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COMPLEX ANALYSIS OF MORPHOLOGICAL CHARACTERS OF GAMASID MITE *VARROA DESTRUCTOR* (PARASITIFORMES, VARROIDAE)

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Complex Analysis of Morphological Characters of Gamasid Mite *Varroa destructor* (Parasitiformes, Varroidae). Akimov I. A., Benedyk S. V., Zaloznaya L. M. — The study of seasonal variability of mite *V. destructor* was carried out. The summer generation of mites appears to be characterized by the largest morphological variability whereas the winter one has stable characters. We failed to evolve the complex of morphological characters that would allow us to identify, with high level of reliability, certain phenotype of the mite. Significant stability of morphological characters of *V. destructor* in the course of time was determined. The mean values of the length and width of the body allow to consider the Ukrainian population of *Varroa* mite, which parasitize the honey bee *Apis mellifera* Linnaeus, as the Korean haplotype of *Varroa destructor* Anderson et Trueman, 2000.

Key words: *Varroa destructor,* gamasid mites, morphology, seasonal variability.

Комплексный анализ морфологических признаков гамазового клеща *Varroa destructor* (Parasitiformes, Varroidae). Акимов И. А., Бенедик С. В., Залозная Л. М. — Проведено исследование сезонной изменчивости клеща *V. destructor*. Установлено, что летняя генерация клещей характеризуется наибольшей дисперсией признаков, а зимняя — наибольшей их стабильностью. Не удалось выделить комплекс морфологических признаков, который бы позволял с высоким уровнем достоверности идентифицировать определенный фенотип клеща. Выявлена значительная стабильность морфологических признаков *V. destructor* во времени. Подтверждено, что по среднему значению длины и ширины тела, паразитирующий на медоносной пчеле *Apis mellifera* Linnaeus в Украине, клещ варроа относится к описанному корейскому гаплотипу *Varroa destructor* Anderson et Trueman, 2000.

Ключевые слова: *Varroa destructor*, гамазовые клещи, морфология, сезонная изменчивость.

Introduction

The ectoparasitic mite *Varroa destructor* Anderson and Trueman, 2000 *(V. jacobsoni* in our works) has tropical origin and initially parasitized the waxy bee *Apis cerana* Fabricius. The shift to the honey bee *Apis mellifera* Linnaeus happened likely in the late 1960s, when *A. cerana* and *A. mellifera* were kept jointly in East Asia (Delfinado, 1963). Then *V. jacobsoni* successfully acclimatized in the wide and different areas of distribution of its new host, and became cosmopolitan species (Griffiths, Bowman, 1981; Akimov et al., 1993; Zhang, 2000). Elucidating the question how, in the expansion, the mite reveals its genetic and phenotypic potential of variability, resulted in a number of study works on intraspecific morphological differentiation of the parasite (Grobov, Shabanov, 1979; Akimov, Yastrebtsov, 1985; Akimov, Zaloznaya, 1986; Zaloznaya, 1988; Delfinado-Baker, Houck, 1989). It is proved that morphological characters of varroa mite characterized by geographic variability that is connected with not only different natural climatic conditions, but also with distinct host species (Delfinado-Baker, Houck, 1989; Anderson, 2000). In addition, there are periods of intensive reproduction of mite (in summer) and non-reproduction (in winter) under temperate climate conditions were observed that is an abnormal phenomenon of varroa, because it is reproducing all the year round in the area of its origin (Malaysia–Indonesia–New Guinea region). That is why the study of morphological adaptation of the parasite to season change is of significant interest. The first studies concerning this issue were carried out by I. A. Akimov, L. M. Zaloznaya, V. M. Efimov and Yu. K. Galaktionov (1988, 1989, 1990).

The purpose of this study was the complex analysis of seasonal morphological differentiation of females *V. destructor* using the multivariate statistics method.

Material and methods

The study of morphological variability of *Varroa destructor* was carried out on adult females, collected in two bee colonies of private apiary in Radomyshl of Zhitomyr Region in different seasons of 2001–2003. Fifteen samples, each of 40–70 mites, were examined. The samples G, J (the X month), H (the II month), I, K, L (the III–V months), M (the VI–VII months) were collected in the colony ♂1, and the samples A, B, E, (the IX–X months), C (the II month), D (the III month), N, O (the VI month) from the colony ♂ 2. Total amount is 741 mites.

At first, every mite was characterized by 22 quantitative characters; 13 of them were bilaterally symmetric. Later, some of characters depending on the position of mites on slide, were excluded from consideration. These are the length of the anal setae, the length of the anal shield, the length of the peritremal tubes, the number of the setae on the second segment of the palps, the number of the pores on the sternal shield.

As a result, 17 morphological characters of females were treated statistically, as follows: 1 — length of dorsal shield; 2 — width of dorsal shield; 3 — width of pleyral shield; 4 — length of pleyral shield; 5 — width of lateral shield; 6 — larger width of sternal shield; 7 — length of genital shield; 8 — width of genital shield; 9 — width of the basis of gnathosoma; 10 — distance between 1st pair of sternal setae; 11 — distance between 1st and 2nd of sternal setae; 12 — number of setae on sternal shield; 13 — distance between anal setae; 14 — length of macrochaeta of trochanter of IV leg; 15 — length of tarsus of IV leg; 16 — distance between 1st and 2nd hypostomal setae; 17 — distance between 2nd and 3rd hypostomal setae.

In addition to absolute measurements of mite, the length to the width ratios of the same organ were used that allow to draw conclusions about variability of organ shape (Filippova, Musatov, 1996). The measurements are as follows: 18 — length / width ratio of dorsal shield; 19 — length / width ratio of pleyral shield; 20 — length / width ratio of genital shield.

The principal component method, analysis of variance and linear discriminant analysis were performed to evaluate variability and compare samples. All characters were standardized prior to the principal component method analysis (Zhivotovskij, 1991). Pictorial model of eight samples (two samples of each season, collected from two colonies at the same time) was used to establish significant dependency of characters on the seasonal factor (Plochinskij, 1961). These samples from all seasons were analyzed by the discriminant analysis method.

Calculations were made using IBM PC, by the use of the Statistica. 6 for Windows (StatSoft, Inc., USA) and SPSS. 11 for Windows (DiaSoft, Inc., USA) software.

Results

Performed analysis resulted in discrimination of 5 principal components that describe 61% of the total variance. The scatter of seasonal samples occurred in space of the first three principal components (fig. 1). Significant contributions of characters 1, 2, 3, 4, 5, 6, 7, 8 in the first principal component (30.7% of the total variance) (tabl. 1) indicate that its establishment is connected with the variability of shields, and it may be interpreted as dimensional. Characters 10, 11 that put significant contributions (but with opposite signs) in the second principal component (8.8%) characterize the distance between sternal setae, i. e., the oblongness of its front part. So that, they may be regarded as female characters that indicate body size oscillations in longitudinal-transverse direction. That is why the second principal component may be considered a component of variation of longitudinal-transverse axis of mite. The third principal component (8.1%) that was established by characters 15, 16, connected with variability of the size of legs and the distance between the 1st and 2nd hypostomal setae, i. e., also with the size of the coxa of I leg (chelicera).

Scatter plot of seasonal samples in space of three principal component (fig. 1) indicates that the groups of summer and spring samples are the most detached from each other, where separation is on the second principal component. When centers of the samples groups projected on its axis, the summer samples are disposed at the beginning of the axis, with the spring samples on the end. Hence we may affirm that summer mites are characterized by less sizes of the body in its longitudinal-transverse direction if compared with spring mites. The winter and autumn samples stand on intermediate position, the winter samples are situated closer to spring ones.

The analysis of ranges of standard deviation and mean values of the absolute measurements of shields and their proportion (fig. 2–4) indicates that the summer mites are characterized by the maximum variability of characters in regard to specimens of

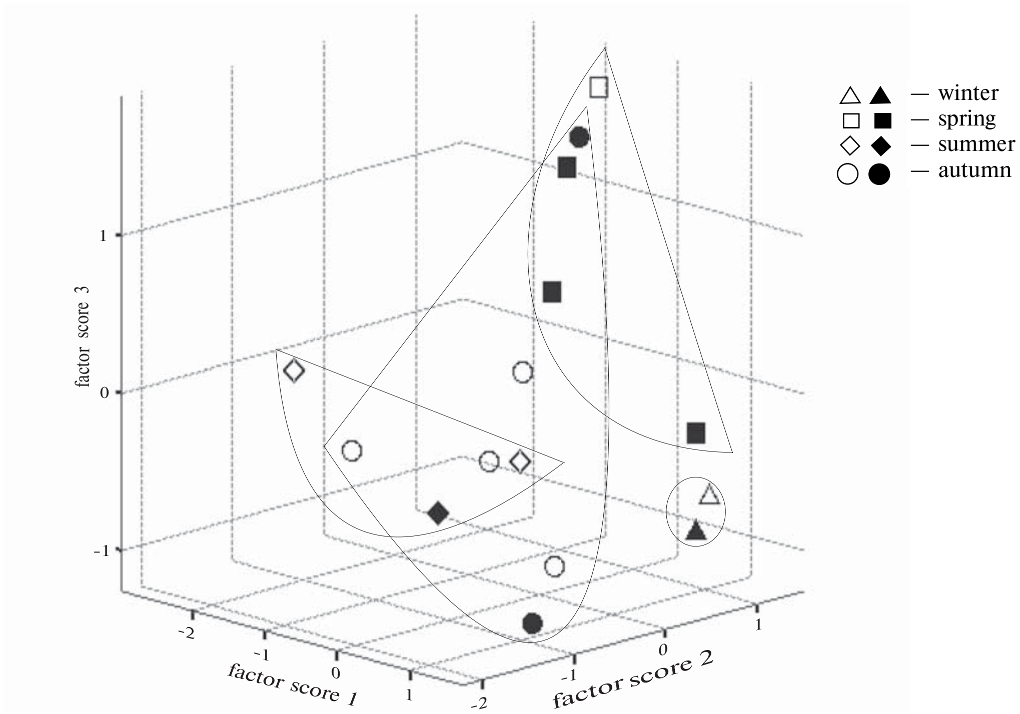

Fig. 1. Scatter plot of seasonal samples of female mite in space of three principal components (blank signs refer colony N 8, black signs to colony N 1).

Рис. 1. Диаграмма рассеяния сезонных выборок самок клеща в пространстве трех главных компонент (незакрашенные маркеры относятся к пчелосемье № 8, закрашенные — к пчелосемье № 1).

other seasonal samples. Moreover, they have the largest mean value of the shield length to width ratio with lesser mean value of the width and insignificantly larger mean value of the length in comparison with the other seasonal samples. The winter and spring mites show enlargement of the width of the genital and pleural shields with almost the same value of the length in regard to the other samples. So, the summer mites are characterized by diminution of measurements of shields in transverse direction as compared with the winter and spring mites that is verified by the results of aforesaid analysis as well.

Table 1. Contributions of characters of seasonal samples' objects in the I, II and III principal components
Таблица 1. Вклады признаков объектов сезонных выборок в I, II и III главные компоненты

Characters	Principal components			Characters	Principal components		
	I	II	III		I	II	III
1	0.78*	−0.05	0.05	10	0.13	−0.84*	0.09
2	0.87*	0.05	0.21	11	0.15	0.85*	0.11
3	0.83*	0.07	0.25	12	0.04	−0.04	0.14
4	0.75*	0.00	−0.08	13	0.09	0.06	−0.21
5	0.59*	−0.20	−0.39	14	0.26	0.01	−0.22
6	0.80*	0.04	0.08	15	0.41	−0.01	0.56*
7	0.76*	−0.04	−0.09	16	0.08	0.00	0.75*
8	0.84*	0.08	0.18	17	0.14	0.03	−0.02
9	0.14	0.06	−0.22	λ, %	30.7	8.8	8.1
				Cumulative variability, %	30.7	39.5	47.6

Note. λ — percent of variance; * — factor loading = or > |0,6|.

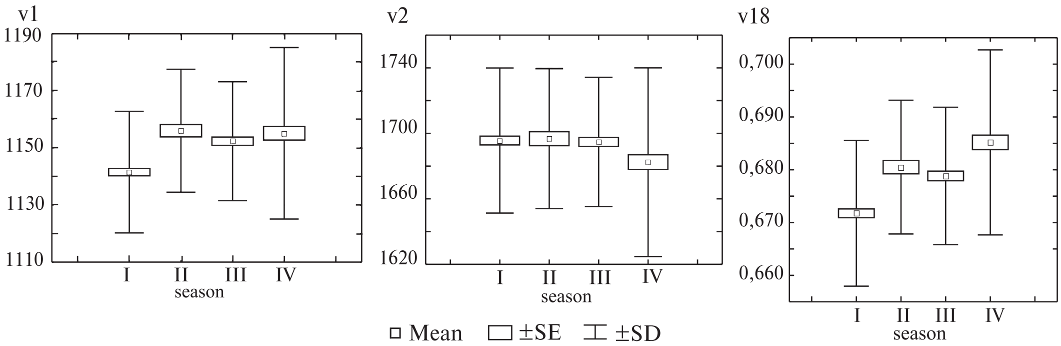

Fig. 2. Mean value (Mean), standard error of mean (SE) and standard deviation (SD) of length (v1) and width (v2) of the dorsal shield and their ratios (v18). By the axis X: I — autumn; II — winter; III — spring; IV — summer.

Рис. 2. Среднее значение (Mean), стандартная ошибка среднего (SE) и стандартное отклонение (SD) длины (v1) и ширины (v2) дорсального щита и их соотношение (v18); по оси X: I — осень; II — зима; III — весна; IV — лето.

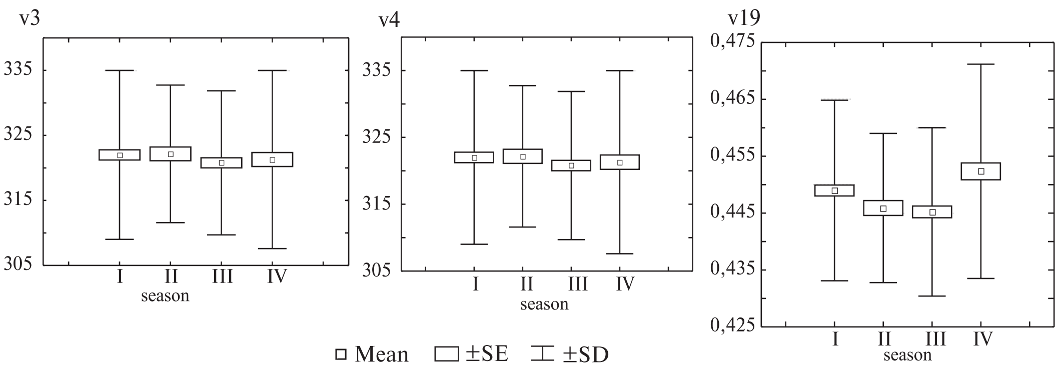

Fig. 3. Mean value (Mean), standard error of mean (SE) and standard deviation (SD) of length (v4) and width (v3) of the pleyral shield and their ratios (v19). By the axis X: I — autumn; II — winter; III — spring; IV — summer.

Рис. 3. Среднее значение (Mean), стандартная ошибка среднего (SE) и стандартное отклонение (SD) длины (v4) и ширины (v3) плейрального щита и их соотношение (v19); по оси X: I — осень; II — зима; III — весна; IV — лето.

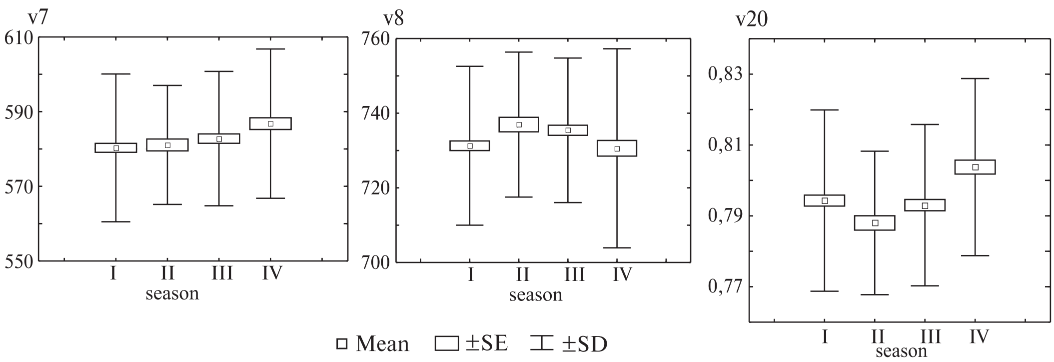

Fig. 4. Mean value (Mean), standard error of mean (SE) and standard deviation (SD) of length (v7) and width (v8) of the genitoventral shield and their ratios (v20). By the axis X: I — autumn; II — winter; III — spring; IV — summer.

Рис. 4. Среднее значение (Mean), стандартная ошибка среднего (SE) и стандартное отклонение (SD) длины (v7) и ширины (v8) генитовентрального щита и их соотношение (v20); по оси X: I — осень; II — зима; III — весна; IV — лето.

Table 2. Characters and the shield length/width ratio by which the distinction of seasonal samples is statistically significant (P < 0.01) (one-way ANOVA)

Таблица 2. Признаки и соотношения длины и ширины щитов, по которым статистически достоверно (P < 0,01) отличаются сезонные выборки (one-way ANOVA)

Samples	Colony	Season	C winter	D spring	O summer	E autumn	H winter	L spring	M summer	J autumn
C	8	winter		18	19,20	18	–	–	20	18
D	8	spring	–		18, 9, 20	–	–	–	18	–
O	8	summer	2, 3, 5, 15, 16	2, 3, 5, 11, 5, 16		18	19, 20	19, 20	–	18, 19,20
E	8	autumn	1, 5, 15, 17	9, 5, 15, 16	2, 3, 11, 16,		18, 20	18, 19	18	–
H	1	winter	–	9	3, 5, 9, 11, 15, 16	1,5, 15, 16		18	20	18
L	1	spring	–	14	2, 3, 5, 11, 14, 15, 16	1,5, 15, 16, 17	14		–	18
M	1	summer	9, 5, 6	9, 15, 16	9, 11, 16	1	5, 15, 16	3, 9, 14, 15, 16		–
J	1	autumn	1, 9, 15	15	2, 3, 14, 16	9	1, 9, 15	1, 15	1, 9, 16	

Note. The left of diagonal — characters, the right of diagonal — ratios.

When pairs of studied seasonal samples are compared (tabl. 2), the summer samples proved to have the largest number of statistically significant distinction in characters as compared with all other samples, though the distribution of these characters is widely overlapped (fig. 2–5). They significantly differ from the spring and winter samples in the width of dorsal, pleyral and lateral shields, width of the basis of the gnathosoma, distance between the 1st and 2nd sternal setae, length of the of the IV leg tarsus, distance between the 1st and 2nd hypostomal setae. They significantly differ from the autumn samples in the length and width of the dorsal shield, width of the pleyral shield, distance between the 1st and 2nd sternal setae, the length of the macrochaeta of the IV leg trochanter, the distance between the 1st and 2nd hypostomal setae. The summer samples also significantly differ from the other in length to width ratio of the dorsal, pleyral and genital shields. The winter and spring mites are most similar

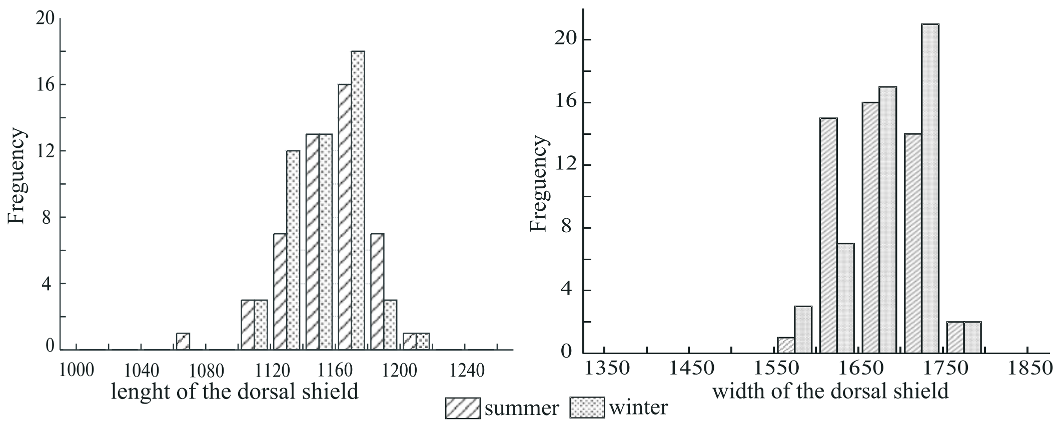

Fig. 5. Distribution frequency of length (*A*) and width (*B*) of the dorsal shield of summer and winter samples.

Рис. 5. Частота распределения длины (*A*) и ширины (*B*) дорсального щита клещей летних и зимних выборок.

Table 3. Correlation coefficients of variables with discriminant functions (P<0.01)
Таблица 3. Коэффициенты корреляции признаков с дискриминантными функциями (P < 0,01)

Characters	DF I	DF II	Characters and ratios	DF I	DF II
1	0.03	0.40*	5	−0.36	−0.12
2	−0.22	−0.11	6	0.04	−0.15
3	−0.29	−0.05	9	0.11	−0.28
4	−0.05	−0,19	10	0.03	0.09
5	0.33	−0.16	11	0.15	−0.19
6	−0.04	−0.13	12	−0.04	0.12
7	0.10	−0.15	13	0.03	0.04
8	−0.15	0.05	14	0.19	−0.22
9	−0.11	−0.23	15	0.50*	0.42*
10	−0.03	0.08	16	0.66*	−0.07
11	−0.14	−0.15	17	−0.00	−0.29
12	0.04	0.10	18	−0.30	0.65*
13	−0.03	0.04	19	−0.28	−0.09
14	−0.18	−0.18	20	−0.32	−0.22
15	−0.45*	0.44*			
16	−0.62*	0.02			
17	−0.01	−0.27			
Cumulative variability, %	71	95	Cumulative variability, %	71	95

Note. * r = or > |0,4|

morphologically and differ significantly from each other in the width of the basis of the gnathosoma, the length of the macrochaeta of the IV leg trochanter, and length to width ratio of the dorsal shield. The exception is the pair of samples C—L that does not significantly differ in any character. Concerning the autumn samples, they are lying in intermediate position between the summer and the spring and winter samples.

Certain distinction is shown to exist between the pairs of the summer, autumn and spring samples, but not winter samples from different colonies. This may indicate significant stability of characters of the winter mites.

The results of discriminant analysis (tabl. 3, beginning from r = |0.4|, P < 0.01) indicate that the length of the IV leg tarsus, distance between the 1st and 2nd hypostomal setae (on the first discriminant function (D 1), length of the dorsal shield and length to width ratio of the dorsal shield (on the second discriminant function (DF 2), length of the IV leg tarsus, distance between the 1st and 2nd hypostomal setae (on DF 1), length of the dorsal shield, and length to width ratio of the dorsal shield (on D 2) mostly contribute to establishment of distinction among seasonal samples. Simultaneous analysis of the absolute values of the length and width of shields and their proportion was not carried out, for in this case the length and width of shields would have been in use twice, and would have resulted in artificial increase in weight of characters (Filippova et al., 1990).

Table 4. Squared Mahalanobis Distances
Таблица 4. Расстояние Махаланобиса

Season	№	1	2	3	4
Autumn	1	0	2,20	1,17	3,06
Winter	2	2,46	0	0,79	3,95
Spring	3	1,30	0,86	0	4,32
Summer	4	3,97	4,51	4,81	0

In the two-dimensional distribution of discriminant functions (fig. 6, *A*, *B*), clear separation of seasonal samples does not occur, though the summer specimens appear to be quite detached.

The analysis of values of squared Mahalanobis distances (tabl 4) also indicate that the summer samples significantly differ from all others, namely they dif-

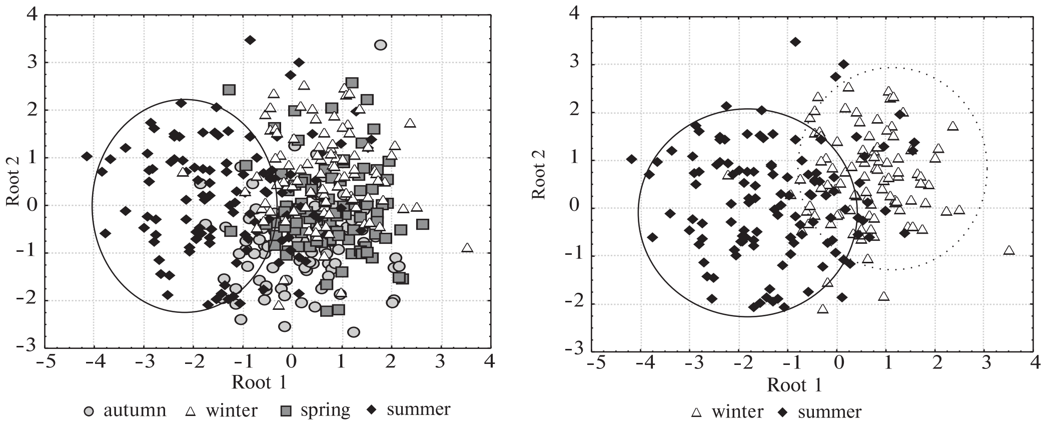

Fig. 6. Distribution of specimens of seasonal samples on area of two discriminant functions by the complex of characters and rations of length and width of shields.

Рис. 6. Распределение экземпляров сезонных выборок в плоскости двух дискриминантних функций по комплексу признаков и соотношений длины и ширины щитов.

fer most considerably from the spring samples, and are the least different from the autumn ones. There was insignificant morphological distinction between the winter and spring mites.

A search for the complex of characters that would significantly improve diagnostic capability of morphological characters, both on the group as a whole and on individual seasonal samples (Magowski et al., 2000) by the means of discriminant analysis, does not bring an expected result. The removal of the characters 12, 8, 2 from the analysis improves the accuracy of identification only by 2% (62% in comparison with 60% when all 17 characters are used). Further diminution of the number of characters in different combinations and change of absolute values of the length and width of shields to their ratio is resulted in decrease of diagnostic capabilities of discriminant analysis. Such a low level of forecasting ability of the given combination of characters may be explained by the fact that every seasonal sample is quite variable by characters that do not allow to collect similar samples for analysis.

Discussion

Studied seasonal samples of *V. destructor* differ from each other statistically reliably. The summer generation of mites is particularly distinguishable in the degree of characters variability. On the contrary, the winter generation is characterized by the stability of morphological characters.

The maximum similarity of characters of the winter and spring generations found in the course of study can be explained by the fact that qualitative structure of mite population during this period changes insignificantly. The point is that the mite mortality does not exceed 6–8% during the wintering (Sal'chenko, 1977; Lange et al., 1977, Fries, Perez-Escala, 2001), and the emergence of sealed brood in February–March does not result in successful reproduction of the parasite (Piletskaya, 1992; Akimov et al, 1993; Martin, 2001).

During intensive reproduction (late spring and summer) maximum diversity of mites may be observed, that explains the highest distinction of the summer samples from the other ones. Variability of most characters of females increases simultaneously from spring till autumn, whereas in winter period it decreases. In particular, the summer generation of mites differs from the winter generation in general decrease of the body measurements and decrease of the measurements of the shields on its ventricular surface, i. e., by decrease in the degree of its sclerotization, decrease of transversal measurements of the body, length of the IV leg tarsus, distance between the 1st and 2nd hypostomal setae. However, ranges of these characters widely overlap. That is why we failed to distinguish clearly between the summer and winter phenotypes (morphotypes) of the mite and pick out the complex of characters that would allow us to identify the definite morphotype with high level of reliability.

Like the other authors (Milushev et al., 1979; Rademacher, 1985), we also noted the mass natural mortality of varroa during the end of August—September that was possibly connected with the completion of life cycle of the summer generation and selection of the wintering generation. This phenomenon is assumed to be connected with different viability of the summer and winter mites during different seasons. During the wintering selection is directed upon elimination of individuals that worse adapted to phoresia on bee, and during the summer selection upholds individuals that better adapted to reproduction (Zaloznaya, 1988; Akimov et al., 1989; 1990; 1993). It may also be assumed that mass natural mortality of the summer generation of mites connected with the fact that, because of intensive reproduction, the summer females exhaust their resources more quickly than the winter females. As a result, their lifetime becomes 2—3 months shorter than that of the winter mites (Lange et al., 1976; Petrova et al., 1982).

One of the main features of the mite biology is the strict synchronization of its reproductive cycle with vital functions of the host, first of all with brood development. The time of its appearance depends, apart from climate conditions, on the microclimate conditions of a colony, race peculiarities, and the age of a queen (Akimov et al., 1993). This fact, to a considerable extent, clarifies why in some colonies (even at the same apiary) some difference in the dynamic of their development may be observed. That is why both the dynamic of mite population development and the increase in its variability may considerably differ in different colonies at the same time. This causes the distinction of pairs of the same seasonal samples, namely those of the summer, autumn, and spring from different colonies. On the contrary, similarity of the winter samples shows that minimum variance of characters is typical for the winter phenotype of the mite. It means that the winter form may figure as a model sample for the comparison of various populations of varroa mites from distinct colonies or ever local population of the parasite.

In general, it is worth noting the considerable conservatism of the morphological characters of *V. destructor* in the course of time. Twenty-five years after the latest study of seasonal variability of mite more then 250 generations of the parasite changed, but its general statistic indexes remain stable.

Hence we may assume that *Varroa* keeps on evolving functionally, adjusting itself to different races of bees and to the new climate conditions. This assumption is strengthen by the interest in study of biochemical interactions between the parasite and host (Hanel, Koeniger, 1986; Rosenkranz et al., 1993; Martin et al., 2001; Colin et al., 2001). It is possible that not only morphologically distinct forms but also the adaptation of outwardly similar forms on the physiological level may serve as a mobilizing reserve.

The obtained results also suggest that the studied mite is identified as *V. destructor* Anderson & Trueman, 2000, in particular, its Korea haplotype by the mean value of the length and width of the body (1149.46 mem. (± 0.8 mem.) and 1692.6 mem. (± 1.70 mem.) accordingly). However, in the course of the study we came across the mites with the least mean value of the length and width of the body 1037.5 mem., 1400.0 mem. accordingly that is typical of *V. jacobsoni* (Anderson, Trueman, 2000). This variation of characters may be considered as a display of the reaction norm under certain conditions (Yablokov, Yusufov, 1989), since the frequency of occurrence of the individuals has made 0.1% from total amount of sample. However, as this study demonstrated, the length and width of the body significantly differ from each other even in the same population of mite during different seasons. That is why we assume that only two morphological characters are not enough for a valid description. The complex approach with use of morphological, genetic and physiological studies is necessary.

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