Article

Wing characters for morphological study on the honey bee (*Apis mellifera* L.) populations among six provinces of Iran

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Received 12 May 2020; *Accepted 20 June 2020*; *Published 1 December 2020*

Abstract
The honey bee is the most useful insect in the environment for its pollination and productions. The Apis genus has some species and subspecies all over the world and *Apis mellifera* is the most famous species among them. Many characters such as morphological and genetical variability were used to discriminate *A. mellifera* races that one of the useful morphological characters is wing perimeter which we use in the current study. Honey bee samples were collected from six provinces of Iran and transfer into 96 % alcohol. About 60 worker bees were used to the slide preparation from each population. Slides were prepared from the right forewing of samples, and digital images of forewings were photographed by using a CCD video camera (Sony, Dinolite 2). ImageJ software was used to measure the perimeter of forewing as a new character, cubital index, length and width of forewing, A4- D7- G18 angles and then data were analyzed by R package, PAST and SPSS software. The results has shown that there are significant differences between the forewings in various populations and the highest and the lowest forewing perimeters were obtained from Isfahan and West Azerbaijan respectively. Also, the correlation between wing perimeter and other characters of the forewing was evaluated. Wing perimeter is an important character that can affect on foraging behavior and flight ability, ultimately this feature will influence on the colony fitness. Also, this character can be used as a new method for discriminating honey bee populations based on morphological measurements.

Key words *Apis mellifera*; morphometric; wing perimeter; Dino Lite; honey bee.

Arthropods
ISSN 2224-4255
URL: http://www.iaees.org/publications/journals/arthropods/online-version.asp
RSS: http://www.iaees.org/publications/journals/arthropods/rss.xml
E-mail: arthropods@iaees.org
Editor-in-Chief: WenJun Zhang
Publisher: International Academy of Ecology and Environmental Sciences

1 Introduction

Flyer animals are showing the diversity of body shapes, structures and a great variation in flight performance (Kemp et al., 2008). Studies on insect can give the information about the relationship between morphology and flight characteristics (reviewed by Srygley, 1994; Dudley, 2000).

The social Hymenopterans colony contains a queen and many adults derived from the queen (Kovacs and Goodisman, 2012). The flight capacity is the major factor of the efficacy of foraging behavior. Honey bee has the ability to fly, foraging effort and return to the colony contribute to the fitness of the colony (Vance, 2009). Several factors may effect on flight capacities, such as: parasite and disease loads, and exposure to insecticides (Potts et al., 2010; Faghani and Rahimian, 2018)

Apis mellifera is one of the most successful species in the animal kingdom which adopted to a wide range of environmental condition (Ruttner, 1986); The dispersion of honey bee resulted in more than 24 different subspecies that distinguished with differences in morphology, behavior, physiology, biochemical processes, and susceptibility to diseases (Ruttner, 1988b).

Honey bee's wings are one of the most frequently measured insect body parts (Ruttner, 1988a). This character is influenced by factors such as temperature, diet and cell size (Shingleton et al., 2005).

Measuring honey bee's wing in biogeography, genetic (Bruckner, 1976), development (Smith et al., 1997) and ecology (Hihhinson & Barnard, 2004) issue have been used in previous studies. The observed differences in wing's shapes could be caused by environmental variation (Villemant et al., 2007). The loss of wing area can destroy flight performance and may reduce fitness (Vance and Roberts, 2014).

The kinematic and aerodynamic capacities of flying are vital for enhanced flight efforts during behaviors, including food carriage (Feurerbacher et al., 2003).

Prior theoretical and empirical researches indicated that it is practicable to make predictions about the relationship between morphology, lift production, power output and take off ability (Marden, 1987). There is obscurity in variation of flight morphology either due to specific ecological conditions (habitat type) or to specific behavioral features (food preferences) (Irschick and Garland, 2001, Darvishzadeh et al., 2015).

Iran has a good capacity for the development of beekeeping with climatic conditions and the existence of various honey bee meadow. However, we have rare researches about wing perimeter and flight performance of honey bee. Morphometric methods represent a potent investigative tool and represent good indicators of relationship between populations. It is significant to identify the variation among Iranian honey bee populations in order to help to understand the flight capacity of honey bees.

On the forewing of honey bees, some characters such as cubital index, length and width of wing, A4, D7 and G18 angles can be used for morphological measurements and these characters affect on the total wing perimeter. Therefore, the main aim of this study was to analyze the size of the forewing, its correlation with other distance characters on forewing and its role on the flight capacity of honey bee from different climate of Iran. Also, in this research we want to separate the Iranian honey bee population from six provinces based on wing characteristics as a new method for honey bee races identification.

2 Materials and Methods

2.1 Honeybee samples

For this study, the honey bees were collected from six provinces of Iran, including: North Khorasan (37.4710° N, 57.1013° E), West Azerbaijan (37.4550° N, 45.0000° E), Ardabil (38.4853° N, 47.8911° E), Isfahan (32.6546° N, 51.6680° E), Semnan (35.2256° N, 54.4342° E) and Kermanshah (34.4576° N, 46.6705° E). About 60 worker bees from each province (one apiary from each province) were used to prepare slides, and this number was collected from five hives. The samples were stored in 96% alcohol until the time of the preparation of slides.

2.2 Slide preparation

Slides were prepared from the fore right wing of each bee and then placed into the incubator (40°C) for 48 hours to dry completely. After fixing samples on the slides, photos were taken of each sample by using Dino

Capture software and Dino-Lite camera. ImageJ software was used to measure the perimeter of the wings (Fig. 1).

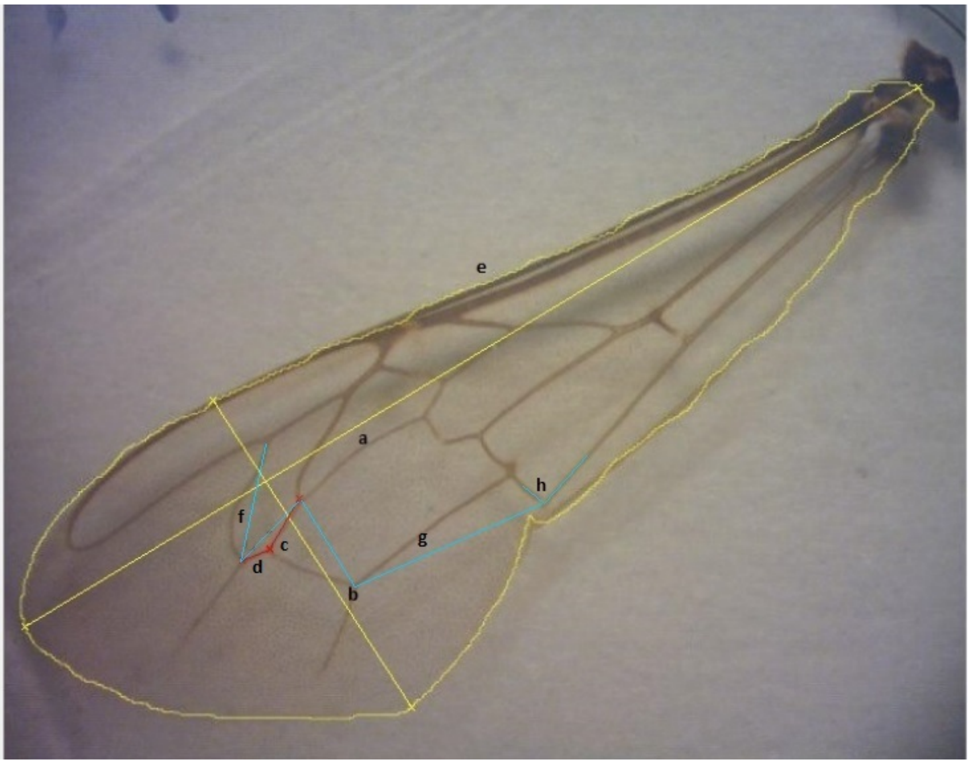

Fig. 1 Right forewing of honey bee. Wing perimeter was measured by ImageJ software. **a**: Forewing length, **b**: Forewing width, **c/d**: cubital index, **e**: total wing perimeter, **f**: A4 angle, **g**: D7 angle, **h**: G18 angle.

2.3 Data analysis

ImageJ software was used to measure seven characters including cubital index, length and width and A4- D7- G18 angles of 60 forewing images from each province that provided by Dino-Lite camera. Data were analyzed by using the R package V.3.1.1, PAST and SPSS V. 20 software. The differences between wing characters of the various populations were determined by one-way analysis of variance (ANOVA) and all of the averages were compared with the Tukey test. Also, to analyze the differences between populations, cluster analysis base on mahalanobis distances (D_2) and Discriminant Function Analysis (DFA) were used.

3 Result

There were statistically significant differences between the perimeter of the forewings in various populations ($F = 97$; $df = 5,174$; $p < 0.001$). Binary competition between treatments is shown in Fig. 2 and in this graph, the treatments which their confidence interval includes zero is not a significant difference. Whenever differences between treatments indicates the mean, it is compared by Tukey test.

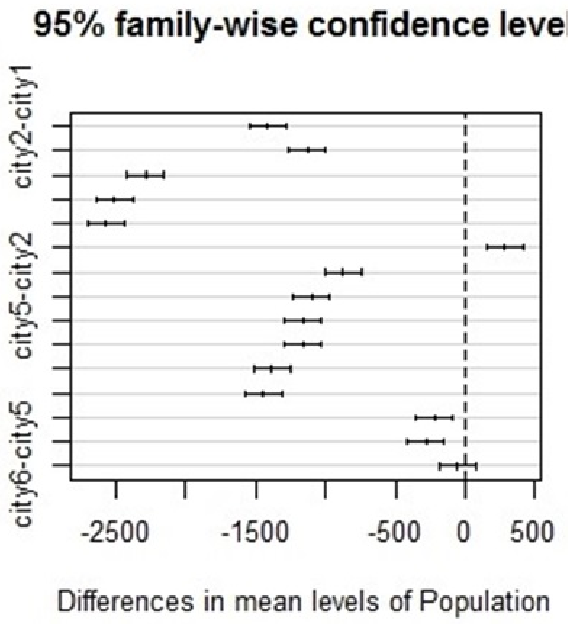

Fig. 2 Perimeter of forewings of *Apis mellifera* in various population. Letters indicated significant differences between perimeter of forewing in various population based on Tukey test (p<0.001).

Comparison of the mean perimeter of forewing in various populations show that the highest and lowest means were for Isfahan (city1) and West Azerbaijan (city6), respectively (45.01, 21.05 mm)(Fig. 3). Also, the mean of wing perimeters, cubital index, wing length and width in each population were shown in Table 1.

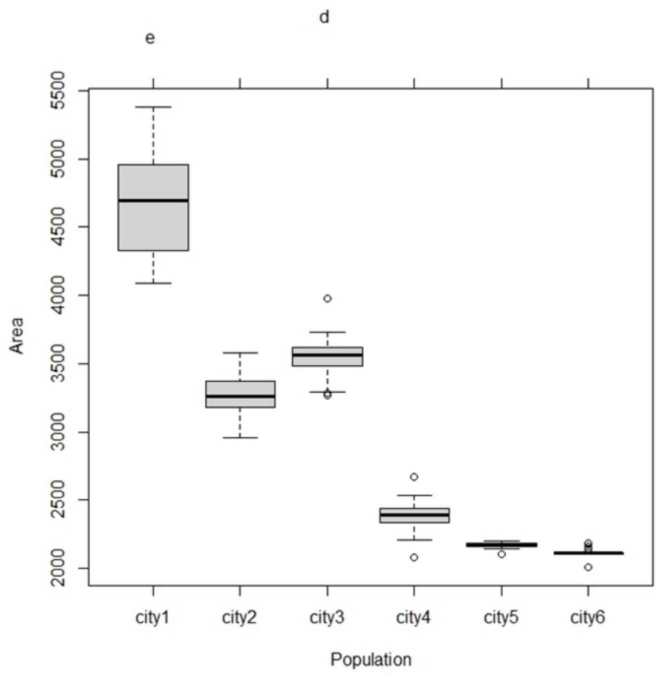

Fig. 3 Means of forewing *Apis mellifera* collected from various locations. City1: Isfahan, city2: North Khorasan, city3: Ardabil, city4: Kermanshah, city5: Semnan and city6: West Azerbaijan.

Table 1 Mean square of forewing characters such as wing perimeter, cubital index, wing length and width for each population.

	Wing Perimeter	Cubital Index	ForeWing L.	ForeWing W.	A4 angle	D7 angle	G18 angle
Isfahan	46.92	2.040	9.386	3.048	31.403	98.179	92.702
N. Khorasan	32.72	2.087	9.256	3.080	31.673	95.50	92.543
Ardabil	35.52	1.987	9.169	3.112	31.263	100.98	94.522
Kermanshah	23.85	1.951	9.236	3.183	30.747	97.311	90.748
Semnan	21.64	2.060	9.181	3.131	31.294	96.379	91.920
W. Azerbaijan	21.12	1.992	9.040	3.076	32.185	104.253	92.582

Based on cluster analysis, Isfahan population has the most differences among other provinces. North Khorasan and Ardabil are in one group and three other populations, including Kermanshah, Semnan and west Azerbaijan are in another group (Fig. 4).

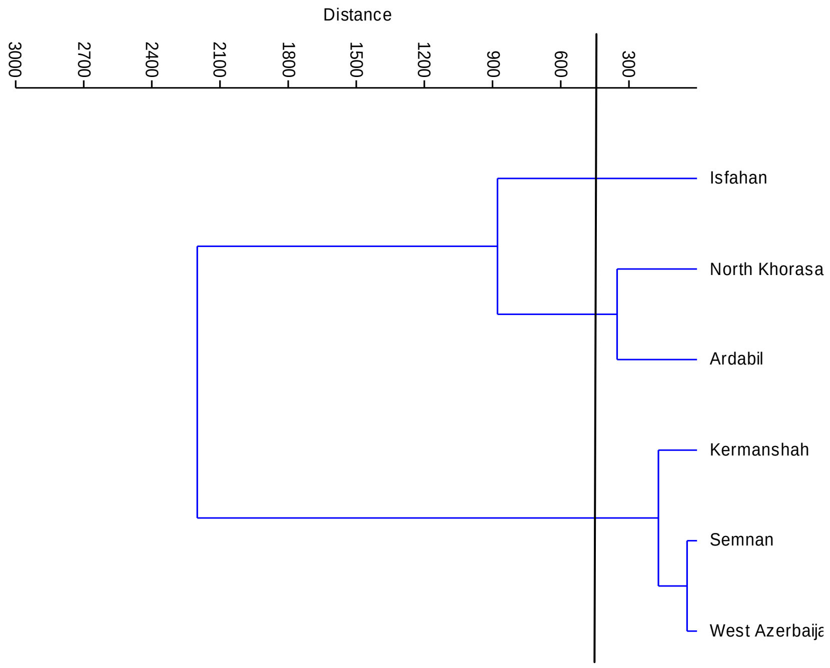

Fig. 4 Cluster analyses based on WARD method by means of total wing perimeter, cubital index, length and width of forewing mesurement. The vertical line is benchmark for population categories.

In addition to the cluster analysis, the DFA analysis was performed to show the correlation between populations in the studied provinces (Table 2). Also, in order to the Table 2, Isfahan population is completely separate from other provinces and it doesn’t have any overlap with other population. Ardabil and North Khorasan have 6.7 % overlap, Semnan and West Azarbaijan have 13.3 % overlap. It is notable that kermanshah provinces don’t have any overlap with others.

Table 2 classificatin results (n (%)) of discriminant analysisthat showing overlap between population based on colony means.

Classification Results[a]								
Locality	popultion	Predicted Group Membership						Total
		Isfahan	N.Khorasan	Ardabil	Kermanshah	Semnan	W.Azerbaijan	
Count	Isfahan	60 (100)	0	0	0	0	0	60 (100)
	N.Khorasan	0	56 (93.3)	4 (6.7)	0	0	0	60 (100)
	Ardabil	0	6 (10)	54 (90)	0	0	0	60 (100)
	Kermanshah	0	0	0	60(100)	0	0	60 (100)
	Semnan	0	0	0	0	52 (86.7)	8 (13.3)	60 (100)
	W.Azerbaijan	0	0	0	0	4 (6.7)	56 (93.3)	60 (100)

a. 93.9% of original grouped cases correctly classified.

As illustrated in the canonical discriminant functions graph in Fig. 5, Isfahan province is located separately from other provinces that the population is not concentrated. Also, the North Khorasan and Ardabil provinces are in the middle region and Semnan, Kermanshah and West Azarbaijan, with a slight difference, are in the left part of the graph.

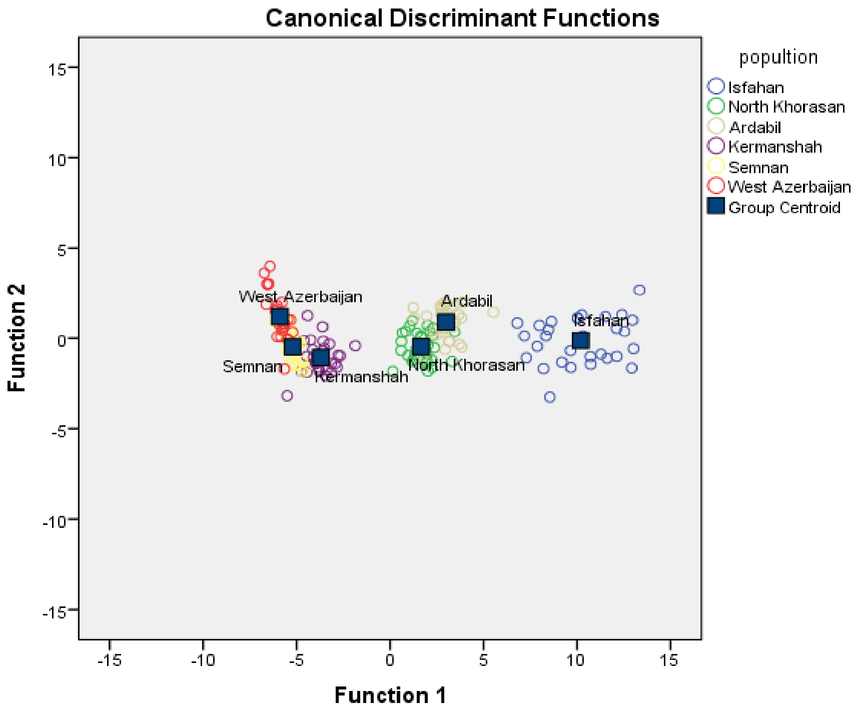

Fig. 5 Distribution of 6 populations from North Khorasan, West Azerbaijan, Ardabil, Isfahan, Semnan and Kermanshah based on the plane of discriminant function 1 and 2 derived from discriminant tanalysis of 7 morphological characters.

Mahalanobis distances (D_2) comparing the size of average multivariate distances between honey bees from six localities for all 7 characters differed ($p < 0.001$) from each other (Table 3). Mahalanobis distances also confirmed the separation of the three group of provinces. D_2 results showed that Isfahan population was more distant from other populations. The shortest mahalanobis distance (D_2 = 2.72) was between samples from Semnan and Kermanshah, whereas the longest distance was between samples from Isfahan and West Azerbaijan (D_2 = 26.51). Overall, our results showed that Isfahan population is independent of other provinces.

Table 3 Mahalanobis distances from discriminant analysis comparing the size of average multivariate distances between localities

	Isfahan	North Kh.	Ardabil	Kermanshah	Semnan	West Az.
Isfahan	0	14.45	11.88	23.17	25.35	26.51
North Khorasan		0	6.47	9.27	11.13	14.54
Ardabil			0	12.81	14.85	14.92
Kermanshah				0	2.72	7.81
Semnan					0	7.97
West_Azerbaijan						0

4 Conclusion

Our aim was to compare morphometric characters of Iranian honey bees that converge in three climate of Iran. Honey bees from whole part of Iran have been classified previously by some morphological characters (Alpatov, 1929; Ruttner, 1988; Ftayeh et al., 1994; Tahmasbi et al., 1996) but in this study we focused on fore wing and one new character.

One of the most effective morphological features for measuring insect flight success is the wing perimeter because it includes all of the important attributes of the wing for flight; In general, the ability to flight depends on wing perimeter. The wing perimeter and flight capacity have positive correlation with each other, also the relation between wing perimeter and length of wing can affect on flight capaiciy that is shown in Table 1 for Isfahan population which has the greatest wing perimeter and wing length in comparision to other provinces (Casey et al., 1985; Byrne et al., 1988). Environmental conditions affect the worker bee size (Ruttner, 1988). Size in worker body depends on foraging activity and herbal resource utilization (Roubik and Ackerman, 1987; Baumgartner and Roubik, 1989), although honey bees with great body size should pay more cost for locomotors capacity (Fleming et al., 2007; Punzo, 1982). A bee with more wing perimeter can spend more time in flight; this is an important feature for foraging behavior (Vance and Roberts, 2014). Wing characters clearly influence the foraging behavior of a worker honey bee. Bees with speared wing perimeters can fly further and have the power to fly more to increase the colony fitness to supply their needs. The worker bees with long flights are able to inspect more flowers and collect more nectar and pollen. So, one of the most important morphological criteria in determining the fitness of the honey bee subspecies is their wing characters.

Environmental and genetic factors are involved in honey bee morphological differences (Farshine Adl et al., 2007; Higginson and Barnard 2004; Brückner, 1976). The results has shown that the wing perimeter is significant difference between collected populations in Iran. On the other hand, all of the samples obtained from hives were native to Iran, so the samples may have been genetically belong to a subspecies. Therefore, we can say that the difference is related to the geographical conditions of the samples. The samples collected from West Azerbaijan were different from the other provinces and the wing perimeter in this province was nearly twice bigger than other samples. The difference can be attributed to several factors, including difference in geographical coordinates, difference in temperature (Isfahan is in central part of Iran and located in close to the desert but Azerbaijan is located in the North West of Iran with cold climate), difference in vegetation, and finally, if we relate these differences with genetic factors, we can conclude that the Azerbaijan province in the border of Iran and maybe in it’s regions honey bee populations were mixed with other subspecies of neighbor countries (Ozdil, 2012). Although the studies in neghbour countries show that , 95% of the population of Iranian honeybees were in a range and different from the subspecies in the neighboring countries, such as Turkey (Kence, 2009).

Although wing perimeter may be influenced by distant characters, but the indexes can follow different way that we can see in Semnan provinces with less wing perimeter and great cubital index.

The cluster and discriminant function analysis have shown that the population of North Khorasan and Ardabil can be in one group. It can be explained that both provinces in northern parts of Iran are associated with the nordic countries. The main race in these countries is Caucasian honey bee.

Kermanshah and West Azerbaijan in Iran's western border are similar to the neighbor countries in western parts of Iran with a genetic mixing population. On the other hand, Isfahan’s population has the most differences from other evaluated provinces because their populations are in central part of Iran, which is far from any borderline countries. Although the Kermanshah’s population didn’t show overlap with other populations, but it is grouped in west provinces of Iran in order to canonical discriminant graph that is accepted by other new studies in Iran (Rahimi et al., 2017)

Generally, in the multivariate analysis of variance it is confirmed that there is a significant difference between the populations of the provinces in terms of wing size. It is notable that the cubital index has the least change in comparision to the other characters and it does not have a significant effect on the population. Also, the total wing perimeter with smaller Wilks' Lambda is more important for the independent variable to the discriminant function (Table 4).

Table 4 A summary of the multivariate analysis of variance between the four groups derived from the discriminate function analysis.

Tests of Equality of Group Means					
	Wilks' Lambda	F	df1	df2	Sig.
Wing perimeter	.032	1056.030	5	174	.000
Cubital index	.965	1.265	5	174	.281
Fore wing L.	.683	16.160	5	174	.000
Forewing W.	.722	13.428	5	174	.000
A4	.871	5.173	5	174	.000
D7	.851	6.109	5	174	.000
G18	.754	11.351	5	174	.000

Although the wing characters is precise enough to prepare useful information for a conclusion about honey bee races or it’s fitness, but if we want to classify subspecies it is advisable to use other morphological methods such as: geometric morphometric or classical morphometric, along with the wing perimeter to increase the accuracy (Dadgostar and Nozari, 2018).

In general, it is preferable to use different methods for categorizing the honeybee’s subspecies. However, new methods such as molecular techniques can be more accurate for identifying subspecies and haplotypes of the honey bees (Magnus et al., 2014). On the other hand, morphological changing is the reflection of changes in genetics of the honeybees that have been taken place for so many years.

The importance of classifying honey bee subspecies is to comparing traits in different locations and populations. Also, by comparing the size of the traits with previous studies, it was found that how much changes has occurred over the years in each population of honey bees. In addition, we can identify the size of the traits in other subspecies or close populations in neighboring countries by knowing the size of the traits, with the probability of genetic mixing or alteration of the bee races into the country.

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