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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

PHENETIC VARIATION IN HONEY BEE (*Apis mellifera*) POPULATION OF THE TORATAU GEOPARK, THE REPUBLIC OF BASHKORTOSTAN

Başkortostan Cumhuriyeti Toratau Jeoparki Bal Arısı (*Apis mellifera*) Popülasyonunda Tergit Rengi Değişimi

Salavat T. SAGITOV[1, 3], Rustem A. ILYASOV*[2, 4, 6], Vener N. SATTAROV[3], Yuliya R. ABDRAKHIMOVA[1], Valery N. DANILENKO[4], Nailya R. GAZIZOVA[5], Amilya V. SATTAROVA[3], Dmitry V. BOGUSLAVSKY[6]

[1]Regional Branch of the Russian Geographical Society in the Republic of Bashkortostan, Ufa, RUSSIA, E-posta: salavatst@list.ru,ORCID No: 0000-0002-7211-1004, E-posta: abdrakhimova.rgo@internet.ru, ORCID No: 0009-0004-8690-5472.

[*2]Laboratory of Molecular Genetics, Scientific Educational Center in Bashkir State Agrarian University, Ufa, RUSSIA, Corresponding author / Yazışma Yazarı E-posta: apismell@mail.ru, ORCID No: 0000-0003-2445-4739.

[3]Department of Bioecology and Biological Education, Faculty of Natural Geography, Bashkir State Pedagogical University named after M. Akmulla, Ufa, RUSSIA, E-posta: wener5791@yandex.ru, ORCID No: 0000-0001-6331-4398, E-postal: amilywener@gmail.com, ORCID No: 0009-0004-0793-2414.

[4]Vavilov Institute of General Genetics, Russian Academy of Sciences, Moscow, RUSSIA, E-posta: valerid@vigg.ru, ORCID No: 0000-0001-5780-0621.

[5]Ufa Scientific Research Institute of Occupational Medicine and Human Ecology, Ufa, RUSSIA, E-posta: nelli.ga012@gmail.com, ORCID No: 0000-0003-4287-8594.

[6]Koltsov Institute of Developmental Biology, Russian Academy of Sciences, Moscow, RUSSIA, E-posta: boguslavsky@rambler.ru, ORCIDNo: 0000-0001-9601-640X.

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ABSTRACT

A phenetic analysis of the honey bee population of the Toratau Geopark (Russia) was performed. Over 1,000 worker and drone bee samples were collected from 250 colonies in 59 apiaries on the territory of the Toratau Geopark (Gafuriysky, Ishimbaysky, Meleuzovsky, and Sterlitamaksky districts of the Republic of Bashkortostan). Six phenes in worker bees and four phenes in drone bees were recognized. The phenes E, 1R, 2R, and 3R in workers and Is, I, and O-gray in drones were predominant in the honey bee population of the Toratau Geopark, which were associated with subspecies of the C-lineage. These phenes can be used as indicators of introgressive hybridization in the local dark European honey bee population. The phenes allow for quick evaluation of certain honey bee colonies hybridization states.

Keywords: Honey bee, phenes, Dark European bee, The Republic of Bashkortostan, Toratau Geopark

ÖZ

Toratau Jeoparkı'ndaki (Rusya) bal arısı popülasyonunun fenetik analizi yapılmıştır. Toratau Jeoparkı topraklarındaki (Başkurdistan Cumhuriyeti'nin Gafuriysky, Ishimbaysky, Meleuzovsky ve Sterlitamaksky bölgeleri) 59 arılıktaki 250 koloniden 1.000'den fazla işçi ve erkek arı örneği

toplanmıştır. İşçi arılarda altı fen ve erkek arılarda dört tergit rengi tespit edilmiştir. İşçilerde E, 1R, 2R ve 3R ve erkek arılarda Is, I ve O-gri fenleri Toratau Jeoparkı'ndaki bal arısı popülasyonunda baskındı ve bunlar C soyunun alt türleriyle ilişkiliydi. Bu tergit rengi, yerel koyu Avrupa bal arısı popülasyonunda içsel melezleşmenin göstergeleri olarak kullanılabilir. Bu tergit renkleri, belirli bal arısı kolonilerinin melezleşme durumlarının hızlı bir şekilde değerlendirilmesine olanak sağlamaktadır.

Anahtar Kelimeler: **Bal arısı, tergit renkleri, Kara Avrupa arısı, Başkurdistan Cumhuriyeti, Toratau Jeoparkı**

GENİŞLETİLMİŞ ÖZET

Çalışmanın amacı: Başkurdistan Cumhuriyeti Toratau Jeoparkı'ndaki işçi ve erkek arıların dağılımının değerlendirilmesidir**.**

Giriş: Batı Avrupa ülkelerinin topraklarında yaşayan bal arıları arasında en yaygın alt tür koyu Avrupa, koyu orman veya Orta Rus arısıdır (*Apis mellifera mellifera*). Şu anda, bu alt tür esas olarak Güney Urallar, Batı Sibirya ve orta Rusya'da hayatta kalmış ve yerel popülasyonlar oluşturmuştur. Tarihsel olarak, Urallar ve Başkurdistan Cumhuriyeti'nin (Rusya) doğal ve iklimsel bölgesi, şiddetli kışlar, bal bitkilerinin zengin bir tür bileşimi, ıhlamur ormanlarının bolluğu ile ayırt edildi ve bununla bağlantılı olarak cumhuriyetin arıları, koyu Avrupa arısının özel bir Başkurt popülasyonu olarak tanımlandı.

Gereç ve yöntem: 2018 yılında, Başkurdistan topraklarında, bölgesel olarak 4 belediye bölgesini kapsayan Toratau Jeoparkı oluşturuldu: Gafuriysky, Ishimbaysky, Meleuzovsky ve Sterlitamaksky. Jeoparkın gelişmiş düzenleyici çerçevesi, rezerv bölgesinin yakın konumu ve doğal ve iklimsel koşullar, Başkurt nüfusunun arılarına dayalı arıcılığın geliştirilmesi için ideal koşulları oluşturmuştur. Bal arısı popülasyonunun fenetik analizi gerçekleştirilmiştir. Toplam 2.000 arı toplanmıştır (1.000 işçi arı ve 1.000 erkek arı). Her bölgede 250 koloniden seçim yapılmıştır. Bu çalışmada Ruttner yöntemi kullanılmıştır.

Bulgular: Oda işleme, M. Akmulla'nın adını taşıyan Başkurt Devlet Pedagoji Üniversitesi Biyoekoloji ve Biyolojik Eğitim Bölümü temelinde gerçekleştirilmiştir. Yüzde ve oranların hesaplanması, grafik çizimi Statistica 12.0 (StatSoft Power Solutions, Inc. USA) programında yapılmıştır. İşçi arılarda altı tergit rengi, erkek arılarda ise dört tergit rengi bulunmuştur. İşçilerde E, 1R, 2R ve 3R ve erkek arılarda Is, I ve O-gri tergit renkleri Toratau Jeoparkı bal arısı popülasyonunda baskındır ve bunlar C-soyunun alt türleriyle ilişkilidir.

Tartışma ve sonuç: Bu tergit renkleri, yerel koyu Avrupa bal arısı popülasyonunda içsel melezleşmenin göstergeleri olarak kullanılabilir. Bu tergit renkleri, belirli bal arısı kolonilerinin melezleşme durumunu hızlı bir şekilde değerlendirmenizi sağlar. Safkan arıların varlığı, koyu Avrupa arısının Başkurt popülasyonunun korunması ve çoğaltılmasına yönelik önlemlerin uygulanması için fırsatlar sunmaktadır. Yerel koyu Avrupa arılarını melezleşmeden korumak acildir. Çünkü insan yardımı olmadan yerel koyu orman arısı gen havuzunun saflığını geri kazanılamaz.

INTRODUCTION

In the process of the evolution, about 30 subspecies of the honey bee (*Apis mellifera*) were formed in the Old World, extended in a wide range of climatic conditions (Ilyasov et al. 2020). The ability of honey bees to adapt well, the human consumption of bee products and the use of honey bees in plant pollination contributed to the wide anthropogenic spread of honey bee colonies to almost all around the world. However, despite the high adaptability and the large distribution area, the number of honey bee populations in the world is decreasing annually, which leads to a decrease of a genetic diversity, the adaptability of the populations and the biodiversity of biomes (Rua et al. 2009).

Among the honey bees inhabiting the territory of Western European countries, the dark European bee *Apis mellifera mellifera* is common. The dark forest, Central Russian, or dark European bee is an indigenous subspecies of the honey bee of the western and northern regions of Europe and Russia, evolutionarily formed in forest conditions. Currently, this honey bee subspecies has been preserved mainly in the Southern Urals, Western Siberia and the central part of Russia, forming local populations named by territorial affiliation: Altai, Arkhangelsk, Vladimir, Bashkir (Ilyasov et al. 2021; Petrov 1980; Petrov 2004). Historically, the natural and climatic

zone of the Ural and Bashkortostan has been distinguished by severe winters, a rich species composition of honey plants, and an abundance of linden forests, in connection with which the bees of the republic were singled out as a special Bashkir population of the dark European bee (Shafikov and Avetisyan 1976; Shafikov 1978; Abdulov and Shafikov 2004; Shafikov and Baimuratov 2002). Kozhevnikov (1931) wrote: “In the forests of Bashkiria and the Urals, we found the remains of a primal dark European bee, which currently is the greatest treasure in terms of genetics. It must be protected in every possible way from hybridization, and on its basis mass breeding of the basic dark European bee should be based, which, over thousands of years of natural selection in the harsh mountain climate, has developed endurance and vitality" (Kozhevnikov 1931). The Bashkir population of the dark European bee is characterized by the dark color of the body of individuals, only 1-2 phenes, the tergites of which do not have signs of yellowness, the sternites are colored from dark gray to dark brown without yellowness; the end of the abdomen in working individuals is more blunt than in bees of other breeds; the body shape of a sitting bee is squat (Krivtsov 1995; Krivtsov and Grankin 2004).

The Republic of Bashkortostan is located in the east of the European part of Russia, in the basin of the Belaya and Ural rivers, extends from north 56°31′N 54°31′E to south 51°34′N 57°12′E and from west 55°07′N 53°08′E to east 54°52′N 60°00′E. Apart from that the republic occupies a wide strip of the western Urals stretching from the north to the south (Figure 1). The Perm and Sverdlovsk regions are adjacent to the republic from the north, the Chelyabinsk Region from the east, the Orenburg Region from the south, the Republic of Tatarstan and the Udmurt Republic from the west.

Due to the presence of many natural areas (forest, mountain forest, forest-steppe, steppe), the Republic of Bashkortostan is a unique territory with a rich species composition of flora and fauna. In this regard, there are many conservation areas located here, for instance: The National Park «Bashkiria», the Nature Reserve «Altyn Solok», and the State Natural Biosphere Reserve «Shulgan-Tash». The latter was created for the purpose of preserving and breeding the Burzyan wild living honey bee (Bashkir honey bee ecotype), which is listed in all editions of the Red Book of the Republic of Bashkortostan (2004, 2014) and has the status of category 4 (population not defined by status). In addition, in 2018, not far from the Shulgan-Tash Nature Reserve, the Toratau Geopark was created, geographically covering 4 municipal districts: Gafuriysky, Ishimbaysky, Meleuzovsky and Sterlitamaksky. The main function of the Geopark is the preservation of the geological, biological, historical, and cultural heritage of the republic. At the same time, the developed regulatory framework of the Geopark, the close location of the reserve territory and natural and climatic conditions have created ideal conditions for honey bees breeding of the Bashkir population. However, at the beginning of the XXI century, due to the increase of the import of honey bees of the unknown origin into the Republic of Bashkortostan, the genetic pressure of other subspecies of honey bees began to pose a serious threat to both Bashkir and Burzyan populations. Therefore, if in the 80s of the last century the influx of other genetic material in the reserve did not exceed - 1% and 2-3% of the total number of honey bee colonies, then in recent years this marker is 10-12% (Abramova 2014; Koroleva et al. 2019; Mannapov et al. 2019; Ruttner 2006; Sabirdzhonova and Sattarov 2021). The result of these processes is that at present moment, the Burzyan bee population is surrounded by hybrid honey bee colonies and the structure of the Bashkir bee population is gradually and irrevocably being lost. In this case, of course, it is necessary to monitor the diversity of the honey bees’ breed in the buffer zone near the locality of honey bees kept in the conservation area.

When the honey bee breeds are being characterized, a different number of exterior indicators are used. Some of them are associated with the linear morphometric measurements, others express the ratio of some quantities to others, the third are characterized by area, the fourth proceed from the topographic incompatibility of the individual points and lines, the fifth are determined by mass, color, pubescence. Speaking of breed-defining features, the color of honey bees should be taken into account. Despite the fact that it plays an important role in determining the breeds of honey bees, scientists do not always pay attention to this feature. Only in some studies, the assessment of honey bees is given together with the honey bee coloring, although it has been proved long ago that the appearance of yellow coloration on the chitinous covers of the abdomen of the European dark bee indicates the processes of hybridization (Abramova et al. 2014; Koroleva et al. 2019; Mannapov et al.

2019; Ruttner 2006; Sabirdzhonova and Sattarov 2021; Sagitov et al. 2022a; Sagitov et al. 2022b).

Previously reported, the color variations of the cuticle of the insects are exclusively diverse and that this feature serves as a reliable sign for determining the species and various geographical forms of the studied animals (Chashchukhin and Lapteva 2011; Chashchukhin and Lapteva 2009). They also noted that the presence of yellow coloring on the tergites of the abdomen is a peculiarity differential of honey bees of many southern subspecies. The appearance of this colouring in the European dark bee indicates, first, the processes of hybridization. Taking into account the importance of maintaining the purity of the gene pool of honey bee subspecies populations (Sabirdzhonova and Sattarov 2021; Sagitov et al. 2022a; Sagitov et al. 2022b; Chashchukhin and Lapteva 2011), it was noted that the system of creating the areas of "pure" breeding should be based on principles that consider the contribution of drones and queens in maintaining the plasticity and stability of population structures. As it is known, the selection at the apiary must be carried out both on the maternal and paternal lines (Sagitov et al. 2022a; Chashchukhin and Lapteva 2011; Cherevko and Avetisyan 2007; Chibilev 2011; Sharygin and Krivtsova 2018). The purpose of the study is evaluation of worker and drone bee phenes distribution in the Toratau Geopark of the Republic of Bashkortostan.

MATERIALS AND METHODS

The collection of 2000 worker bees and drones from 59 apiaries that are part of the Gafuriysky (53°53'41"N. 56°28'07"E), Ishimbaysky (53°28'37"N. 56°30'43" E.), Meleuzovsky (52°57'00"N. 55°55'59" E) and Sterlitamak (53°37'59" N 55°57'00" E) districts, was used as the material for this study (Figure 1).

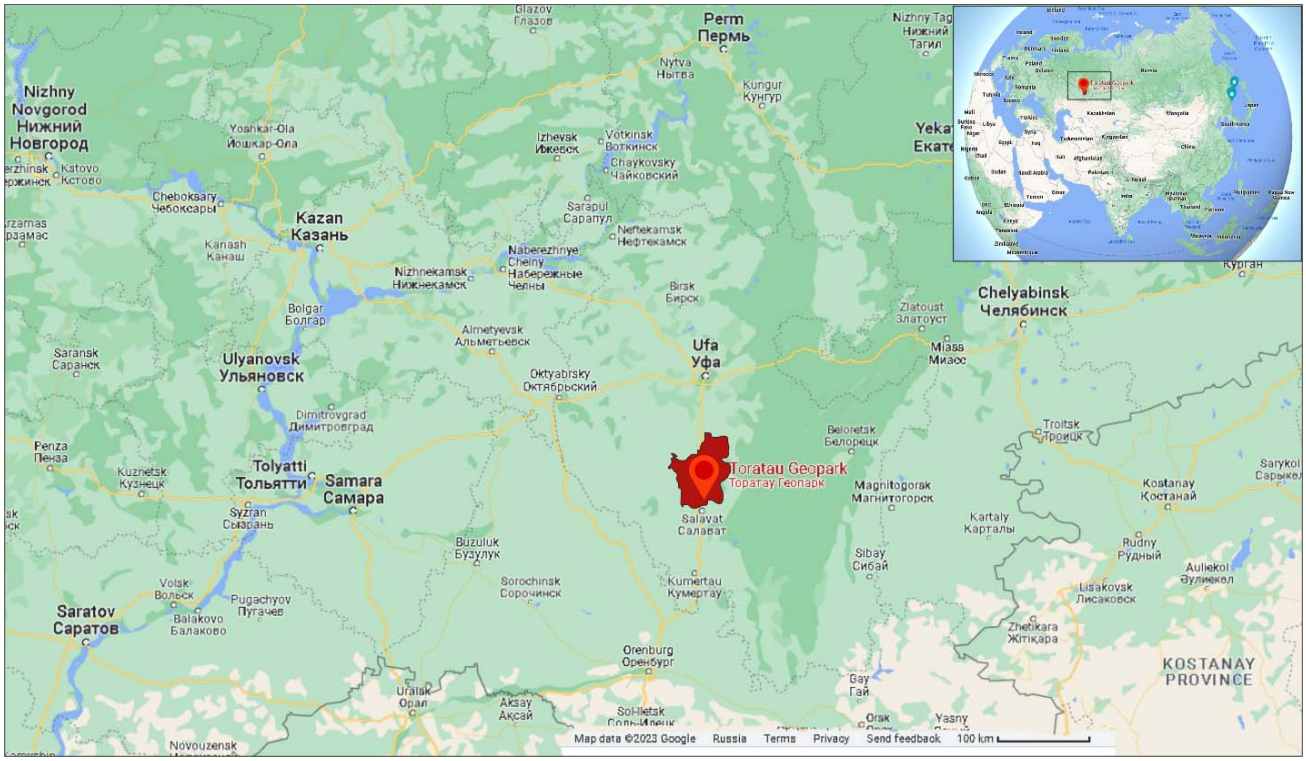

Figure 1. Geographical location of the Toratau Geopark in the Republic of Bashkortostan.

In total, 2,000 bees were collected (1,000 worker bees and 1,000 drones). In each district, the selection was carried out from 250 colonies. The Ruttner's method (Ruttner 2006) used in this work. The cameral treatment was carried out based on the department of Bioecology and Biological Education

of the Bashkir State Pedagogical University named after M. Akmulla. The calculation of percentages and proportions, and construction of graphs were performed with Statistica 12.0 (StatSoft Power Solutions, Inc. USA).

RESULTS

In the course of researches, 6 phenes of worker bees were identified: O, e, E, 1R, 2R and 3R (Figure 2). As we can see, the phenetic structure of the honey bees on this territory is characterized by high heterogeneity, since the aboriginal population would differ in the presence of only one (O) or two (O and e) phenes.

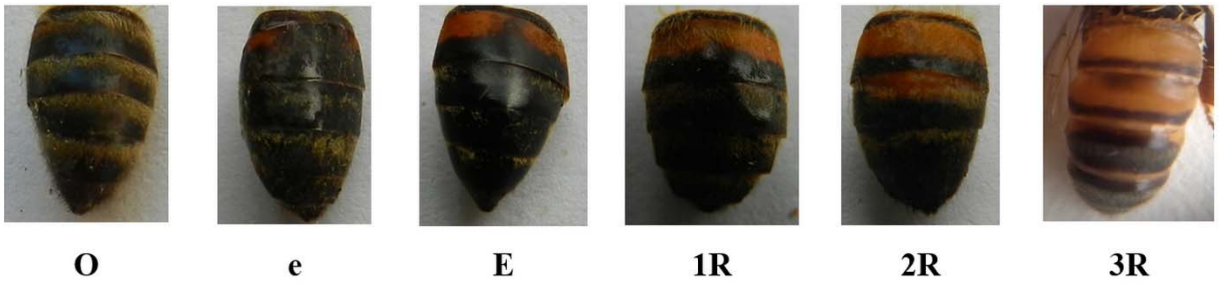

Figure 2. The detected phenes of the worker bees in the Toratau Geopark.

According to the obtained results, it can be seen that the honey bee colonies with phenes 2R and 3R predominate in almost all apiaries. At the same time, the occurrence (%) was: in the Gafuriysky district from 4.8 to 100 (2R) and from 50 to 100 (3R), in the Meleuzovsky district from 50 to 100 (2R) and from 14.3 to 100 (3R); in the Ishimbaysky district from 9.1 to 100 (2R) and from 6.7 to 100 (3R) and in Sterlitamaksky district from 5 to 92.3 (2R) and from 12.5 to 95 (3R) (Table 1).

Table 1. The phenes of worker bees in the Toratau Geopark

Locality, apiary	No. of colonies, pcs.	Worker bee phenes (pcs. / %)					
		O	e	E	1R	2R	3R
Gafuriysky district							
Kutluguza	15	-	-	-	-	2 (13.3)	13 (86.7)
Krasnousolsky	8	-	-	-	-		8 (100)
Uzbjakovo	16	-	-	-	-	-	16 (100)
Tolparovo	11	-	-	-	-	-	11 (100)
Tashly							
apiary 1	26	-	-	-	-	13 (50)	13 (50)
apiary 2	18	17 (94.4)	-	-	-	1 (5.6)	-
Tolparovo:							
apiary 1	17	-	-	-	-	3 (17.6)	14 (82.4)
apiary 2	11	-	-	-	-	11 (100)	-
Mendim:							
apiary 1	24	-	-	-	-	24 (100)	-
apiary 2	26	-	-	-	-	10 (38.5)	16 (61.5)
Tabynsk	21	20 (95.2)	-	-	-	1 (4.8)	-
Cowardy:							
apiary 1	17	-	-	-	-	1 (5.9)	16 (94.1)

apiary 2	12	-	-	-	-	12 (100)	-
Ibragimovo	13	-	-	-	-	-	-
Kyzyl Yar	15	-	-	-	-	4 (26.7)	-
Total	250	37 (14.8)	0	0	24 (9.6)	82 (32.8)	107 (42.8)
Meleuzovsky district							
Araslanovo	18	-	-	-	-	-	18 (100)
Alexandrovka:							
apiary 1	22	-	-	-	-	13 (59.1)	9 (40.9)
apiary 2	19	-	-	-	-	12 (63.2)	7 (36.8)
Meleuz:							
apiary 1	28	-	-	-	8 (28.6)	16 (57.1)	4 (14.3)
apiary 2	4	-	-	-	-	4 (100)	-
Aptrakovo	15	-	-	-	-	11 (73.3)	4 (26.7)
Pokrovka	15	-	-	-	-	10 (66.7)	5 (33.3)
Nugush	10	-	-	-	-	5 (50)	5 (50)
Zirikovo	30	-	-	-	10 (33.3)	20 (66.7)	-
Abitovo	30	-	-	-	-	20 (66.7)	10 (33.3)
Klenovaya gora	23	-	-	-	11 (47.8)	12 (52.2)	-
Kutlubulatovo	8	-	-	-	2 (25)	-	6 (75)
Smak	5	-	-	-	-	3 (60)	2 (40)
Sugar Factory Nursery	5	-	-	-	-	5 (100)	-
Voskresensko e	18	-	-	-	-	9 (50)	9 (50)
Total	250	0	0	0	31 (12.4)	140 (56)	79 (31.6)
Ishimbaysky district							
Makarovo:							-
apiary 1	5	-	3 (60)	-	-	2 (40)	
apiary 2	19	-	-	-	-	5 (26.3)	14 (73.7)
apiary 3	10	-	-	-	-	3 (30)	7 (70)
Gumerovo:							
apiary 1	8	-	-	-	-	1 (12.5)	7 (87.5)
apiary 2	8	-	-	-	-	8(100)	-
Sargaevo:							
apiary 1	17	-	-	-	-	4 (23.5)	13 (76.5)
apiary 2	5	-	-	-	-		5 (100)
Isyakaevo:							
apiary 1	35	-	-	10 (28.6)	18 (51.4)	4 (11.4)	3 (8.6)
apiary 2	22	-	-	-	-	10 (45.5)	12 (54.5)
Asiyalanovo	30	16 (53.3)	12 (40)	-	-	-	2 (6.7)
Verkhneitkulov o							
apiary 1	10	-	1 (10)	-	-	9 (90)	-
apiary 2	11	-	1 (9.1)	-	-	8 (72.7)	2 (18.2)
Armetovo	14	-	-	-	-	7 (50)	7 (50)
Asiyalan	11	-	-	-	-	1 (9.1)	10 (90.9)
Urazbaevo	14	-	-	-	-	7 (50)	7 (50)

Podgornaya	17	-	-	-	-	13 (76.5)	4 (23.5)
Kinzebulatovo	14	-	-	-	5 (35.7)	2 (14.3)	7 (50)
Total	250	16 (6.4)	17 (6.8)	10 (4)	23 (9.2)	84 (33.6)	100 (40)
Sterlitamaksky district							
Pokrovka	28	-	-	-	6 (21.4)	12 (42.9)	10 (35.7)
Berezovka	28	-	-	-	-	15 (53.6)	13 (46.4)
Karmaskaly	23	-	-	-	-	7 (30.4)	16 (69.6)
Spasskaya	26	-	-	-	-	12 (46.2)	14 (53.8)
Burikazganovo	25	-	-	-	-	16 (64)	9 (36)
Talachevo	15	-	-	-	-	5 (33.3)	10 (66.7)
Pokrovka	8	-	-	-	-	7 (87.5)	1 (12.5)
Aigulevo	15	7 (46.7)	-	-	1 (6.7)	5 (33.3)	2 (13.3)
Spasskaya	26	2 (7.7)	-	-	-	24 (92.3)	-
Kuganak	20	-	-	-	-	1 (5)	19 (95)
Kosyakovka	9	-	-	-	-	1 (11.1)	8 (88.9)
Strelkovka	27	-	-	-	-	9 (33.3)	18 (66.7)
Total	250	9 (3.6)	0	0	7 (2.8)	114 (45.6)	120 (48)
Total	1000	62 (6.2)	17 (1.7)	10 (1)	85 (8.5)	420 (42)	406 (40.6)

Honey bee colonies with the Dark European bee phenes - O and e were identified in all districts except Meleuzovsky. At the same time, only in the Ishimbaysky district there were honey bee colonies with both phenes. In the apiaries of the Gafuriysky and Sterlitamaksky districts, honey bees were found only with a phene - O: about: 14.8% and 3.6%, respectively. Purebred colonies were found in the apiaries of settlements: Tashly (94.4% or 17 colonies out of 18) and Tabynsk (95.2% or 20 out of 21 colonies) in the Gafuriysky district. Honey bee colonies with European dark bee phenes were noted in 4 apiaries: Makarovo (apiary 1) – 60% or 3 out of 5 colonies studied; Asiyalanovo – 28 colonies (93.3%) out of 30; Verkhneitkulovo (apiary 1 and 2) - 1 colonies out of 10 (10%) and 11 (9.1%) colonies in the Ishimbaysky district. In Sterlitamaksky district honey bees with a purebred phenes are registered in two localities: Aigulevo (colonies or 46.7% of 15 colonies) and Spasskaya (2 colonies – 7.7% of 27).

Honey bees with phenes E were found in apiaries of only 2 districts (%): Ishimbaysky - 4 and Sterlitamaksky district - 1. The situation with the phenes 1R was similar to honey bees with phenes 2R and 3R, i.e. 1R was noted in apiaries of all districts, but in minimal numbers compared to 2R and 3R: Gafuriysky – 9.6%, Meleuzovsky – 12.4%, Ishimbaysky – 9.2% and Sterlitamaksky – 2.8%. The occurrence of honey bee colonies with different phenes in apiaries is shown in Figure 3.

In quantitative terms, honey bee colonies with phenes 2R and 3R prevailed both: by districts and by the explored territory: Gafuriysky - 2R (32.8%) 3R (42.8%), Meleuzovsky - 2R (56%) 3R (31.6%), Ishimbaysky - 2R (33.6%), 3R (40%), Sterlitamaksky - 2R (45.6%), 3R (48%) and in the whole examined territory - 2R (42%), 3R (40.6%). The occurrence of phenes (O and e) related to the Dark European bee was 7.9%, which is significantly lower than the quantitative composition of colonies with phenes 1R (8.5%), 2R (42%), 3R (40.6%). The occurrence of colonies with phenes E was the lowest – 1% (10 colonies out of 1000).

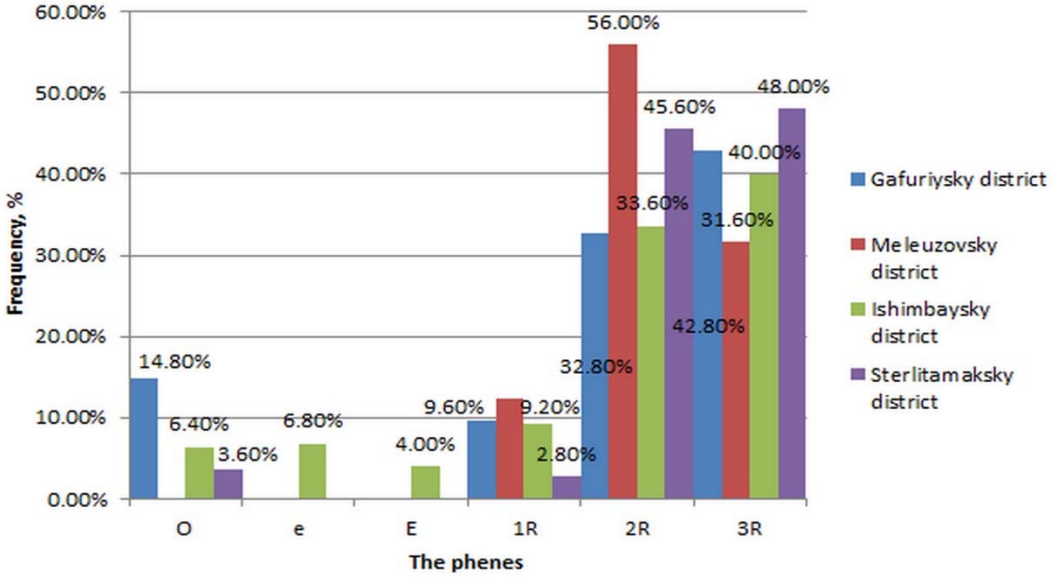

Figure 3. Distribution of the worker bee phenes in the Toratau Geopark.

The phenes of the drones that are found in the honey bee colonies in the apiaries of the Gafuriysky, Ishimbaysky, Meleuzovsky and Sterlitamaksky districts, which are part of the Toratau Geopark, are shown in Figure 4. As we can see, the phenetic structure of drones is also characterized by high heterogeneity (Is, I, O – dark and O- grey), as well as worker bees, because the purebred population of the Dark European bee is characterized only by the O-dark phene.

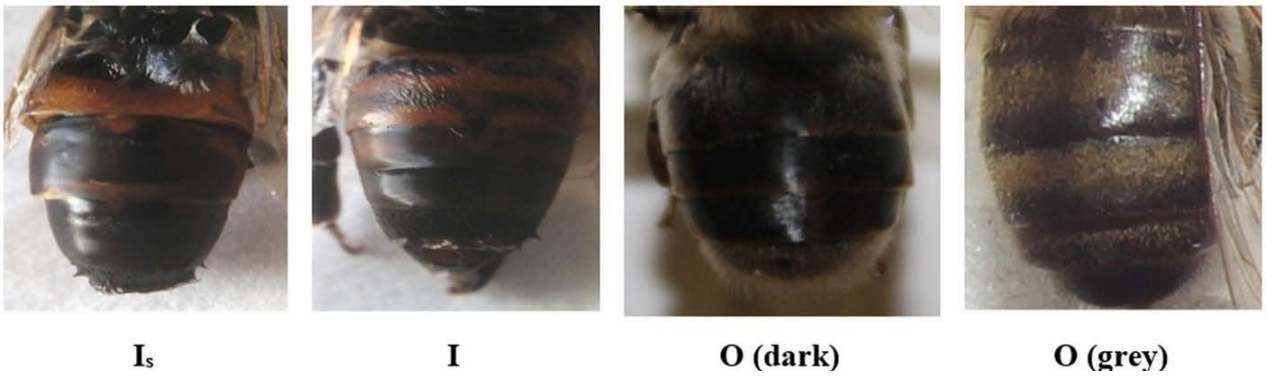

Figure 4. The detected phenes of the drone bees in the Toratau Geopark.

Drones with phenes Is and I prevailed in almost all apiaries, %: in the Gafuriysky district from 28.6 to 100 (I_s) and from 12.5 to 100 (I); in Meleuzovsky – from 33.3 to 100 (I_s) and from 28.6 to 60 (I); in Ishimbaysky – from 20 to 100 (I_s), 25 to 50 (I) and in the Sterlitamaksky district - from 37.1 to 100 (I_s) and from 40 to 57.1 (I) (Table 2).

Table 2. The phenes of the drone bees in the Toratau Geopark

Locality, apiary	No. of colonies, pcs	Drone bee phenes (pcs. / %)			
		I_s	I	O - dark	O - grey
Gafuriysky district					
Kutluguza	15	5 (33.3)	10 (66.7)	-	-
Krasnousolsky	8	-	8 (100)	-	-
Uzbjakovo	16	12 (75)	2 (12.5)	-	2 (12.5)
Tolparovo	11	11 (100)	-	-	-
Tashly					
apiary 1	26	13 (50)	13 (50)	-	-
apiary 2	18	-	-	16 (88.9)	2 (11.1)
Tolparovo:					
apiary 1	17	8 (47.1)	9 (52.9)	-	-
apiary 2	11	11 (100)	-	-	-
Mendim:					
apiary 1	24	12 (50)	10 (41.7)	-	2 (8.3)
apiary 2	26	10 (38.4)	8 (30.8)	-	8 (30.8)
Tabynsk	21	6 (28.6)	5 (23.8)	10 (47.6)	
Kowardy:					
apiary 1	17	5 (29.4)	-	6 (35.3)	6 (35.3)
apiary 2	12	12 (100)	-	-	-
Ibragimovo	13	6 (46.2)	7 (53.8)	-	-
Kyzyl Yar	15	5 (33.3)	10 (66.7)	-	-
Total	250	116 (46.4)	82 (32.8)	32 (12.8)	20 (8)
Meleuzovsky district					
Araslanovo	18	9 (50)	9 (50)	-	-
Alexandrovka:					
apiary 1	22	10 (45.5)	12 (54.5)	-	
apiary 2	19	11 (57.9)	8 (42.1)	-	-
Meleuz:					
apiary 1	28	12 (42.8)	8 (28.6)	-	8 (28.6)
apiary 2	4	4 (100)	-	-	-
Aptrakovo	15	5 (33.3)	7 (46.7)	-	3(20)
Pokrovka	15	10 (66.7)	5 (33.3)	-	-
Nugush	10	5 (50)	5 (50)	-	-
Zirikovo	30	10 (33.3)	15 (50)	-	5 (16.7)
Abitovo	30	12 (40)	18 (60)	-	-
Klenovaya gora	23	10 (43.5)	11 (47.8)	-	2 (8.7)
Kutlubulatovo	8	8 (100)	-	-	-
Smak	5	5 (100)	-	-	-
Sugar Factory Nursery	5	5 (100)	-	-	-
Voskresenskoe	18	9 (50)	9 (50)	-	-
Total	250	125 (50)	107 (42.8)	0	18 (7.2)
Ishimbaysky district					
Makarovo:					
apiary 1	5	-	-	5 (100)	-
apiary 2	19	6 (31.6)	6 (31.6)	6 (31.6)	1 (5.2)
apiary 3	10	2 (20)	-	7 (70)	1 (10)
Gumerovo:					

apiary 1	8	-	-	7 (87,5)	1 (12.5)
apiary 2	8	3 (37.5)	2 (25)	2 (25)	1 (12.5)
Sargaevo:					
apiary 1	17	12 (70.6)	5 (29.4)	-	-
apiary 2	5	5 (100)	-	-	-
Isyakaevo:					
apiary 1	35	15 (42.8)	10 (28.6)	5 (14.3)	5 (14.3)
apiary 2	22	11 (50)	11 (50)	-	-
Asiyalanovo	30	8 (26.6)	8 (26.6)	6 (20.2)	8 (26.6)
Verkhneitkulovo					
apiary 1	10	5 (50)	-	5 (50)	-
apiary 2	11	11 (100)	-	-	-
Armetovo	14	10 (71.4)	4 (28.6)	-	-
Asiyalan	11	11 (100)	-	-	-
Urazbaevo	14	7 (50)	5 (35.7)	2 (14.3)	-
Podgornaya	17	8 (47.1)	8 (47.1)	1 (5.8)	-
Kinzebulatovo	14	5 (35.7)	6 (42.9)	2 (14.3)	1 (7.1)
Total	250	119 (47.6)	65 (26)	48 (19.2)	18 (7.2)
Sterlitamaksky district					
Pokrovka	28	12 (42.9)	16 (57.1)	-	-
Berezovka	28	14 (50)	14 (50)	-	
Karmaskaly	23	11 (47.8)	10 (43.5)	-	2 (8.7)
Spasskaya	26	12 (46.2)	14 (53.8)	-	-
Burikazganovo	25	13 (52)	12 (48)	-	-
Talachevo	15	7 (46.7)	6 (40)	-	2 (13.3)
Pokrovka	8	8 (100)	-	-	-
Aigulevo	15	6 (40)	6 (40)	3 (20)	-
Spasskaya	26	10 (38.5)	12 (46.2)	4 (15.3)	-
Kuganak	20	10 (50)	10 (50)	-	-
Kosyakovka	9	5 (55.6)	4 (44.4)	-	-
Strelkovka	27	10 (37.1)	12 (44.4)	-	5 (18.5)
Total	250	118 (47.2)	116 (46.4)	7 (2.8)	9 (3.6)
Total	1000	478 (47.8)	370 (37)	87 (8.7)	65 (6.5)

Honey bee colonies with drone phenes of the European dark bees (O – dark) were found in all districts except Meleuzovsky. Similar situation was observed while evaluating worker bees in these areas. In Ishimbaysky district, drones with phenes O – dark were detected in the largest number of honey bee colonies, compared to other districts, %: 19.2 (Gafuriysky – 12.8; Sterlitamaksky – 2.8).

In the Gafuriysky district, purebred drones were registered in three apiaries: Tashly (apiary 2) – 88.9%, Tabynsk – 47.6% and Kowardy (apiary 2) – 35.3%. In Ishimbaysky district, drones with a phenes O – dark were found in 11 apiaries, %: Makarovo (three apiaries) – from 31.6 to 100; Gumerovo (2 apiaries) – from 25 to 87.5; Isyakaevo (1 apiary) – 14.4; Asiyalanovo – 20.2; Verkhneitkulovo (1 apiary) – 50; Urazbaevo and Kinzebulatovo by 14.3; Podgornaya – 5.8. Honey bees with a phene O - grey were found in apiaries of all districts, %: Gafuriysky - from 8.3 to 35.3; Meleuzovsky – from 8.7 to 28.6; Ishimbaysky – from 5.2 to 26.6, Sterlitamaksky – from 8.8 to 18.5.

In general, honey bee colonies with I_s phenes prevailed in the Meleuzovsky district – 50%, and honey bee colonies with I drones dominated in the Sterlitamaksky district – 46.4%. In the minimum number, the occurrence of I_s drones was 46.4% in the Gafuriysky district, and phene I in the Ishimbaysky district was 26% (Figure 5).

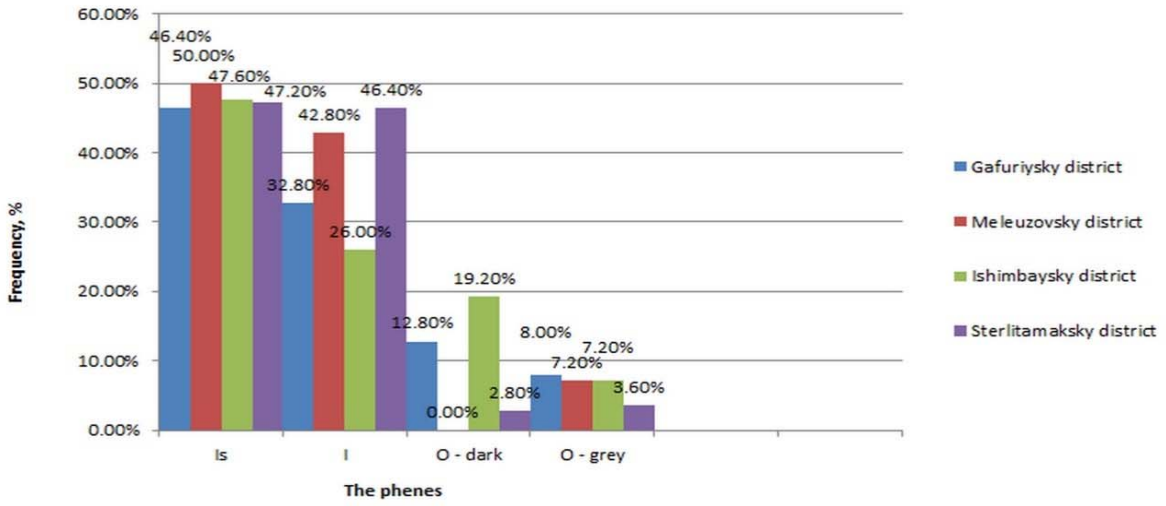

Figure 5. Distribution of the drone bee phenes in the Toratau Geopark.

According to the occurrence of colonies with drones O – grey, the Sterlitamaksky district was different, where they were represented at a minimum – 3.6%. In the Gafuriysky district, this indicator was the maximum compared to other districts – 8%. In the remaining two districts, the honey bee colonies with drones O – grey were represented in the same number – 7.2%.

DISCUSSION

The Bashkir dark European bees originally is characterized by the dark color of the body of individuals, only 1-2 phenes, the tergites of which do not have signs of yellowness, the sternites are colored from dark gray to dark brown without yellowness (Krivtsov 1995; Krivtsov and Grankin 2004). The conducted studies revealed a high phenetic heterogeneity of apiaries in the territory of the Toratau Geopark for worker bees (6 phenes) and drones (4 phenes). At the same time, the dominant content of worker bees' colouring-forming phenes (E, 1R, 2R, 3R) that do not meet the standards of the Dark European bee (*A. mellifera mellifera*) has been established, which, of course, indicates the processes of hybridization. Some occurrence of honey bee colonies with a European bark bee's phene in three districts (Gafuriysky – 14.8%, Ishimbaysky – 13.2%, Sterlitamaksky – 7.9%) is an indicator of the presence of a certain proportion of the gene pool of the local Bashkir bee population. However, maintaining the rate of hybridization in the near future may lead to a complete loss of the gene pool of purebred honey bees in this area. The honey bee colonies purebredness were checked using 9 microsatellite markers by Ilyasov R. A. in spring 2023 (unpublished yet).

The high occurrence of drones with phenes (Is, I, O – gray) that do not meet the requirements of the standard of the Dark European bee characterizes the processes of changing the structure of the population of native honey bees. It should also be noted that the results obtained in this research reveal the presence of southern subspecies in the colonies of queen bees. Unfortunately, purebred drones were not registered from the studied areas in Meleuzovsky district, however in Sterlitamaksky district their occurrence was only 8.7%. Of course, the presented facts require further analysis and annual monitoring of breed in apiaries. Though, in general, the occurrence of purebred drones on the explored territory shows the preservation of the biopotential of the Bashkir population of the European dark bees, which opens up opportunities for the implementation of measures aimed at the conservation and breeding of this population (Chashchukhin and Lapteva 2011; Chashchukhin and Lapteva 2009; Cherevko and Avetisyan 2007; Chibilev 2011; Sharygin and Krivtsova 2018). Taking into account the fact that the Toratau Geopark was created to preserve the geological, biological, historical, and cultural heritage of the republic, it is

necessary to create a network of breeding reproducers for breeding the European dark bee in the explored areas, as well as to provide state support to those apiaries where colonies of this subspecies are kept. It should also be mentioned that the most important areas of the sustainable ecosystem development strategy include the conservation of biodiversity in the context of control and management of the resources. At the same time, experts note that in order to implement scientific-based programs for the conservation of the biodiversity, it is necessary to apply various methods of its assessment both for the entire population of plants and animals, and for individual rare and vulnerable biological species. The assessment of biodiversity with geographical reference makes it possible to assess the uniqueness of regional biomes and ecosystems, their role and place, the conservation status, the boundaries of habitats and factors negatively affecting populations. Such assessments make it possible to plan optimal conservation strategies and develop necessary measures for the protection and restoration of endangered species of flora and fauna (Koroleva et al. 2019).

Conclusions

In the modern world, the most important direction of the strategy for sustainable development of ecosystems is the conservation of biodiversity in terms of control and management of resources. At the same time, experts note that in order to implement scientifically based biodiversity conservation programs, it is necessary to apply a set of assessment methods at different spatial scales. Biodiversity assessment makes it possible to determine the value and uniqueness of regional biomes and ecosystems, the role and place of conservation status, the boundaries of habitats, and factors that negatively affect their population. Such an assessment makes it possible to optimally plan environmental strategies and develop the necessary measures for the protection and restoration of endangered species of flora and fauna.

The results of the assessment of honeybee phenes on the territory of the Geopark Toratau (Republic of Bashkortostan, Russian Federation) indicate that the predominant content in the color of worker bees that form phenes (E, 1R, 2R, and 3R) that do not meet the standards of the dark European bees (*A. m. mellifera*) indicates hybridization processes. The presence of colonies with the Dark European bee phenotypes in the Toratau Geopark are an indicator of the presence of a certain proportion of the gene pool of the Bashkir bee population. The presence of purebred bees opens up opportunities for the implementation of measures aimed at the conservation and reproduction of the Bashkir population of the dark European bee. There is an urgent need to protect local dark European bees from hybridization, as without human help, the local dark forest bee cannot restore the purity of its gene pool.

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Ethical issues: The research on honey bees have no ethical issues.

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