

Apis mellifera Mitochondrial DNA (mtDNA), A Review Paper

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Abstract. This review paper presents a comprehensive analysis of the mitochondrial DNA (mtDNA) of *Apis mellifera*, commonly referred to as the Western honey bee. The present study aims to examine the genetic diversity, population structure, and evolutionary history of a species of insect that holds economic significance. This study elucidates the genetic determinants that impact the adaptive capacity, behavioral patterns, and overall well-being of honey bees through the examination of mitochondrial DNA (mtDNA) sequences derived from diverse honey bee populations across the globe. Furthermore, this study examines the potential implications of mitochondrial DNA (mtDNA) research on various aspects of beekeeping practices, conservation initiatives, and the comprehension of honey bee biology. This paper serves as a significant asset for researchers, beekeepers, and conservationists with a keen interest in exploring the genetic characteristics of *Apis mellifera*

1 Introduction

The presence of a distinct form of DNA, known as mitochondrial DNA (mtDNA), has been identified within the mitochondria of *Apis mellifera*, commonly known as the honey bee. The transmission of this trait is maternally inherited, indicating that it is passed down from the mother bee to her offspring. The investigation into the mitochondrial DNA of *Apis mellifera* provides valuable insights into the genetic variability, population dynamics, and evolutionary trajectory of honey bees [1,2]. The utilization of mitochondrial DNA (mtDNA) analysis has proven instrumental in the identification and categorization of diverse subspecies and lineages within the species *Apis mellifera*. This approach has greatly contributed to the advancement of their taxonomic classification and enhanced our comprehension of their spatial dispersion. According to the provided references [1,3] it is evident that the subject matter under discussion has been The utilization of mitochondrial DNA (mtDNA) sequences by researchers has facilitated the examination of the genetic affinities among various honey bee populations, consequently leading to an enhanced comprehension of their migratory patterns and the exchange of genetic material [1,4] it is evident that the subject matter under discussion has been extensively researched The high mutation rate of mitochondrial DNA (mtDNA) facilitates the identification of genetic variations and the investigation of evolutionary mechanisms within relatively short temporal frameworks [1,4] The genetic diversity and population dynamics of *Apis mellifera* have been substantially influenced by various human activities, including beekeeping practices and habitat fragmentation. In the investigation of these impacts, the

utilization of mitochondrial DNA (mtDNA) analysis has proven to be an invaluable tool [1,5]. It is evident that there is a significant body of research supporting the comprehension of the mitochondrial DNA (mtDNA) of *Apis mellifera*, which holds significant importance in the context of conservation endeavors, as it facilitates the discernment of genetically unique populations that may require tailored conservation strategies [1,6]. Review articles on *Apis mellifera* mtDNA offer a thorough and extensive examination of the present understanding, methodologies, and discoveries in this particular domain. Consequently, these articles contribute to the advancement of our comprehension regarding the genetics and evolution of honey bees [1,6].

This review paper provides a comprehensive overview of the mtDNA of *Apis mellifera*, covering topics such as genetic diversity, population structure, evolutionary history, and the implications of mtDNA research for beekeeping and conservation. It serves as a valuable resource for researchers, beekeepers, and conservationists interested in the genetic aspects of Western honey bees.

Genetic Diversity of *Apis mellifera* mtDNA

1. Overview of the different mtDNA haplotypes and their distribution

The transmission of mitochondrial DNA (mtDNA) occurs exclusively through the maternal lineage, rendering it a valuable tool for the investigation of maternal lineages and the inference of population history. The utilization of mitochondrial DNA (mtDNA) is of utmost importance in the examination of genetic diversity and evolutionary patterns within honey bee populations. Recent studies have successfully elucidated

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the genetic diversity of honey bee populations through the meticulous analysis of mitochondrial DNA (mtDNA) haplotypes. In research conducted in Russia, the maternal lineage of honey bees from 19 regions was determined, and the dark forest bee population in Russia was classified into two distinct groups [7]. The mitochondrial marker tRNA^{Leu}-COII was employed as a tool for this investigation. In a separate investigation carried out in Argentina, the prevalence and geographic distribution of Africanized bees in the province of Buenos Aires were determined utilizing an mtDNA cytochrome b polymorphism [8]. The present study conducted an investigation of the genetic diversity of Turkish honeybees by examining the variation at four diagnostic restriction sites and the sequence of a noncoding intergenic region in the mitochondrial DNA (mtDNA) of 84 colonies [9]. The analysis resulted in the identification of four distinct haplotypes. A study was conducted in Iran to investigate the genetic composition of honey bee populations (*Apis mellifera* meda) at two specific mitochondrial regions. The study identified three previously unreported composite genotypes (haplotypes) [10]. The investigation of mitochondrial DNA (mtDNA) is of paramount importance for the comprehensive understanding of the genetic diversity and evolutionary dynamics within honey bee populations [8-11].

2. Factors influencing genetic diversity within honey bee populations

The impact of management practices in beekeeping on honey bee populations' genetic diversity has been a subject of concern [12]. Activities such as the extensive relocation of hive stocks, introductions to novel regions, implementation of breeding programs, and the trading of queens have been identified as potential threats to genetic diversity in both wild and managed honey bee populations [12]. Furthermore, the process of urbanization has the potential to exert an impact on the genetic diversity observed within both feral and managed honey bee colonies [13]. Additional factors that contribute to the phenomenon under investigation encompass natural selection, feralization, and the ramifications of anthropogenic activities such as habitat destruction, environmental contamination, and climate alteration [14]. The occurrence of inbreeding within populations can also lead to a depletion of heterozygotes, which serves as an indicator of a reduction in genetic diversity [15]. Hence, the implementation of conservation strategies aimed at safeguarding the genetic diversity of honey bee populations becomes imperative. These strategies encompass refraining from interregional exchange, refraining from selectively breeding solely local subspecies for beekeeping purposes, and granting colonies the autonomy to determine the succession of queens in the subsequent generation [12].

Population Structure and Evolutionary History

The population structure and evolution of honeybees, specifically *Apis mellifera* and *Apis cerana*, can be understood through the following key points.

1. Genetic Diversity and Population Structure

Honeybees exhibit high genetic diversity, with distinct populations structured differently [16-17]. This population structure can be influenced by various factors, including geographical proximity, ecological conditions, and historical events [16,18].

2. Demographic History and Adaptation

Studies on human-managed honeybees have shown that they exhibit distinct population-based genomic changes, suggesting a history of adaptation to their managed environment [17]. However, there has been a lack of analysis regarding the evolutionary history of wild honeybees, particularly their adaptation to their natural environment [17,18].

3. Evolutionary History and Adaptation

Wild honeybees, specifically those in the SWIO, display a genetic clustering that suggests a similar insular evolution of both hosts and parasites [19]. This clustering is also observed in other archipelagos, further supporting a shared ancestral population [19].

4. Role of Colonization and Population Expansion

The honeybees' population structure and evolution have been influenced by their colonization history and population expansion [20,21]. For instance, the honeybees in Puerto Rico exhibit a unique genetic structure due to their distinct colonization history [20].

5. Role of Parasites

The microsporidian parasite exhibits a similar genetic clustering as the honeybee hosts, suggesting a shared evolutionary history [19].

6. Future Directions

Understanding the population structure and evolution of honeybees is crucial for maintaining genetic diversity, which is vital for maintaining the health and productivity of honeybee colonies [16,21]. Further studies focusing on wild honeybees and their evolutionary history are needed to gain a comprehensive understanding of honeybee genetics [16,17].

7. Analysis of population structure using mtDNA markers

The examination of population structure in honeybees through the analysis of mitochondrial DNA (mtDNA) loci involves the investigation of genetic diversity and differentiation among different populations of honeybees. The present mode of analysis illuminates the

evolutionary history, genetic variability, and population dynamics of honeybees.

A pair of scholarly articles have been identified that pertain to the examination of population structure in honeybees through the utilization of mitochondrial DNA (mtDNA) markers. The inaugural investigation carried out in Slovenia employed mitochondrial and nuclear DNA analyses to examine the genetic composition of the Carniolan bee population. The mitochondrial DNA (mtDNA) analysis conducted on honeybees from Slovenia revealed a limited degree of genetic diversity within the COI and COII regions. The highly homogeneous structure of the population was observed through microsatellite analysis [22]. The subsequent investigation conducted in Türkiye focused on the examination of the population structure of honeybees across diverse regions through the utilization of microsatellite markers. The findings of this study suggest that the overall structure of the population remained intact, as evidenced by the presence of gene flow between different clusters. The investigation successfully delineated four discrete clusters, each of which aligns with the respective geographic distributions of four distinct subspecies of honeybees [23].

Based on the findings presented, it is evident that the utilization of mtDNA markers holds significant value in the examination of honeybee population structure, thereby facilitating a more comprehensive comprehension of their genetic diversity and differentiation. The aforementioned analyses provide valuable insights pertaining to the evolutionary dynamics and conservation strategies concerning honeybee populations.

7.1 Insights into the evolutionary relationships among honey bee populations

Two publications on the evolutionary connections between populations of honey bees may be found in the search results. The initial investigation focused on evaluating the genetic diversity of honey bee populations in Turkey by utilizing the ND5 region of the mitochondrial genome. The investigation conducted in this study has successfully identified five previously unreported haplotypes pertaining to the ND5 gene segment of Turkish honeybees. Furthermore, the findings of this study have shed light on the existence of limited genetic similarity among populations of managed honey bees [24]. The investigation of the evolutionary relationships of the Western honey bee was conducted in the second study, utilizing mitochondrial genomes as the primary source of genetic information. The primary objective of this study was to establish a robust phylogenetic framework for the Western honey bee, with the aim of facilitating the investigation of the evolutionary patterns underlying economically significant traits within this species. The investigation revealed that the associations among the different subspecies of *A. mellifera* exhibit an ambiguous nature, characterized by conflicting outcomes [4].

Based on the findings presented, it is evident that further investigation is warranted in order to gain a

comprehensive understanding of the evolutionary connections among different honey bee populations. The utilization of mitochondrial DNA markers, specifically the ND5 region and mitochondrial genomes, has proven to be a valuable tool in elucidating the intricate evolutionary history and genetic diversity of honey bee populations. Further investigation is necessary in order to establish a dependable phylogeny of subspecies within the honey bee species, as well as to gain a comprehensive understanding of the factors that contribute to their genetic differentiation and evolutionary processes.

7.2 Historical events shaping the genetic structure of *Apis mellifera*

The genetic composition of global populations of *Apis mellifera* has been substantially influenced by historical occurrences. Notwithstanding this, several investigations have been conducted to assess the genetic variability and demographic patterns of honeybee populations in Turkey, Algeria, and Europe, among other geographical areas. These studies have employed diverse genetic markers, including microsatellites, mitochondrial DNA, and whole-genome sequencing, to achieve their research objectives. The aforementioned studies have successfully identified discrete subspecies of honeybees and have provided valuable insights into the considerable genetic diversity and population differentiation within these subspecies. A study conducted in Turkey utilized microsatellite markers to investigate the genetic divergence observed among honeybee populations. The findings revealed significant levels of genetic divergence, which align with the established notion of Anatolia serving as the genetic hub for honeybees in the Middle Eastern region [25]. The present study aimed to assess the genetic diversity and population structure of various subspecies of *Apis mellifera* through the utilization of complementary sex determiner (CSD) gene analysis in both Algeria and Europe. The study conducted an investigation into the presence of polymorphisms within the CSD gene across six distinct subspecies of *Apis mellifera* [26]. The available literature suggests that honeybee populations demonstrate considerable levels of genetic diversity, characterized by variations in the frequency and distribution of alleles as well as the extent of population differentiation. Further investigation is necessary in order to establish a dependable phylogeny of subspecies within the honeybee species as well as gain a comprehensive understanding of the factors that contribute to their genetic differentiation and evolutionary processes.

Genetic Factors Influencing Adaptation and Behavior

1. Examination of mtDNA variations associated with specific traits

Numerous inquiries have been conducted to examine the correlation between variations in mitochondrial DNA

(mtDNA) and specific phenotypic traits across diverse taxa. A research investigation pertaining to bovine species, for instance, has successfully identified specific genetic variations known as single-nucleotide polymorphisms (SNPs) that are closely linked to various milk production traits. Notably, the study has highlighted the significance of two specific SNPs, namely NUB1 and SLC24A2, in relation to these characteristics [27]. A separate investigation was conducted to explore the genetic association between mitochondrial DNA copy number and growth and carcass characteristics in beef cattle [28]. The association between mitochondrial DNA (mtDNA) haplotypes and phenotypic traits, including coat color and endurance performance, has been established in pigs [29,30]. The Korean population has observed a correlation between physical performance and athlete status with specific mitochondrial DNA (mtDNA) haplogroups [31]. Furthermore, an extensive study on the human genome was conducted, which aimed to identify specific regions associated with nucleotide metabolism, platelet activation, and megakaryocyte proliferation. This investigation also shed light on the causal relationship between mitochondrial function and mortality, as supported by previous research [32]. The collective body of evidence from these studies suggests a significant correlation between variations in mitochondrial DNA (mtDNA) and distinct phenotypic traits, thereby providing valuable insights into the underlying genetic mechanisms governing these particular characteristics.

2. Example of the Examination of mtDNA variations associated with specific traits in honeybees

2.1 Genetic Structure of Carniolan Bee Population

The genetic structure of the Carniolan bee population was investigated in a study conducted in Slovenia, employing mitochondrial and nuclear DNA analyses. The study revealed that Slovenian honeybees exhibit minimal genetic differentiation in the COI and COII regions of their mitochondrial DNA (mtDNA). The researchers also discerned particular mitochondrial DNA (mtDNA) haplotypes that are linked to specific populations. For instance, they observed the presence of the C2C haplotype in bee samples collected from Slovenia, Croatia, Poland, Germany, and the Czech Republic [33].

2.2 Haplotype Diversity in USA Honey Bee Populations

The present study aims to investigate the diversity of haplotypes within the United States of America (USA). The analysis focuses on examining the genetic variations and distribution patterns of haplotypes across different regions of the country. By utilizing a comprehensive dataset comprising a diverse range of individuals from various ethnic backgrounds, this

research endeavors to shed light on A subsequent investigation utilized mitochondrial DNA (mtDNA) loci to examine the genetic diversity of honey bee populations within the geographical boundaries of the United States. The investigation successfully delineated three distinct maternal evolutionary lineages, namely lineage C originating from the northern Mediterranean, lineage M originating from the western Mediterranean, and lineage A originating from Africa. The study revealed that the subspecies *A. m. ligustica* and *A. m. carnica*, found in the United States, exhibit a notable reliance on two distinct haplotypes (C1 and C2j) belonging to the same lineage [34].

2.3 Genetic Divergence of Turkish Honeybee Subspecies

The investigation titled "Genetic Divergence of Turkish *Apis mellifera* Subspecies" conducted in Turkey employed the sequencing of the ND5 mitochondrial segment as a means to ascertain the genetic divergence exhibited by various subspecies of *Apis mellifera* indigenous to Turkey. A total of five distinct haplotypes were successfully identified for the ND5 gene segment in the population of Turkish honeybees. The examination of the ND5 region yielded insights into the genetic diversity of honeybee populations in Turkey, as evidenced by the observed genetic distances and variations [24].

The aforementioned studies provide evidence regarding the correlation between particular mitochondrial DNA (mtDNA) variations and discrete populations and subspecies of honeybees. The examination of mitochondrial DNA (mtDNA) variations presents an opportunity to elucidate the intricate genetic architecture, expansive diversity, and evolutionary interrelationships among different populations of honeybees. Further investigation is warranted to explore the distinct characteristics and functional implications of mitochondrial DNA (mtDNA) variations in honeybees.

Directions

1. Potential areas for further research on *Apis mellifera* mtDNA

The utilization of mitochondrial DNA (mtDNA) analysis presents a versatile approach for the assessment of honeybee health through various methodologies. The present discourse presents a number of prospective avenues for further inquiry pertaining to the mitochondrial DNA (mtDNA) of *Apis mellifera*.

1.2 Disease resistance

Further investigation can be undertaken to ascertain the mitochondrial DNA (mtDNA) haplotypes that are

correlated with heightened resistance against diseases such as Varroa mites. This endeavor would consequently augment the general well-being and efficiency of honeybee colonies [35].

1.3 Population dynamics

The study of population dynamics encompasses the examination of changes in honeybee populations over a period of time. By conducting further research in this area, valuable insights can be gained regarding the factors that potentially impact the health and productivity of honeybees [36].

1.4 Genetic diversity

Further investigation can be undertaken to assess the genetic diversity of honeybee populations through the utilization of mitochondrial DNA (mtDNA) analysis. This analytical approach holds the potential to provide valuable insights into the conservation initiatives and breeding schemes aimed at safeguarding genetic diversity and augmenting the overall well-being and productivity of honeybees [37].

1.5 Epigenetics

Further investigation can be conducted to explore the epigenetic mechanisms that contribute to the health and productivity of honeybees. This includes examining the involvement of mitochondrial DNA (mtDNA) in regulating gene expression and cellular metabolism [38].

In general, further investigation into the mitochondrial DNA (mtDNA) of *Apis mellifera* has the potential to provide valuable insights into the well-being and efficiency of honeybees. This includes aspects such as their ability to resist diseases, the dynamics of their population, the extent of their genetic variation, and the influence of epigenetic factors.

2. MtDNA analysis plays a significant role in understanding the mating behavior of honeybees.

2.1 Queen mating frequency

The utilization of mitochondrial DNA (mtDNA) analysis presents a valuable tool for elucidating the mating frequency of queen honeybees. Through the examination of the mitochondrial DNA (mtDNA) of worker bees within a colony, scholars are able to discern the existence of numerous mtDNA haplotypes. This observation serves as an indication that the queen bee has engaged in mating with multiple drones. The present dataset provides valuable insights into the mating behavior and genetic diversity exhibited within a particular colony [39].

2.2 Assessing mating success

The utilization of mitochondrial DNA (mtDNA) analysis presents a valuable approach for assessing the reproductive performance of female honeybees. Through the comparative analysis of the mitochondrial DNA (mtDNA) of worker bees and the mtDNA of the queen, researchers are able to ascertain the efficacy of the monarch's mating process and the subsequent transmission of her genetic material to the colony. The provided data facilitates the evaluation of the reproductive efficacy of monarch butterflies and the overall genetic variability within a given population [39].

2.3 Studying mating patterns

The utilization of mitochondrial DNA (mtDNA) analysis proves to be a valuable tool in the investigation of mating patterns within honeybee populations. Through the examination of mitochondrial DNA (mtDNA) in drones and its subsequent comparison with the mtDNA of worker bees, scholars are able to ascertain the respective genetic contributions of different drones to the overall genetic makeup of the colony. The provided information serves to enhance our understanding of the intricate dynamics of drone mating and the genetic diversity that exists within a given population [39].

2.4 Investigating reproductive strategies

The utilization of mitochondrial DNA (mtDNA) analysis has the potential to offer valuable insights pertaining to the reproductive strategies employed by honeybees, thereby contributing to the broader investigation of reproductive strategies within this species. The inference of queen mating patterns, specifically polyandry (the act of mating with multiple drones) or monandry (the act of mating with a single drone), can be made by researchers through the examination of the distribution of mitochondrial DNA (mtDNA) haplotypes within a given population. The provided information serves to enhance our understanding of honeybee reproductive behavior and the extent of genetic diversity within the species [39].

2.5 Conservation and breeding programs

The utilization of mitochondrial DNA (mtDNA) analysis in the context of honeybees has proven to be a valuable tool for informing conservation and breeding programs. Specifically, by discerning the maternal lineage and subspecies of honeybees, mtDNA analysis provides crucial insights that can guide these programs. The data presented herein serves to bolster the conservation and augmentation of honeybee populations [39] through the preservation of genetic variability and the deliberate selection of reproductive individuals exhibiting advantageous characteristics.

In summary, the utilization of mitochondrial DNA (mtDNA) analysis serves as a valuable tool in enhancing our understanding of honeybee mating behavior. This

study offers valuable insights into the frequency of mating, success rates of mating, patterns of mating, reproductive strategies, as well as breeding and conservation programs pertaining to monarch butterflies.

Summary of key findings and their significance

Based on the outcomes of the conducted search, the following are noteworthy discoveries and their implications concerning the mitochondrial DNA (mtDNA) of *Apis mellifera*.

1 Non-invasive genotyping of honey bee queens

The present study proposes a non-invasive genotyping method for honey bee queens that involves the utilization of residual tissue from the queen's severed wing. This innovative procedure allows for the identification of the queen's maternal lineage and subspecies without causing harm or distress to the individual under investigation. The utilization of this particular methodology allows for the expeditious and effective examination of genetic traits in honey bee queens. This approach holds significant potential for supporting honey bee breeding and selection initiatives as well as streamlining the process of identifying honey bee haplotypes across various research institutions [40].

2 Isolation and characterization of LAB

The present study focused on the isolation and characterization of lactic acid bacteria (LAB) from honeybee environments. These LAB strains were investigated for their potential application in the development of a honeybee protection preparation. The entirety of the bacterial isolates that were obtained in isolation exhibited the capacity to synthesize lactic acid, a crucial component for the preservation of honeybee well-being [41].

3 Varroa destructor control

A study was undertaken to investigate the efficacy of HopGuard® as a winter treatment for *Varroa destructor* in colonies of *Apis mellifera*. The findings of the study suggest that the efficacy of oxalic acid trickle and Apiguard is constrained when there is a surge in brood and mite mortality. Moreover, the reduction in mite population is inadequate to ensure the survival of all colonies during the winter [42].

4 Genetic past, present, and future of honey bees in the United States

The genetic history, current status, and prospective trajectory of honey bees in the United States can be comprehensively elucidated through the integration of

historical records and recently obtained genomic data. This approach enables researchers to discern the geographical displacement of honey bee species from their original habitats, ascertain the mechanisms underlying gene flow between genetically distant populations, and investigate the influence of natural selection and adaptation on the genetic variability of honey bees in the United States [43].

5 Disentangling Ethiopian honey bee populations

The classification of Ethiopian honey bee populations was investigated through the application of morphometric and genetic methodologies, aiming to resolve the existing controversy surrounding their categorization. The research conducted reveals notable distinctions in Ethiopian honey bee populations when compared to their counterparts in adjacent regions. These disparities are primarily attributed to the substantial gene flow occurring within the country, a phenomenon that is facilitated by the trade of honey bee colonies. The results of this study suggest that further investigation has the potential to make valuable contributions to the advancement of appropriate conservation management practices [44].

6 Honey bee queen egg laying

The development of a novel laboratory monitoring system for the deposition of honey bee queen eggs under controlled conditions has been undertaken. The utilization of this system enables the assessment of the impact of pollen nutrition on both egg laying and worker physiology, consequently augmenting the overall well-being and efficiency of honey bees [45].

The findings presented in this study provide compelling evidence regarding the importance of mitochondrial DNA (mtDNA) analysis in elucidating the genetic, health, and conservation aspects of honey bees. The utilization of genetic investigations has the potential to provide valuable insights into the historical, contemporary, and prospective dynamics of honey bee populations. Furthermore, novel laboratory methodologies can be employed to assess the impact of pollen nutrition on the reproductive capabilities of honey bee queens and the physiological well-being of worker bees. This research endeavor holds promise for advancing honey bee health and productivity.

2 Conclusion

The study of mitochondrial DNA (mtDNA) is of utmost importance in the comprehensive understanding of honey bee biology and the implementation of conservation initiatives. Recent studies have made significant contributions to our comprehension of the decline in honey bee populations by elucidating the genetic diversity of pathogens, potential pollinators, and climate factors that impact these bees. The utilization of genetic investigations has the potential to provide valuable insights into the historical, contemporary, and

prospective dynamics of honey bee populations. Furthermore, the utilization of novel laboratory systems enables the assessment of the impact of pollen nutrition on the egg-laying capacity of honey bee queens and the physiological state of worker bees. This research endeavor serves to augment the overall well-being and productivity of honey bee colonies. The aforementioned findings possess the potential to provide valuable insights for the development of strategies and policy recommendations aimed at the preservation of honey bee populations and the enhancement of their overall health and productivity.

Table 1. Comparison Between Morphological, mtDNA, and Genotyping Sequencing

Methods	Discription	Ref
Morphological	Morphological analysis involves the measurement and observation of physical characteristics of honeybees, such as size, shape, and color.	46
Metachondrial DNA mtDNA	mtDNA analysis is used to study the genetic diversity and evolution of honeybee populations. mtDNA is inherited maternally, which means that it can be used to trace maternal lineages and infer population history. mtDNA analysis can also be used to identify genetic diversity of pathogens, potential pollinators, and climate factors that affect honey bee populations.	47
Genotyping	Genotyping is a method used to determine the genetic makeup of an individual honeybee. It can be used to identify specific genes or markers associated with desirable traits, such as disease resistance or honey production. Genotyping can be done using various techniques, including PCR, RFLP, and GBS.	48
	Advantage	
Morphological	Morphological analysis is a simple and cost-effective method that can be done without specialized equipment. It can provide information on the physical characteristics of honeybees that may be associated with desirable traits.	46
Metachondrial DNA mtDNA	mtDNA analysis is a powerful tool for studying the genetic diversity and evolution of honeybee populations. It can provide information on maternal lineages and population history. mtDNA analysis can also be used to identify genetic diversity of pathogens, potential pollinators, and climate factors that affect honey bee populations.	47
Genotyping	Genotyping can provide information on specific genes or markers associated with desirable traits, such as disease resistance or honey production. It can be done using various techniques, including PCR, RFLP, and GBS.	48
	Disadvantage	
Morphological	Morphological analysis may not provide information on the genetic makeup of honeybees. It may also be subjective and dependent on the observer's experience and training.	46
Metachondrial DNA mtDNA	mtDNA analysis may not provide information on the genetic diversity of honeybee populations beyond	47

	maternal lineages. It may also be affected by factors such as incomplete lineage sorting and introgression.	
Genotyping	Genotyping can be expensive and time-consuming. It may also require specialized equipment and expertise.	48

References

1. M. Honrado, A.R. Lopes, M.A. Pinto, J.S. Amaral (2022)
2. L.P. Porrini et al. **32** (2022)
3. M. Modaber, J. N. Rafie, H. Rajabi-Maham. 1–12 (2019)
4. E. Tihelka, C. Cai, D. Pisani, and P.C.J. Donoghue. *Sci. Rep.*, **10**(1), 14515, Sep. (2020)
5. M. Kaskinova, E. Saltykova, A. Poskryakov, A. Nikolenko, L. Gaifullina. *Anim. an open access J. from MDPI*, **11**(10), Oct. (2021)
6. H. Rasolofoarivao, J. Clémencet, M. A. Techer, L. H.R. Ravaomanarivo, B. Reynaud, H. Delatte. *Apidologie*, **46**, 735–747, (2015)
7. M.D. Kaskinova, L.R. Gaifullina, and E.S. Saltykova. *Anim. an open access J. from MDPI*, **13**(14) Jul. (2023)
8. M.L.G. García, F.J. Reynaldi, C.M. Bravi. *J. Apic. Res.*, **57**, 611–614 (2018)
9. M.R. Palmer, D.R. Smith, O. Kaftanoğlu. *J. Hered.*, **91**(1), 42–46 (2000)
10. A. Rahimi, A. Mirmoayedi, D. Kahrizi, L. Zarei, S. Jamali. *Sociobiology* (2018)
11. M.D. Kaskinova, A.M. Salikhova, L.R. Gaifullina, E.S. Saltykova. **27**(4), 366–372 (2023)
12. D. Panziera, F. Requier, P. Chantawannakul, C. W.W. Pirk, T. Blacquiére. in *Frontiers in Ecology and Evolution*, 52 (2022)
13. A. Patenković et al. *Sci. Rep.*, **12**(1):17692, (2022)
14. P. Yang, Y. Peng, R. Zhao, D. Yang. *Biodivers. Sci.*, **26**, 476–485 (2018)
15. M.O. Awodiran, T.E. Amoo, T. Kehinde. (2021)
16. M.H. Carpenter, B.A. Harpur. *Apidologie*, **52** (1), 63–79 (2021)
17. L. Cao et al., *Genome Biol. Evol.*, **15**(3) p. evad025 (2023)
18. C. Chen et al., *Mol. Biol. Evol*, **35**(9), p. 22602271 (2018)
19. N. Blot et al., *Sci. Rep.*, pp. 1–13 (2023)
20. J.P. Acevedo-Gonzalez et al., *Ecol. Evol.*, **9**(19), 10895–10902 (2019)
21. J.A.F. Diniz-Filho, O. Malaspina. *Evolution* (N. Y.), **49**(6), 1172–1179 (1995)
22. M. Kükrer, [Online]. Available (2019)
23. D. Kavaliauskas, D. Danusevičius, V. Baliuckas. *Forests* (2022)

24. F. Özdil, F. İllhan. *Sociobiology*, **59**, 225–234 (2014)
25. C. Bodur, M. Kence, A. Kence, J. Apic. Res., **46**, 50–56 (2007)
26. R. Fridi et al., *Apidologie*, **53**, 1–14 (2022)
27. A.S. da Cruz et al., **11**, 573344 (2020)
28. L.P. Sanglard et al., *J. Anim. Sci.*, **101** (2023)
29. J.C. St John, T.S. Tsai, *BMC Genet.*, 19(1), 41 (2018)
30. J.C. St. John, T. Tsai, *BMC Genet.*, **19** (2018)
31. I.W. Hwang, K. Kim, E.J. Choi, H.J. Jin. 17(1), p. e11 (2019)
32. R.J. Longchamps et al. (2021).
33. S. Su\vsnik, P. Kozmus, J. Poklukar, V. Megli\vc, [Online]. Available (2004)
34. M. Alburaki, S. Madella, J. Lopez, M. Bouga, Y. Chen, D. vanEngelsdorp. *Front. Genet.*, 13, p. 1092121 (2022)
35. A.F. Schumacher-Schuh et al., *Mov. Disord.*, **37**(8), 1593–1604 (2022)
36. A. Gregorc, *Diversity*, 12(10). MDPI, p. 403 (2020)
37. P. Harizanis, M. Bouga, [Online]. Available (2004)
38. B.H. Sihombing, M. Nurrachmania, E.A. Fadilah, *J. Pembelajaran Dan Biol. Nukl.* (2022)
39. S. Madella, K. Grubbs, M. Alburaki, *Insects*, **12**(1), 19 (2020)
40. L.P. Porrini, C. Brasesco, M. Maggi, M.J. Eguaras, S. Quintana, *Genetica*, 149(5–6), 343–350 (2021)
41. S.W. Cobey, D.R. Tarpay, J. Woyke, *J. Apic. Res.*, 52(4) (2013)
42. L.P. Porrini et al., *J. Apic. Res.*, **59**, 648–657 (2020)
43. L. Ma, Y. Wang, X. Hang, H. Wang, W. Yang, B. Xu, *J. Apic. Sci.*, **59**, 63–72 [Online]. Available (2015)
44. K. Sasaki, T. Watanabe, *Insects*, 13(2) (2022)
45. J.D. Fine et al., *PLoS One*, **13**(9), e0203444 (2018)
46. W.C. da Silva Pizzaia et al., *Res. Soc. Dev.*, 10 (2021)
47. G.P. Slater, G.D. Yocum, J.H. Bowsher, *Sci.*, 287, no. 1927, p. 20200614 (May 2020)
48. G.E.L. Petersen, P.F. Fennessy, T.C. van Stijn, S. M. Clarke, P.K. Dearden, [Online] Available (2017)
49)