent declines, particularly importation restrictions, which this study's findings emphasise as urgent. The introductions threshold which will allow the restoration of *A. m. ruttneri* traits is uncertain. However, clear hybridisation patterns have emerged, indicating that the genetic integrity of the Maltese and Gozitan honey bee populations is at existential risk.

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Supplementary Material

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