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CLONAL STRUCTURE OF SOME WEEVIL SPECIES (COLEOPTERA, CURCULIONIDAE) FROM CENTRAL UKRAINE

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Clonal Structure of Some Weevil Species (Coleoptera, Curculionidae) from Central Ukraine. Nazarenko, V. Yu., Morozov-Leonov, S. Yu. — The clonal structure of the populations of nine weevil species (family Curculionidae) from central Ukraine was analyzed. Clonal diversity varied extensively among studied species. The level of clonal variation of some species (*Otiorhynchus ligustici, O. raucus, Liophloeus tessulatus*) is high, within some other species (*O. tristis, Tropiphorus micans*) it is low. The constant heterozygosity of lot of genes has been demonstrated that it may be a proof of the hybrid origin of the studied weevil populations. The asymmetry of some obtained electrophoretic spectra was observed. This can be a consequence of their polyploid nature. The significant interpopulation differentiation of most of the species studied was demonstrated.
Key words: Curculionidae, weevils, allozyme, clonal structure, hybridization, genetic variation, evolutionary potential.

Introduction

The phenomenon of parthenogenesis in natural populations of animals is known long ago (Avise, 2008). Parthenogenesis being an unusual method of inheritance is still widespread among animals (Simon et al., 2003). That phenomenon allows us to assume the evolutionary advantages of this reproduction mode. This problem is relevant for example for insects (Normark, 2003). In particular, parthenogenetic forms are known for weevils (Stenberg et al., 1997, 2000). The abundance and the wide distribution of parthenogenetic forms in this family allow us to expect the presence of significant evolutionary advantages of parthenogenesis within weevil populations. Evaluation of these benefits requires the application of quantitative criteria for assessing the level of parthenogenetic forms genetic variability. Previous studies have shown the clonal structure of several populations from a lot of weevil species of the family Curculionidae (Mazur et al., 2016; Morozov-Leonov, Nazarenko, 2016; Takenouchi, 1983, 1986; Tomiuk, Loeschcke, 1994; Stenberg et al., 2000, 2003; Stenberg, Lundmark, 2004; Kajtoch et al., 2012; Suomalainen, Saura, 1973). It is interesting that the constant heterozygosity of the studied genes has been demonstrated more than once. In addition, many studied weevil populations had a polyclonal genetic structure. This may be resulted from the hybrid origin of at least a part of the clonal forms of weevils. In this case, the detected polyclonality can indicate multiple hybridization acts between Mendelian parental species. This hypothesis of the hybrid origin of several clonal forms within weevils can be tested in two ways.

1. It can be confirmed by more detailed studying of genetic structure of other populations belonging to the previously studied species.

2. It can be also confirmed by the genetic structure study of parthenogenetic populations belonging to other weevil species that were not analyzed before.

To test this hypothesis, we set the following tasks:

A) Description of the genetic structure of samples from populations supposed to be parthenogenetic of several weevil species from Ukraine;

B) Detection of constant heterozygosity by the studied genes;

C) Quantitative estimation of the genetic variability levels of the studied populations;

D) Interspecies comparison of calculated genetic variation indicators.

Material and methods

The material for this work was collected in 2016–2017. Samples were taken in 16 localities on the territory of Central Ukraine (decimal latitude and longitude of each data collection point are indicated in parentheses).

No. 1 — vicinity of Pivni (50.004, 29.733); No. 2 — near Bila Tserkva (49.808, 30.059); No. 3 — Novi Petrivtsi (50.631, 30.446); No. 4 — Muromets island (50.514, 30.544); No. 5 — Kyiv (50.421, 30.550); No. 6 — Teremky (50.362, 30.454); No. 7 — Kruhlyk (50.306, 30.416); No. 8 — near Hutir Vilny (50.323, 30.514); No. 9 — Hutir Vilny (50.318, 30.518); No. 10 — Lisnyky (50.294, 30.529); No. 11 — Pidhirtsi (50.245, 30.550); No. 12 — Baryshivka (50.363, 31.279); No. 13 — near Rankovyy (50.236, 31.724); No. 14 — Plav locality (50.230, 31.731); No. 15 — Panfily (50.225, 31.741); No. 16 — Radychiv (51.772, 33.071).

The sample size and total number of specimens for each species studied are shown in tables 1, 3, 4. Samples of five or more individuals were analyzed separately (tables 3, 4). In addition, combined samples of each species were analyzed (table 4).

Nine species belonging to four tribes of 2 subfamilies were selected as model.

Subfamily Entiminae. Four species of the tribe Otiorhynchini: *Otiorhynchus ligustici* (Linnaeus, 1758) (*O. l.*), *O. raucus* (Fabricius, 1777) (*O. r.*), *O. ovatus* (Linnaeus, 1758) (*O. o.*), *O. tristis* (Scopoli, 1763) (*O. t.*).

Table 1. Distribution of electrophoretic phenotypes from the populations of the studied species in Central Ukraine

O. ligustici						
Clone	No.1	No.2	No.4	No.7	No.8	No.9
Ol1	1					
Ol2						1
Ol3						1
Ol4					1	
Ol5					1	
Ol6					1	
Ol7					7	2
Ol8			1		1	
Ol9			1		2	5
Ol10			12	1	3	22
Ol11		2				
Ol12		3				
Ol13	1					
Ol14		4				

E. ovulum				
Clone	No.10	No.11	No.12	No.13
Eo1	11	7	5	
Eo2				15
Eo3		3		

L. tessulatus					
Clone	No.2	No.3	No.5	No.6	No.9
Lt1					1
Lt2	1		2	1	
Lt3		1	1		
Lt4			2		
Lt5			2		

O. raucus						
Clone	No.6	No.7	No.9	No.13	No.15	No.16
Or1			3		1	
Or2			8			
Or3			1		1	
Or4	3	7		4		6
Or5			2		5	
Or6						2

O. ovatus						
Clone	No.3	No.6	No.7	No.11	No.13	No.16
Oo1				1		
Oo2	1					
Oo3		2	1			25
Oo4				3	1	1

O. tristis			***T. micans***	
Clone	No.4	No.10	Clone	No.5
Ot1	11		**Tc1**	5
Ot2		1	**Tc2**	1

P. inustus						
Clone	No.3	No.10	No.11	No.12	No.13	No.14
Pi1	1					
Pi2	2					
Pi3	40		8		3	1
Pi4			7			
Pi5				1		
Pi6		3				

Table 2. Genes that demonstrated the constant heterozygosity in populations of studied weevil species

Species	Constant heterozygosity	Gene							
		Es-1	Es-2	Es-3	Es-4	Mdh	Aat	Pt	Sod
Otiorhynchus ligustici	In some samples	—	■	■	—	■	■	■	—
	In all samples	—	■	■	—	■	■	■	—
Otiorhynchus raucus	In some samples	—	■	—	—	■	■	—	—
	In all samples	—	■	—	—	■	■	—	—
Otiorhynchus ovatus	In some samples	—	—	■	■	—	—	—	—
	In all samples	—	—	■	■	—	—	—	—
Otiorhynchus tristis	In some samples	—	—	■	—	■	—	■	—
	In all samples	—	—	■	—	■	—	■	—
Tropiphorus micans	In some samples	—	—	—	—	—	—	■	—
	In all samples	—	—	—	—	—	—	■	—
Eusomus ovulum	In some samples	—	—	—	■	—	—	—	—
	In all samples	—	—	—	■	—	—	—	—
Liophloeus tessulatus	In some samples	—	—	■	—	—	■	■	—
	In all samples	—	—	■	—	—	■	■	—
Polydrusus inustus	In some samples	—	■	—	—	—	—	—	—
	In all samples	—	■	—	—	—	—	—	—

One species of the tribe Tropiphorini: *Tropiphorus micans* Boheman, 1842 (*T. m.*).

One species of the tribe Sciaphilini: *Eusomus ovulum* Germar, 1824 (*E. o.*).

Subfamily Polydrosinae. Two species of the tribe Polydrosini: *Liophloeus tessulatus* (Müller, 1776) (*L. t.*), *Polydrusus inustus* Germar, 1824 (*P. i.*).

We selected the species having such features.

1. The specimens of each species are of sufficient size to analyze their proteins variability using gel electrophoresis.

2. All species on the list are flightless. It is known that just such all parthenogenetic weevils are (Lachowska et al., 2008).

3. Data obtained by other researchers have demonstrated that many of Western European populations of the listed species are partly or completely parthenogenetic.

More concretely, are known to be parthenogenetic such taxa as *O. ovatus* (Takenouchi, 1965), *E. ovulum* (Mazur et al., 2016), *P. inustus* (Kajtoch, Lachowska-Cierlik, 2009; Kajtoch et al., 2012). It is also known that some species (*O. ligustici, O. raucus*) are with asexual as well as bisexual populations (Heijerman, Hodge, 2005).

4. In addition, it is known that at least some Western European populations of these genetic forms contain triploid specimens, which makes possible their parthenogenetic reproduction only. Triploid specimens

Table 3. Variation of quantitative indices of the level of clonal diversity of weevil populations

	Species									
	O. l.				*O. r.*				*O. o.*	*O. t.*
Locality	No. 2	No. 4	No.8	No. 9	No. 7	No. 9	No. 15	No. 16	No. 16	No.4
N_{clon}	3	3	7	5	1	4	3	2	2	1
N_{eff}	2.79	1.34	3.88	1.87	1.00	2.51	1.81	1.60	1.08	1.00
H_{clon}	0.72	0.27	0.79	0.48	0.00	0.65	0.52	0.43	0.08	0.00
G_{eff}	0.22	0.03	0.19	0.03	0.00	0.12	0.14	0.09	0.00	0.00
n	**9**	**14**	**16**	**31**	**7**	**14**	**7**	**8**	**26**	**11**

	Species							
	T. m.	*E. o.*				*L. t.*	*P. i.*	
Locality	Total	No. 10	No. 11	No. 12	No. 13	No. 5	No. 3	No. 11
N_{clon}	2	1	2	1	1	4	3	2
N_{eff}	1.38	1.00	1.72	1.00	1.00	3.77	1.15	1.99
H_{clon}	0.33	0.00	0.47	0.00	0.00	0.86	0.14	0.53
G_{eff}	0.08	0.00	0.08	0.00	0.00	0.46	0.00	0.07
n	**6**	**11**	**10**	**5**	**15**	**7**	**43**	**15**

are detected in populations of such taxa as *O. ligustici, O. ovatus* (Lokki, Saura, 1980), *O. raucus*, *E. ovulum* (Lachowska et al., 2008).

We have studied the electrophoretic variability of such proteins as esterase (Es-1, 2, 3, 4), malate dehydrogenase (Mdh), aspartate aminotransferase (Aat), muscle protein (Pt) and superoxide dismutase (Sod). The thoracic segments of every weevil (or animals as a whole in the case of small specimens) were frozen during 12 hours, then used in the acrylamide electrophoresis. Sample preparation, electrophoretic analysis of enzymes and non-enzyme proteins, the data interpretation were performed by standard methods (Mezhzherin, Peskov, 1992).

The following parameters were calculated for samples studied.

Observed number of clones (N_{clon}). Determined by direct count of electrophoretically distinct multilocus phenotypes.

Effective number of clones (N_{eff}). Is calculated by using formula Neff = 1 / Σ (Pi^2), where Pi — the frequency of the i-phenotype in a sample (Parker, 1979).

Clonal heterozygosity (Het). Is calculated by a formula similar to the the expected heterozygosity one for one gene H = (1 — Σ (Pi^2))* n / (n-1) where Pi is a frequency of the i-phenotype in a sample, n is a sample volume (Nei, Roychoudhury, 1974).

Clonal diversity index (G_{eff}). It is calculated according to the formula G_{eff} = (Neff-1) / (n-1) (Dorken, Eckert, 2001).

Frequency of local (identified only in one population) clones (P_{loc}). This parameter was calculated from formula Ploc = Σ (Pi) where Pi is a frequency of the i-clone detected in a single sample only.

Results

Identified clones. The number of different clones in the samples from the populations of each studied taxon of weevils is shown in table 1. The designations of the identified clones and their electrophoretic phenotypes are given in the Appendix.

Electrophoretic spectra of studied proteins. Of the eight genes studied, two (Es-1, Sod) do not exhibit an intraspecific variability that may be electrophoretically detected (table 2). Other genes encode proteins represented as homozygous or heterozygous electrophoretic spectra. At the same time, at least some of the heterozygous spectra of each protein (except Aat) are asymmetric, which was noted earlier (Morozov-Leonov, Nazarenko, 2016).

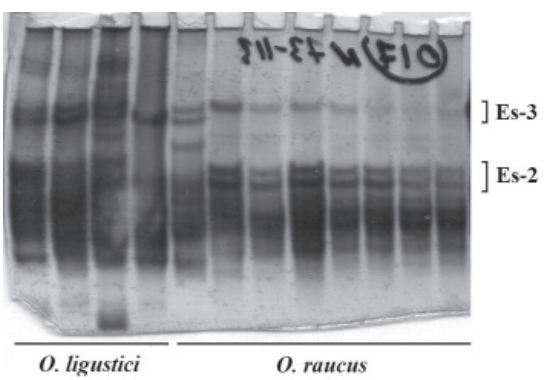

Fig. 1. The electrophoretic spectra of the esterases in the *Otiorhynchus* specimens.

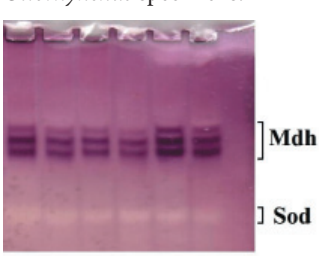

Fig. 2. The electrophoretic spectra of the malate dehydrogenase and superoxide dismutase in the muscle of *Otiorhynchus ligustici* specimens.

Constant heterozygosity. Among the samples studied by us, cases of the constant heterozygosity were repeatedly found when all specimens in the sample were heterozygous for one or more genes (table 2, Appendix).

Subfamily Entiminae. Tribe Otiorhynchini. *Otiorhynchus ligustici* and *O. raucus* specimens show constant heterozygosity only in some samples. In case of detection, the constant heterozygosity in their populations was revealed by 3–5 genes from the studied 8 (Es-2, 3, Mdh, Aat, Pt) (figs 1–3).

Specimens of *O. ovatus* show a constant heterozygosity for two genes (Es-3, 4) (fig. 3). All the studied specimens of this form have heterozygous phenotype $Es\text{-}4^{66/85}$.

Specimens of *O. tristis* demonstrate a constant heterozygosity by three genes (Es-3, Mdh, Pt). In this case, all studied individuals of this form have heterozygous phenotypes for the Es-3 gene ($Es\text{-}3^{54/74}$ and $Es\text{-}3^{76/91}$) (fig. 4).

Tribe Tropiphorini. Specimens of *T. micans* show constant heterozygosity in some samples of one gene (Pt).

Tribe Sciaphilini. Specimens of *Eusomus ovulum* demonstrate a constant heterozygosity for the Es-4 gene in samples Nos. 10, 12, and 13 (fig. 5).

Tribe Polydrosini. Specimens of *Liophloeus tessulatus* show constant heterozygosity in some samples by three genes (Es-3, Aat, Pt), specimens of *Polydrusus inustus* — by one gene (Es-2) (fig. 6).

Intrapopulation clonal structure. The number of identified clones and their numbers vary widely, demonstrating both intraspecific and interspecific differences (table 1).

Tribe Otiorhynchini. In samples from *O. ligustici* populations, the number of identified clones varies from 3 (Nos. 2, 4) to 7 (No. 8). The most common clone in populations of this form is Ol10. It is found in 4 samples of 6, its average frequency is 0.52. The average frequency of clones Ol7, Ol9 is 0.11–0.12. The total frequency of other clones is an average of only 0.25. Clones Ol11, Ol12 (Es-3^{112}, Pt$^{103/103/110}$, Pt$^{103/110}$) are found in a single sample (No. 2, Bila Tserkva).

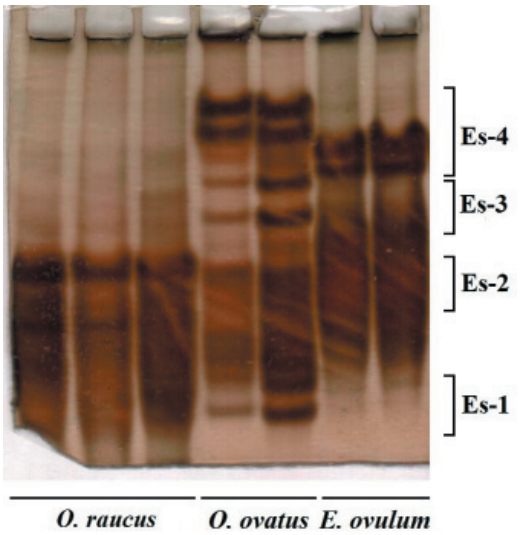

Fig. 3. The electrophoretic spectra of the esterases in the *Otiorhynchus* specimens.

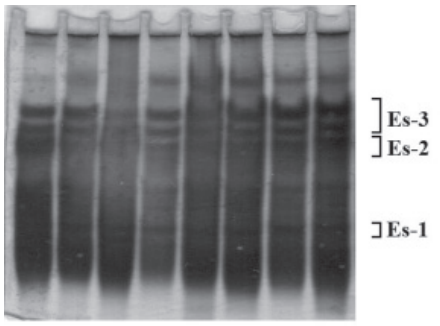

Fig. 4. The electrophoretic spectra of the esterases in the *Otiorhynchus tristis* specimens.

Samples Nos. 2, 8 of *O. ligustici* are characterized by a relatively high level of clonal heterozygosity index (**Het** = 0.72–0.79). The heterozygosity of sample No. 4 is significantly lower (**Het** = 0.27, P > 0.95). The heterozygosity of sample No. 9 has an intermediate value (0.48), not demonstrating significant differences from other samples. Values of the clonal diversity index $\mathbf{G}_{\mathbf{eff}}$ fluctuate within the range of 0.03–0.22 and do not demonstrate reliable inter-sample differences.

The number of clones in samples from *O. raucus* populations varies within 2 (No. 16) — 4 (No. 9). The maximum average frequency is the clone Or4, identified in 4 samples of 6 (0.47). The average frequency of the other two clones (Or2, Or5) exceeds 0.10. The total frequency of other clones is on average 0.19. Clones Or4, Or6 (Es-3^{86}, Mdh^{141}, Aat^{18}) are found in samples No. 6, 7, 13, 16. Clones Or1–3, 5 are found only in samples Nos. 9, 15.

The sample No. 7 is represented by a single clone and, respectively, has no heterozygosity. The clonal heterozygosity index of other samples is significantly higher

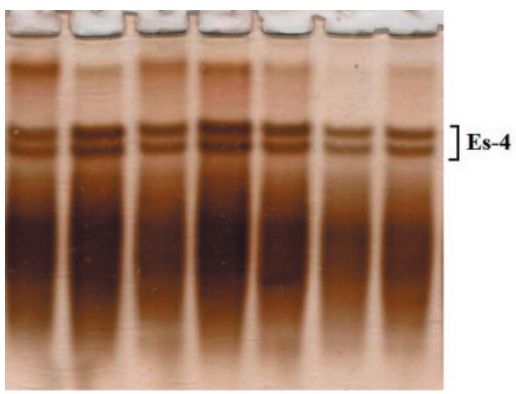

Fig. 5. The electrophoretic spectra of the esterases in the *Eusomus ovulum* specimens.

(**Het** = 0.43–0.65, P > 0.99). Values of clonal diversity index $\mathbf{G_{eff}}$ fluctuate within 0.00–0.14 and do not demonstrate reliable inter-sample differences.

Table 4. Quantitative indicators of clonal variability of the studied weevil species of Central Ukraine

	O. l.	*O. r.*	*O. o.*	*O. t.*	*T. m.*	*E. o.*	*L. t.*	*P. i.*
$\mathbf{N_{clon}}$	14	6	4	2	2	3	5	6
$\mathbf{N_{eff}}$	3.27	3.44	1.51	1.18	1.38	2.20	4.17	1.57
$\mathbf{H_{clon}}$	0.70	0.73	0.35	0.17	0.33	0.56	0.84	0.37
$\mathbf{G_{eff}}$	0.03	0.06	0.02	0.02	0.08	0.03	0.32	0.01
$\mathbf{P_{loc}}$	0.22	0.23	0.06	1.00	—	0.44	0.45	0.21

Appendix

Clone	Gene								n
	Es-1	Es-2	Es-3	Es-4	Mdh	Aat	Pt	Sod	
Otiorhynchini									
Otiorhynchus ligustici									
Ol1	110	95/100	88/100	211	100	89/100	94/100	100	1
Ol2	110	95/100	88/100	211	79/100/100	100	100	100	1
Ol3	110	95/100	88/100	211	79/100/100	100	108	100	1
Ol4	110	100	88/100/112	211	79/100/100	100	100	100	1
Ol5	110	100	88/100/100	211	79/100/100	100	100/108/108	100	1
Ol6	110	100	88/100/100	211	79/100/100	100	100/100/108	100	1
Ol7	110	100	88/100/100	211	79/100/100	100	100/108	100	9
Ol8	110	100	88/100	211	79/100/100	100	100/108/108	100	2
Ol9	110	100	88/100	211	79/100/100	100	100/100/108	100	8
Ol10	110	100	88/100	211	79/100/100	100	100/108	100	38
Ol11	110	100	112	211	100	100	103/103/110	100	2
Ol12	110	100	112	211	100	100	103/110	100	3
Ol13	110	100	100	211	100	89/100	94/100	100	1
Ol14	110	100	100	211	100	100	100	100	4
Otiorhynchus raucus									
Or1	103	95/100	112	100	118/136/136	100	100	100	4
Or2	103	95/100	112	100	136	100/108	100	100	8
Or3	103	95/100	112	100	136	100	100	100	2
Or4	103	95/100	86	100	141	81	100	100	20
Or5	103	95/100	119	100	136	100	100	100	7
Or6	103	100	86	100	141	81	100	100	2
Otiorhynchus ovatus									
Oo1	91	94/100	null	66/85	86	100	96	60	1
Oo2	91	100	100	66/85	84	100	96	60	1
Oo3	91	100	82/100	66/85	86	100	96	60	28
Oo4	91	100	null	66/85	86	100	96	60	5
Otiorhynchus tristis									
Ot1	103	100	76/91	186	93/112	93	100	71	11
Ot2	103	105	54/74	186	112	93	100/111	71	1
Tropiphorini									
Tropiphorus micans									
Tc1	105	89	86	77	100	null	85/89	50	5
Tc2	105	89	86	77	100	null	85	50	1
Sciaphilini									
Eusomus ovulum									
Eo1	91	82	118	100/100/113	105	74	100	60	23
Eo2	91	82	118	100/113	105	74	100	60	15
Eo3	91	82	118	100	105	74	100	60	3
Polydrosini									
Liophloeus tessulatus									
Lt1	103	104	48/73	124	84	86/99	100	59	1
Lt2	103	104	108	124	84	null	100	59	4
Lt3	103	104	108	124	84	74/86	100	59	2
Lt4	103	104	108	124	84	74/86	100/122	59	2
Lt5	103	104	108	124	84	86	100	59	2
Polydrusus inustus									
Pi1	100	94/100/100	105/119	100	86	74	100/108	60	1
Pi2	100	94/100/100	105/119	100	86	74	100	60	2
Pi3	100	94/100/100	100	100	86	74	100	60	52
Pi4	100	94	100	100	86	74	100	60	7
Pi5	100	100	100	100	86	74	100	60	1
Pi6	100	100	119	100	86	74	100	60	3

The only sample of sufficient volume (No. 16) from the population of *O. ovatus* is represented by two clones. The frequency of the more abundant clone Oo3 is 0.96 for this sample and 0.80 for this form on average. The frequency of the second mass clone Oo4 averages 0.14. Thus, the share of the other two clones is only 0.06. The sample number 16 is characterized by low values of clonal heterozygosity index (0.08) and clonal diversity index (0.00).

A sample of the *O. tristis* (No. 4) is represented by a single clone.

Tribe Tropiphorini. The only sample of the *T. micans*, suitable for analysis, is represented by two clones. The frequency of the more abundant clone Tc1 is 0.83. Clonal heterozygosity index is 0.33, the index of clonal diversity is 0.08.

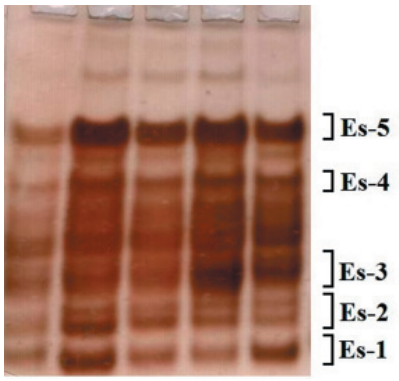

Fig. 6. The electrophoretic spectra of the esterases in the *Polydrusus inustus* specimens.

Tribe Sciaphilini. Sample No. 11 from *E. ovulum* population is represented by two clones. The value of the clonal heterozygosity index in sample No. 11 is 0.47. Other samples of this form are monoclonal and have zero heterozygosity ($P > 0.99$). Values of the clonal diversity index do not show reliable inter-sample differences.

Subfamily Polydrosinae. Tribe Polydrosini. The sample of *L. tessulatus* (No. 5) contains 4 clones. The clone frequency Lt2, Lt4, Lt5 is 0.29. The frequency of the rarer clone Lt3 is 0.14. The average frequency of the most abundant clone Lt2 as a whole is 0.36. Clone Lt1 (Es-$3^{48/73}$) is found in the sample No. 9 only.

The clonal heterozygosity index of sample No. 5 is 0.86, the clonal diversity index is 0.46.

The number of clones in samples of *P. inustus* varies from 2 (No. 11) — 3 (No. 3). Clones Pi3-Pi5 (Es-3^{100}) are found in the samples Nos. 3, 11–14.

Values of clonal heterozygosity index in sample number 3 are 0.14, in sample No. 11–0.53, which is significantly higher ($P > 0.99$). Values of clonal diversity index fluctuate within 0.00–0.07 and do not demonstrate reliable inter-sample differences.

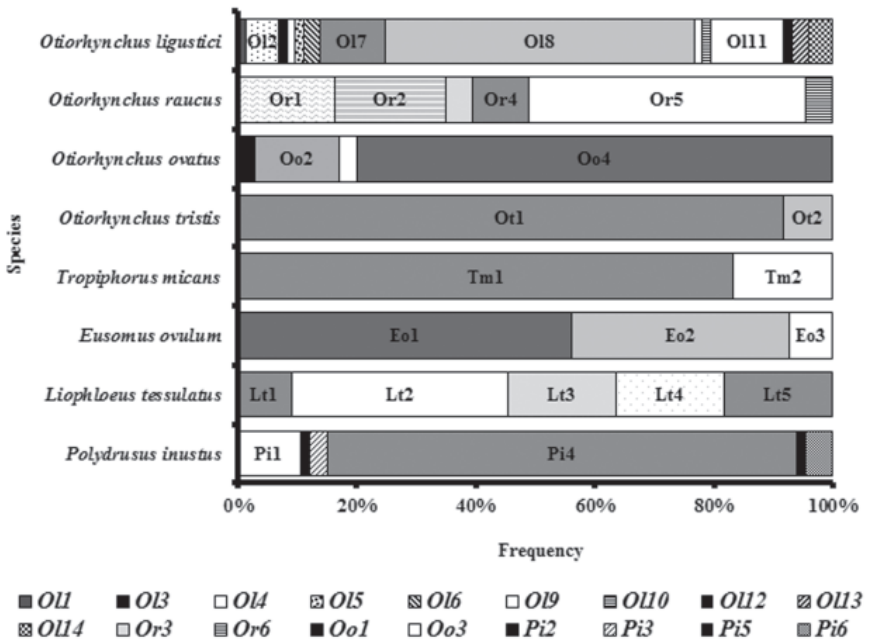

Fig. 7. Polyclonal structure of studied weevil species in Central Ukraine.

Interspecific differences in the level of genetic variability of the studied weevil forms. The number of clones identified within the studied genetic form varies from 2 (*O. tristis*, *T. micans*) to 14 (*O. ligustici*) (table 4, fig. 7). In this case, the inequality of clone frequencies in populations leads to the fact that the effective number of clones is significantly lower than observed and does not exceed 4.17 (*L. tessulatus*).

The level of genetic variability of the studied forms of weevils varies widely — from very low (*O. tristis*) to relatively high (*L. tessulatus*) (table 4, fig. 8). At the same time, none of the studied forms demonstrates significant differences from **all** others in values of genetic diversity quantitative indicators. The clonal heterozygosity index value of the *L. tessulatus* (**Het** = 0.84) was higher than in most of the studied forms (P > 0.95–0.99), except *O. ligustici* (**Het** = 0.70), *O. raucus* (**Het** = 0.73), *E. ovulum* (**Het** = 0.56).

Values of the clonal diversity index vary in more narrow limits. However, they also do not always show reliable differences. The values of this indicator for *L. tessulatus* ($\mathbf{G}_{\mathbf{eff}}$ = 0.32) are significantly higher (P > 0.95–0.99) than for all other forms except *T. micans* ($\mathbf{G}_{\mathbf{eff}}$ = 0.08). The values of the clonal diversity index for *O. ligustici* and *O. raucus* ($\mathbf{G}_{\mathbf{eff}}$ = 0.03–0.06) are significantly lower (P > 0.95–0.99) than in *L. tessulatus*, despite the lack of reliable differences in values of clonal heterozygosity index.

The frequency of local clones varies largely within the studied weevil forms. The value of this indicator in the *O. tristis* is equal to one ($\mathbf{P}_{\mathbf{loc}}$ = 1.00). In practice this means the absence of clones common to all populations. This is significantly higher than in all other forms (P > 0.95). The frequency of local clones does not show significant differences within *O. ligustici, O. raucus, P. inustus* ($\mathbf{P}_{\mathbf{loc}}$ = 0.21–0.23). The frequency of local clones is also similar for *L. tessulatus, E. ovulum* ($\mathbf{P}_{\mathbf{loc}}$ = 0.44–0.45).

Discussion

The reproduction mode of the studied weevil taxa. The result of our study can confirm the assumption that the weevil populations studied by us reproduce parthenogenetically. The constant heterozygosity which we identified in the samples of all the forms studied, most likely is a manifestation of their non-Mendelian inheritance. The basis for such idea can be the

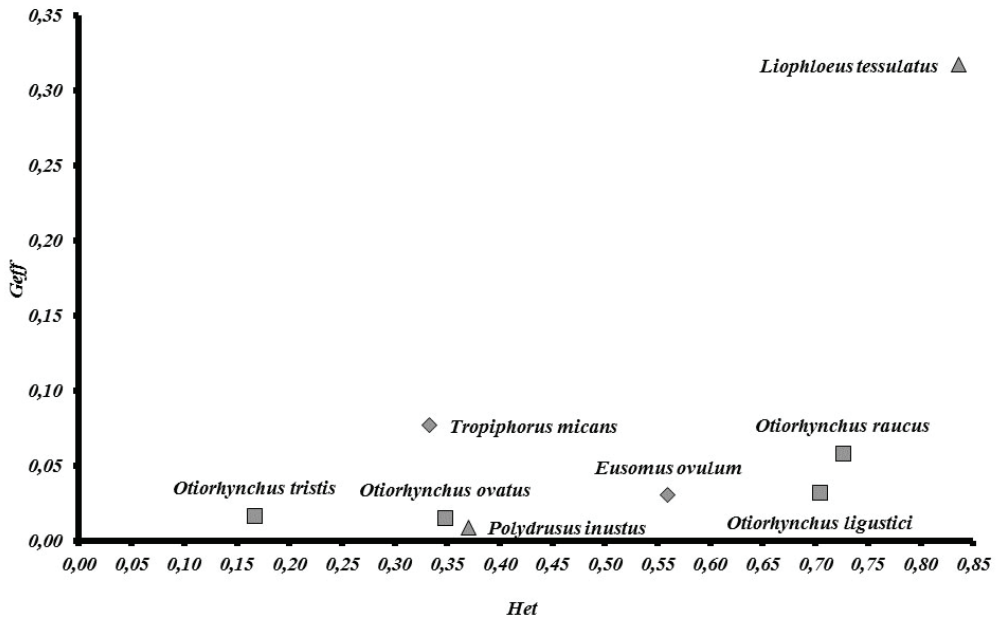

Fig. 8. The values of genetic variability quantitative indicators of the studied weevil populations in Central Ukraine.

Abbreviations. ***Het*** — the clonal heterozygosity, ***Geff*** — the clonal diversity index.

detection of constant heterozygosity in populations of other parthenogenetic forms of weevils (Suomalainen, Saura, 1973). In addition, the hybrid origin of parthenogenetic forms of weevils was previously proven by Kajtoch, Lachowska-Cierlik (2009). The additional argument supporting the assumption of parthenogenetic reproduction of the populations we studied is the presence of triploids in them. The effect of dose of gene, which manifests itself in the form of asymmetric electrophoretic spectra, was revealed by us in samples from populations of some forms. Moreover, triploidity and parthenogenetic reproduction were previously demonstrated by other researchers for some taxa (*O. ligustici, O. ovatus*, *O. raucus*, *E. ovulum*) from our list (Lokki, Saura, 1980; Lachowska, Rozek, Holecova, 2008). In addition, the symmetry of electrophoretic spectra cannot serve as a reliable proof of the diploid nature of the studied organism. Therefore, it can be supposed that all the populations we studied consisting exclusively of flightless specimens are parthenogenetic.

Multiple hybrid origin of the studied weevil parthenogenetic forms. The interpopulation differentiation that we identified, which is especially strong in some forms (*E. ovulum, L. tessulatus*), requires its explanation. The presence of local clones in the studied populations may indicate genetic recombination, which is actually revealed in some parthenogenetic forms (Kajtoch, Lachowska-Cierlik, 2009). However, genetic differences between clones within some of the forms studied by us (Morozov-Leonov, Nazarenko, 2016) reach a level of fixed gene differences. These differences cannot be resulted from recombination. In addition, the appearance of new local clones as a result of mutations cannot be ruled out. However, the high frequency of many local clones (Or2 clone within *O. raucus*, Eo2 within *E. ovulum* etc.) makes their mutant origin few probable. Thus, the most probable explanation of interpopulation differentiation is the origin of the parthenogenetic forms we studied is the result of repeated acts of hybridization of parental species.

The trigger mechanism for the generation of parthenogenetic forms of weevils. The mechanism of generation of such forms is very important among the questions arising from the study of the genetic structure of parthenogenetic forms of animals (in particular weevils), The data obtained by us show that spontaneous hybridization of bisexual weevils seems to occur regularly. Stable inheritance and successful reproduction of hybrid forms are possible in the presence of very specific conditions. The fact that the factor that creates exactly such conditions for weevils is known and studied in detail is very interesting. We keep in mind the effect created by a bacterium of the genus *Wolbachia* (Rodriguero, Lanteri, Confalonieri, 2010; Mazur et al., 2016). Infection with this bacterium induces such effects as parthenogenesis, feminization, and embryonic male killing (Stevens, Giordano, Fialho, 2001). This allows hybrid specimens to successfully reproduce parthenogenetically and simultaneously makes the reproduction of parental bisexual populations impossible. At the moment, it is established that among the species infected with *Wolbachia*, flightless parthenogenetic forms predominate (Lachowska-Cierlik, Kajtoch, Knutelski, 2010). Thus, the most probable is the origin of parthenogenetic forms of weevils as a result of the combined effect of *Wolbachia* infection and multiple interspecific hybridization. As indicated above, according to other researchers, at least one of the parthenogenetic weevils (*E. ovulum*) is infected with *Wolbachia* and is of hybrid origin.

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Scientific responsibilities of the co-authors were shared as follows: Dr. V. Yu. Nazarenko collected samples from natural populations of the studied species of weevils and identified them. Dr. S. Yu. Morozov-Leonov carried out an electrophotetic analysis of proteins of the studied weevils. The samples preparation, the interpretation of the obtained electrophoretic spectra, the mathematical processing of the results obtained and the article writing were carried out by the both co-authors.

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