NAT. CROAT. | VOL. 11 | No 3 | 293–319 | ZAGREB | September 30, 2002

ISSN 1330-0520
UDK 595.78:591.46(497.6)

original scientific paper / izvorni znanstveni rad

VARIATION IN EXOGENOUS AND ENDOGENOUS (GENITALIA) CHARACTERISTICS OF BUTTERFLIES OF THE SPECIES *LEPTIDEA SINAPIS* LINNAEUS, 1758 (*PIERIDAE*, *DISMORPHIINAE*) WITHIN POPULATIONS FROM THE AREA AROUND SARAJEVO

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Lelo, S.: Variation in exogenous and endogenous (genitalia) characteristics of butterflies of the species *Leptidea sinapis* Linnaeus, 1758 (*Pieridae*, *Dismorphiinae*) within populations from the area around Sarajevo. Nat. Croat., Vol. 11, No. 3., 293–319, 2002, Zagreb.

This work describes a precise intrapopulation analysis, from a large sample, of a number of exogenous and macroendogenous characteristics of members of the species *Leptidea sinapis* Linnaeus, 1758. The analysis clearly shows the existence of considerably greater variation in a large number of characteristics than is recorded in the existing literature.

Key words: *Papilionidea, Pieridae, Dismorphiinae, Leptidea, sinapis, reali, duponcheli, morsei major,* variability, variation

Lelo, S.: Varijacija egzofenotipskih i endofenotipskih (genitalni aparat) osobina leptira vrste *Leptidea sinapis* Linnaeus, 1758 (*Lepidoptera*, *Pieridae*) unutar populacije iz šire okolice Sarajeva. Nat. Croat., Vol. 11, No. 3., 293–319, 2002, Zagreb.

U ovom radu je izvršena precizna intrapopulacijska analiza niza egzomorfoloških i makroendofenotipskih svojstava jedinki vrste *Leptidea sinapis* Linnaeus, 1758. Analizom je jasno pokazano postojanje znatno veće varijabilnosti kod velikog broja osobina u odnosu na literaturne podatke.

Ključne riječi: *Papilionidea, Pieridae, Dismorphiinae, Leptidea, sinapis, reali, duponcheli, morsei major,* varijabilnost, varijacija

INTRODUCTION

Leptidea sinapis Linnaeus, 1758 is one of the best known and most widespread species of butterfly. There is a very large number of works devoted to this species,

starting from the earliest work in »Systema Naturae«, and continuing right up to the present day. Following the recognition of the separate genus *Leptidea* Billberg, 1820. a number of species were differentiated, four of which are found in Europe: *Leptidea sinapis* Linnaeus, 1758. *Leptidea reali* Reissinger, 1989. *Leptidea duponcheli* Staudinger, 1871. *Leptidea morsei major* Grund, 1905. Of these four species, all can be found in Bosnia and Herzegovina except *L. duponcheli* Staudinger (SIJARIĆ, 1966. 1980; LELO, 2000).

Leptidea sinapis L. is a very widespread and common species in a large part of Europe south of latitude 66° N. It lives in Fennoscandia, although it is very local in northern regions (within the Artic Circle). It is also found on Mediterranean islands: Majorca, Corsica, Sardinia, Elba, Sicily, Corfu, Levkas, Cephalonia, Zakinthos, Skiros, Thasos, Lesbos, Khios, Samos and Crete. It is known in North Africa in one very narrow zone around the El-Rif locality. It is absent from Scotland, northern England, Holland, north Germany and Denmark, apart from Bornholm. It can be found at altitudes from 0 to 1900 m above sea-level (HIGGINS & RILEY, 1980; 1993; TOLMAN & LEWINGTON, 1997).

The following characteristics can be considered sufficient to identify individuals of this species. The wings of the male are clearly white, and their length varies from 19(?) to 24 mm (HIGGINS & RILEY, 1993). The antennal club is black, the extreme tip being chestnut-brown with a white patch beneath. *First brood*: on the upper side of the forewing is a clearly distinguished grey (?) apical mark, on the underside the costa is grey, and the apex yellowish. The underside of the hind wing is yellowish, with unclear dark markings across the whole of the wing, except for the cell and the area behind it. The female is slightly different: on the upper side of the forewing is an apical mark, but reduced to grey streaks along the veins. *Second brood*: the apical mark on the upper side of the forewing is darker, smaller and rounded. On the underside of the hind wing markings are reduced to obscure yellow-grey marbling (f. *diniensis* Boisduval). In the female the apical mark is greatly reduced, and may even be completely absent (f. *erysimi* Borkenhausen) (HIGGINS & RILEY, 1980. 1993; TOLMAN & LEWINGTON, 1997; FORSTER & WOHLFART, 1955).

The genitalia of the male are characterised by a clearly visible, hard genital capsule with a crenelate tegumen. Uncusi are black and overlap the apical part of the aedeagus. The aedeagus itself varies in length from 1.33 to 1.70 mm (LORKOVIĆ, 1993). Also clearly visible is the saccus, whose length can be around 0.50–0.77 mm.

The shape of the saccus is, according to some authors, of exceptional importance and can represent a basic systematic endogenous characteristic (HIGGINS, 1975). The genitalia of the female are very simple, consisting of a fairly short tube, widened at the top. A unique feature of the Palearctic genus *Leptidea* Billberg within this group of insects is the presence of a rudimentary valve which is no longer held by the abdomen of the female, this role being taken over by the tergite of the eighth segment (LORKOVIĆ, 1930/31).

Butterflies of this species are found from lowland to subalpine regions, where they fly in sunny clearings, at the edges of forests and in clearings on mountains. Most often they are found in areas that have not been claimed for agricultural use.

Males fly in sunny areas, searching for females who are usually hidden in the vegetation — on deciduous trees or on flowers. When the male finds a female of his own species he positions himself with his head facing hers, extending his proboscis with a rhythmic to-and-fro swaying of his head, while the female follows with her head the circular motion of his proboscis. With this the male signals his readiness for copulation, to which the female responds by raising the rear part of her body towards the male. The male then flies on to the female, and copulates for a period of about an hour. A female ready for mating may react to the approach of a male of her own species by almost taking off in flight, or she may remain stationary with her antennae folded until the arrival of the male. The female remains monogamous, and because of this she has plenty of time to lay her eggs (LORKOVIĆ, 1993; WIKLUND, 1977; »TAGFALTER UND IHRE LEBENSRÄUME«, 1997).

The day after mating the female lays her eggs on one of the plants of the family *Papilionaceae*. There are a large number of plants which have been recorded as being used for egg-laying by species of this genus. For *L. sinapis* L. these include *Lathyrus pratense*, and less commonly *L. linifolius* (»TAGFALTER UND IHRE LEBENSRÄUME«, 1997), also *Lotus corniculatus*, but others have also been noted, for example the geni *Vicia*, *Coronilla* and *Astragalus* (FORSTER & WOHLFART, 1955; LORKOVIĆ, 1993). Before laying her eggs the female 'sits' on the plant, and, holding it with her forelegs, carefully examines the plant (»TAGFALTER UND IHRE LEBENSRÄUME«, 1997). Then she lays the eggs, one by one, on the underside of a leaf, so that upon hatching the larvae have food available.

MATERIALS AND METHODS

Collection and preparation of butterflies

The material, specimens from populations of the genus *Leptidea* (Billberg, 1820), was collected during 1998 and 1999 in a wide area around Sarajevo (Tab. 1, Fig. 1). 366 butterflies were collected, 13 of which were badly damaged and could not be taken into consideration. Among the remaining 353 specimens 59 were female (I – 32. II – 27) and 294 male (I – 109. II – 177. III – 8).

Material was collected using classic techniques. Butterflies were caught in an entomological net, and then transferred to a »killing bottle«, i.e. a jar containing absorbent paper saturated with acetic acid and ether in the ratio of 1:3. The saturated absorbent paper was isolated with thick cardboard so that the butterfly specimen would not be soaked and hence ruined. Butterflies were left for 30 minutes in the killing bottle to make sure of the efficacy of the poison, and then either transferred to an entomological envelope, or immediately prepared for transport in a special, hand-made entomological field box as described in (WILLIAMS, 1969).

Butterfly specimens were prepared on hand-made »stretchers«, more precisely on polystyrene which had previously been hollowed out with a hot metal wand. This method of preparation left traces on the prepared material, and hence will not be employed in future without great necessity. The prepared butterflies were dried for 7–14 days, after which they were transferred to a hand-made entomological box and protected with a TUS tablet.

Tab. 1. List of localities in which specimens were collected.

Locality number	Name of locality	Altitude (m above sea level)*
1.	Bembaša	580–600
2.	Gazijin han – kula	940–965
3.	Pašino brdo	920–965
4.	Grdonj – Špicasta stijena	880–900
5.	Grdonj	880–904
6.	Mrkovići	850–900
7.	Gornji Mrkovići	980–1.020
8.	Orlovača	1.200–1.212
9.	Debelj	700–750
10.	Orlovac	750–792
11.	Blekin potok	580–600
12.	Kromolj	600–700
13.	Gornji Kromolj	700–750
14.	Poljine	750–800
15.	Gornje Poljine	900–965
16.	Slatina	560–600
17.	Bare	550
18.	Sokolović kolonija	505–510
19.	Sokolovići – Hrasnica	510
20.	Hrasnica	510–520
21.	Stojčevac	490–500
22.	Ilidža – aleja	480–490
23.	Vrelo Bosne	500
24.	Župča – Breza	520–540

(*Altitude is represented as a range because material was collected from an area around the designated locality.)

Fig. 1. Map of the general area of Sarajevo with the position of the collection sites (sub-population A – blue spots, sub-population B – red spots)

RESULTS AND DISCUSSION

Analysis of individual characteristics

Individual characteristics were analysed in the first instance according to the descriptions given by Lorković (LORKOVIĆ, 1927; 1930; 1993), and then by independently observed properties which could be shown to be significant.

1. Observed characteristics of butterflies

1.1. White mark on ventral surface of antennae

Analysis of collected material showed that in populations of the species *L. sinapis* L. in the area around Sarajevo there are two variants of ventral markings of the antennae. The first variant is typical, i.e. under the anulus of the antenna there is a fairly large white area which continues along the whip of the antenna to the end. The second variant has a similar white area which is extended along the antennal whip, but only half-way (Figs. 2 and 3).

Figs. 2 and 3. Variants of white markings on the ventral surface of the anulus and stem of the antenna in *L. sinapis* L.

Both variants of the observed characteristic appeared in males of all three generations, but only in second generation females. It was ascertained that among the males only 5.10% of the first generation and 0.57% of the second generation, but as many as 50% of the third generation in this population have the »shortened« type of marking on the stem of the antenna. Among the females this feature does not appear among the first generation, and in the second generation it was observed that 7.41% of the specimens showed the second (»shortened«) variant of white antennal marking.

In the literature there are data to show that most butterflies of the genus *Leptidea* have white markings on the antennae. Moreover it has been noted that these mark-

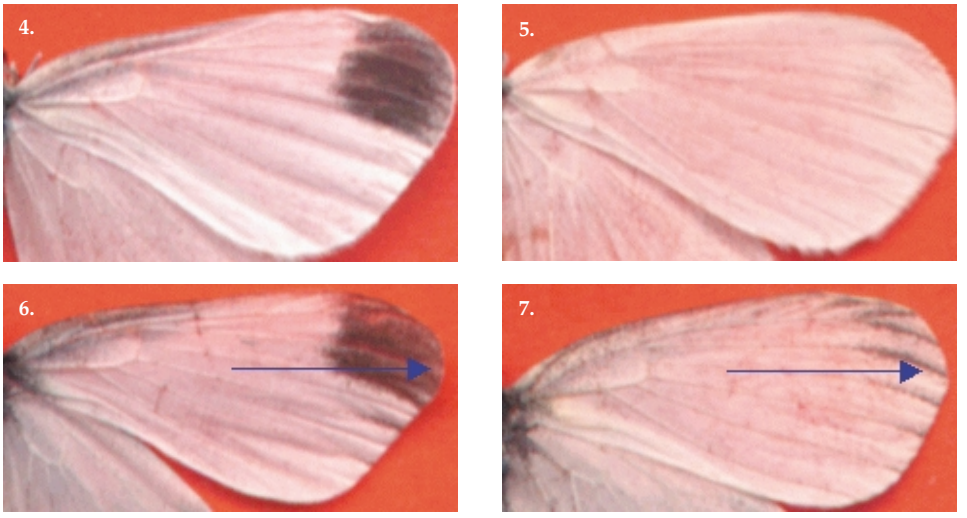
ings are most prominent in *L. sinapis* L., and that they extend from below the top of the antennal club, along the antenna to its base. So we can conclude that the observed variability agrees with the published data.

1.2. Shape of the outer edge of forewing

Examination of the outer edges of the forewing in the species *L. sinapis* L. showed that this characteristic also appears as two variants. It was established that in some individuals the edge of the wing at the end of vein *v6* protrudes somewhat, in relation to vein *v7* (Figs. 4–7).

The variant showing the typical smooth wing edge is generally much more frequent. Among males this feature was found in only 2.75% of the first generation, 0.57% of the second generation and 0.00% of the third generation. Among females »ragged edge of forewing« was more frequent in comparison to males. 19.36% of first generation females showed this feature, with its frequency dropping insignificantly in the second generation: 14.82%.

The appearance of specimens showing »ragged edge of forewing« was particularly surprising because of the currently accepted belief that only members of the species *L. morsei major* Grund have such a characteristic (LORKOVIĆ, 1927; 1930; 1993; HIGGINS & RILEY, 1980; 1993, etc.). However, careful examination established that some specimens of *L. sinapis* L. have this feature, and that other features, especially the apical mark do not support the current description of males or females of the subspecies *L. morsei major* Grund, which directly contradicts the current position of undisputed authorities in this field.



Figs. 4–7. Variants in the shape of the outer edge of the forewing of representatives of *L. sinapis* L. from the surroundings of Sarajevo (male with »smooth« wing edge – 5. and »ragged« edge – 7; female with »smooth« wing edge – 6. and »ragged« edge – 8)

1.3. Length of the right forewing

The length of the right forewing of the butterflies was measured using squared paper with a precision of half a millimetre. The total length was measured from the base of the wing to the tip of the wing at the end of vein *m1* (*v6*) (Fig. 8.).

Fig. 8. Line of measurement for establishing the length of the right forewing of butterflies of the species *L. sinapis* L.

It was established that the length of the right forewing of males varies from 15.5 mm to 22.5 mm. By generation the variation is as follows: I – 17.5 – 22.0 ($\bar{x}$ – 20.243); II – 15.5 – 22.5 ($\bar{x}$ – 20.534); III – 18.5 – 21.0 ($\bar{x}$ – 19.625). Slightly surprising was the finding that female have a smaller range of variation: ♀♀ – 17.0 – 23.0 mm. The variation of this characteristic in females by generation was: 17.0 – 22.5 ($\bar{x}$ – 20.391); II – 17.0 – 23.0 ($\bar{x}$ – 20.982). From these data it can clearly be seen that average wing length is greater in females.

The most frequent length category for the right forewing in both sexes and in most generations was 21.0 mm (♂♂ I – 29.60%, II – 31.24%; ♀♀ I – 31.24%, II – 37.04%). Among the eight third generation males the only length that appeared twice was 19.5 mm, but the frequency of a complex quantitative characteristic cannot be determined with such a small sample.

Further, it was established that the observed values do not agree with the monograph data in HIGGINS – RILEY (1980; 1993) reporting that the range of variation of forewing length is from 19 mm to 24 mm. Moreover they agree with the older work of Lorković (LORKOVIĆ, 1929), which states that the wingspan of *sinapis* L. can be 30–40 mm in the first generation, and 34–42 mm in the second generation. However, one second generation male was collected with a forewing length of 15.5 mm, so that these data do not agree exactly, but are incomparably more realistic.

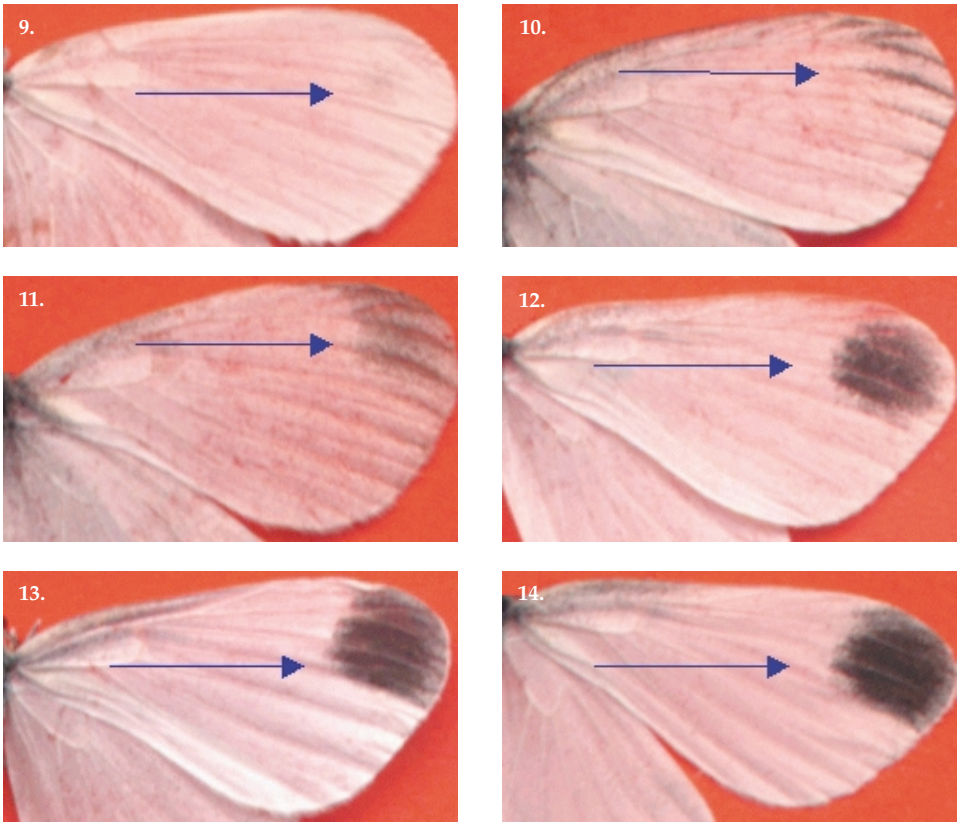
1.4. Apical mark

This exceptionally important morphological feature was analysed according to the detailed description given by Lorković (LORKOVIĆ, 1927; 1930). It was observed that specimens of this species from the area around Sarajevo have 6 distinct phenotypes for this feature (Figs. 9–14):

1 mark non-existent;

2 mark non-existent, just pigmentation of veins;

3 mark non-existent, veins clearly pigmented and tending to join;
4 mark exists, v5-v7(*v8*) joined, but mark nowhere reaches to edge.
5 mark exists, grey-black, black, oval or square;
6 mark exists, bold black; v5-v9 and possibly v10 joined.



Figs. 9–14. Variation in pigmentation of the apical part of the upper side of the forewing in representatives of the species *L. sinapis* L. from the surroundings of Sarajevo

The apical mark is present in males of *L. sinapis* L. within the population around Sarajevo, but it has three variants. In the first generation specimens with grey-black to black marks (variant 4) predominantly appear, and a smaller number have bold black marks (variant 5). In the second generation, and in the third, besides these two varieties there appears a third: a rounded apical mark which touches the outer edge of the forewing (variant 6). In males of all three generations variant 4 is the most common (♂♂ I – 68.81%, II – 49.71%, III – 62.50%).

The apical mark in first generation females is in most cases reduced so that it appears only as pigmentation of the veins (variant 2) which in some cases tends to

join (variant 3), and specimens are rarely found with the typical grey-black or black apical mark (variant 4). Among the second generation specimens without any mark at all (variant 1) occur most frequently, together with specimens with a clear apical mark that does not touch the tip of the wing (variant 6). Specimens with pigmented veins and specimens in which the space between the pigmented veins contains splotchy pigmentation occur rarely. Thus we can say that the following are the most frequent variants: first generation – variant 2 (40.62%) and variant 3 (46.88%), second generation – variant 1 (50.00%) and variant 6 (34.62%).

1.5. Ground colour of the upper side of the wings

It was noted that individuals of this species have different shades of colour on the upper side of the wings. Careful study showed that butterflies of the species. *L. sinapis* L. around Sarajevo show four phenotypes for this feature (Figs. 15–20):

1 milky white;
2 grey-white;
3 dirty white;
4 yellowish white.

Note: borderline examples are often found, for example milky white with some yellow. Such specimens are recorded as being of the closest type (1) their showing a tendency towards another category being marked as (+). Thus such specimens are recorded as: 1+, 2+, 3+.

Figs. 15–20. Colour variation on the upper side of the wings of specimens *L. sinapis* L. from the area around Sarajevo.

First-generation males appear in all four variants. The most frequently observed variant is the dirty white shade (variant 3 – 90.48%), while the other variants, especially variant 4. appear rarely. Among the second generation it was observed that they were all of two variants: variant 1 and variant 3, with variant 1 the more frequent (70.86%). The milky white colour predominated in the third generation as well (87.50%). The spring generation females were likewise found in all four variants, but differed from the males in that the predominant variant was variant 1 (43.75%). Among the summer generation only the yellowish white shade appeared on the upper side of the wings (100.00%).

Clearly a white colour and a dirty white shade are the most frequent variants in colouration of the upper side of the wing in *L. sinapis* L. Lorković observed it more as greyish, while the yellowish shade was noted in several works as characteristic of the second generation (LORKOVIĆ, 1927; 1930). However, other published work (LORKOVIĆ, 1927) describes a typical white colour, rarely marbled with grey, for spring specimens, which in this work are recorded as dirty white (variant 3). Therefore the appearance of a clear grey-white shade is very interesting, although it is rare. Unfortunately the photograph (Fig. 16) does not realistically show the grey shade, which is clearly grey and distinctively different from typical individuals of the first generation (which was shown by long study of the specimens described). Also interesting is the sporadic appearance of a clearer yellow shade on the upper side of the wing, very similar to that found in *L. duponcheli* Staudinger.

1.6. Pigmentation of the upper side of the forewing

Pigmentation of the upper side of the forewing varies widely. More precisely, there is usually pigmentation, mainly in the basal and diskal regions which in some cases is reduced, and we can say that there exist two variants (Figs. 21–22):

1 less pigmentation than described below;

2 basal region pigmented over more than half the cells, more than three quarters of s11 and all of s12.

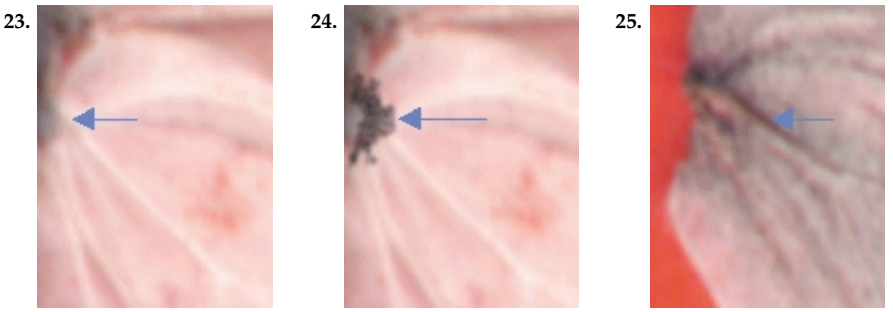
Figs. 21–22. Variation in the pigmentation of the basal part of the upper side of the forewing in representatives of species *L. sinapis* L. from the area around Sarajevo.

Examination of these features in individuals from the Sarajevo population of *L. sinapis* L. showed that among males of all generations the most frequent variant is variant 2 (I – 99.08%, II – 63.28%, III – 100.00%), while among females variant 1 is more frequent: I – 51.61%, II – 100.00%.

1.7. Pigmentation of the upper side of the hind wing

For this feature also a lot of published data can be found, though usually as short comments. For this reason the feature is clearly defined, i.e. it is differentiated into the following variants (Figs. 23–25):

1 pigment absent, or appears as occasional pigmented scales;
2 pigmented basal region, but pigment exists only at the base of veins, generally less pigmentation than in variant 3;
3 strongly pigmented basal region, extending along veins to the diskal region.



Figs. 23–25. Variation in pigmentation of the basal part of the upper side of the hind wing in representatives of species *L. sinapis* L. from around Sarajevo.

In first-generation males the predominant variant was observed to be strong pigmentation of the basal region of the upper side of the hind wing, with the pigment spreading from this region to the diskal region (variant 3 – 97.25%). However, some individuals did not show pigment outside the basal region (variant 2 – 2.75%). Among the second generation the variant 3 variant for this feature was rare (1.70%), while variant 2 was rather more frequent (23.6%), and most frequent was the variant without pigment at the base of the veins or with just a few pigmented scales (variant 1 – 75.14%). Of course the variants described for this feature have degrees of variation; in the photographs (Figs. 23–25) variant 2 appears to have the least pigmentation, in order to differentiate in the simplest way between variant 1 and variant 2. Among third generation males only variant 1 is found (100.00%). First generation females showed an identical pattern of variation for this feature to first generation males, i.e. most frequently was found strong pigmentation of the basal region of the upper side of the hind wing, with the pigment spreading from this region to the diskal region (variant 3), but with a much lower percentage of individuals having this phenotype (62.50%). Pigmentation of just the basal region was somewhat more frequent among females of this generation (37.50%). Among the second generation strongly pigmented individuals rarely appeared (variant 3 – 7.41%), and individuals with minimal pigmentation of the base of the hind wing predominated (variant 1 – 92.59%). The intermediate variant (variant 2) did not appear among females of this generation.

1.8. Ground colour of the underside of the wings

The ground colour of the underside of the wings in individuals of the Sarajevo population of *L. sinapis* L. showed four variants which were categorized as follows (Figs. 26–29):

1 white to pale whitish yellow;
2 grey-white, possibly with a little yellow;
3 yellowish green;
4 ochre shading to green.

Figs. 26–29. Variation in colouration of the underside of the wings of representatives of species *L. sinapis* L. from the area around Sarajevo

Found among first generation males were individuals with grey-white, (variant 2), yellowish green (variant 3) and predominantly yellow with a touch of green (variant 4) as the ground colour of the underside of the hind wing. It was found that variant 3 of this feature appeared most frequently (80.58%), while the other variants appeared rarely (2 – 8.74%, variant 3 – 10.68%). Among the second generation, besides the variants already mentioned there appeared individuals whose ground colour was white, sometimes with touches of yellow (variant 1). In this generation the most frequent variant found was also variant 3 (88.00%), while the others rarely appeared (variant 1 – 7.43%, 2 – 1.14%, variant 4 – 3.43%). In the third generation all individuals had white colouration on the underside of the wing (100.00%). Among females of both generations variants 2, 3 and 4 of this feature were found. In the first generation the most frequent was variant 3 (63.33%), while variant 2 was rare (3.33%). However in the second generation variant 4 of this feature was most frequently found (74.08%), and variant 2 again occurred rarely (3.70%).

1.9. Pigmentation of the underside of the forewing

It was found that the pigmentation of the underside of the forewing had more variants, four to be precise, and in order to make statistical analysis easier, they were categorized as follows (Figs. 30–33):

1 interrupted light green region in *s9*, *s10*, *s1*, pigmentation generally fainter;
2 partially interrupted light green region in *s9*, or *s9*, *s10*, pigmentation starts at the base of *s4*, the first sixth of *s5*, the first quarter of *s6*, the greater part of *s11* and all of *s12*, remaining area not pigmented;
3 light green region unbroken and pigmented almost as in the previous variant or with a few irregularities (richer pigmentation), e.g. pigment in *s3*;
4 typical light green coloured region is olive green, unbroken, pigment strongly expressed, appears at the beginning of *s3*, the first sixth of *s4*, the first quarter of *s5*, a quarter or a third of *s6* and fills *s12*, *s11*, *s10* or half of s*10*, and diffuse pigment covers the remaining area.

Males of the first generation appeared in all three variants of this feature. The most frequently occurring variant was characterized by an unbroken light green coloured region in a clear olive green shade, the pigment itself being strongly expressed and appearing at the beginning of the *s3* area, the first sixth of *s4*, the first quarter of *s5*, a quarter or a third of *s6* and filling *s12*, *s11*, *s10* or half of s*10*. Diffuse pigmentation was observed across the whole remaining area of the underside of the forewing (variant 4 – 44.96%). Less frequently somewhat weaker pigmentation was observed, where the light green region was likewise unbroken and pigmented almost as in the

Figs. 30–33. Variation in pigmentation on the underside of the forewing of representatives of *L. sinapis* L. from around Sarajevo

previous variant but without the diffuse pigmentation on the »free« parts of the wing (variant 3 – 41.28%). In a smaller number of individuals there was a partially interrupted light green region in *s9*, or *s9* and *s10*, while the pigmentation started from the base of area *s4*, the first sixth of *s5*, the first quarter of *s6*, the greater part of *s11* and all of *s12*, and pigment was never found in area *s3* (variant 2 – 13.76%). In the second generation, as well as the variants described above individuals were found with a clearly interrupted light green region in areas *s9*, *s10* and *s11*, and generally weaker pigmentation (variant 1). It could be said that in this generation all variants were almost equally represented except, perhaps, variant 4 (variant 1 – 18.64%, variant 2 – 44.63%, variant 3 – 25.99%, variant 4 – 10.74%). In the second generation only variant 1 (87.50%) and variant 2 (12.50%) were present. Females of both generations followed the same pattern of variation as males of the same generation, but with different relative frequencies of phenotypes. Thus among the first generation females the most frequently observed variant was variant 2 (53.13%), while variant 4 occurred only rarely (6.24%). In the second generation the dominant variant of this feature was variant 1 (70.37%), while, relatively, the other variants were infrequent (variant 2 – 11.11%, variant 3 – 3.70%, variant 4 – 14.82%).

1.10. Pigmentation (drawing) on the underside of the hind wing

Analysis of this characteristic showed that it varies in more variants than was expected on the basis of the extensive published data. Variants were classified as follows (Figs. 34–38):

1 drawing does not exist, pigmentation minimal;
2 design indiscernible, and the whole region described above diffusely pigmented;
3 design discernible, wing is faintly divided into dark and light zones; visible spots in *s4*, *s3*, sometimes in *s2*; edges of the area diffusely pigmented, as are the central parts of areas *s6*, *s7*;
4 drawing clearly discernible, unpigmented zone slightly widened (usually cells on the upper half are not pigmented), *v4* intensely pigmented and clear division of the wing into an upper light and a lower dark region; in the dark region light marks can be seen in *s4* (faintly), *s3*, *s2*, *s1b* (faintly); in the light zone in *s6* and faintly discernible in *s7*, area *s7* faintly diffusely pigmented;
5 drawing unclear because of intense pigmentation, only a small part of the upper region of cells and the first third area *s5* unpigmented, last third of *s5* strongly diffusely pigmented; *s4* strongly pigmented, so that the usual light spot is covered making it only faintly discernible, wing clearly divided into a lower dark and an upper light region; light spot more or less visible in area *s6*, and in *s7* is difficult to make out, or covered by strong pigmentation.

Among first generation males it was observed that variation in this feature is insignificant. A small number of individuals are characterized by a drawing with a more strongly pigmented vein *v4* which faintly divides the wing into a lower dark and upper light zone. Characteristic light spots were found in areas *s4*, *s3*, and

Figs. 34–38. Variation in drawing on the underside of the hind wing of representatives of *L. sinapis* L. from the area around Sarajevo

sometimes in *s2*. The edges of the areas between the wings in these specimens is diffusely pigmented, as are the central parts of areas *s6* and *s7* (variant 3 – 10.19%). The greatest number of individuals are characterized by a strongly pigmented vein v4 which clearly divides the wing into light and dark zones, but the pigment has not filled the areas which usually have light spots (*s2*, *s3*, *s4*, *s6*) so that they show up (variant 4 – 52.78%). It is interesting, however, that more than a third of the examined specimens show very strong pigmentation so that the light spots just mentioned are not generally observable (variant 5 – 37.03%). In the second generation all the above-mentioned variants were noted, but in very small numbers of individuals (variant 3 – 1.13%, variant 4 – 0.57%, variant 5 – 0.57%). In the majority of individuals a rudimentary drawing was observed, i.e. the drawing is not discernible, and in the places where the pigment creates the drawing there is only diffuse pigmentation (variant 2 – 77.96%).

However, in this generation also observed were individuals without any trace of the drawing, or where pigment exists only on a few scales here and there (variant 1 – 19.77%). Among individuals of the third generation only these last two variants were found, the last of which was more frequent (variant 1 – 75.00%, variant 2 – 25.00%). Female specimens showed a range of variation identical to that of the males, and in the first generation even the relative frequencies of the phenotypes (variant 3 – 25.00%, variant 4 – 46.88%, variant 5 – 28.12%). In the second genera-

tion, likewise, variant 2 was found most frequently (70.37%), while we can say that all other variants are observed relatively infrequently (variant 1 – 7.41%, variant 3 – 11.11%, variant 4 – 3.70% and variant 5 – 7.41%).

1.11. Hairiness of the underside of the hind wing

This feature was analysed according to the data published in (LORKOVIĆ, 1927; 1930). It was found that the individuals of the Sarajevo population *L. sinapis* L. vary through three different phenotypes:

1 very little hair, possibly even absent at the base of the veins;
2 a small amount of hair present from the base of the veins to half of the cell;
3 hair present on the veins across the base of the cell, and possibly further.

It was established by analysis that the majority of male individuals, and the majority of females, in the first generation have a very marked covering of hair, which appears from the base of the wing and across the cell almost to the postdistal region (variant 3 – ♂♂ 63.30%, ♀♀ 50.00%). The variant with little hair present (variant 2) and the variant completely without hair (variant 1) occur in equal frequencies (♂♂: variant 1 – 18.35%, variant 2 – 18.35%; ♀♀: variant 1 – 28.13%, variant 2 – 21.87%). An identical pattern of variation among males and females, in the variants found and in the relative frequencies of the phenotypes, was observed in the second generation also. Only the first two variants of hairiness of the underside of the hind wing were found, and the first variant of this feature was the most frequent (♂♂ – 98.87%, ♀♀ – 96.30).

2. Observations of characteristics of the genitalia

2.1. Observations of characteristics of the male genitalia

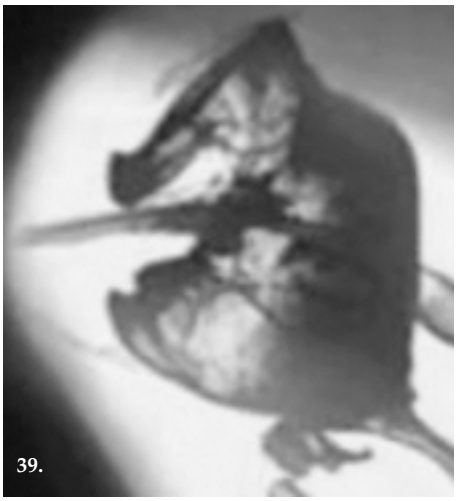
Analysis of the genitalia of male members of the species *L. sinapis* L. was carried out according to descriptions from many published works by Lorković (LORKOVIĆ, 1927; 1930; 1993). The characteristics mentioned in these works were analysed together with some additional ones so that characteristics could be more clearly grouped, characteristics which were noted as being exceptionally important, but without data about their degree of variability.

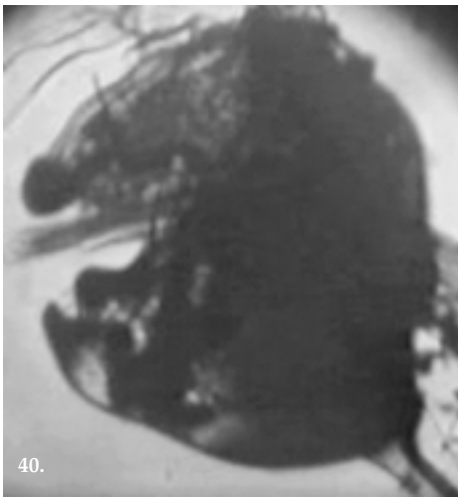
2.1.1. Shape of the genital capsule

It was established that the shape of the genital capsule in males of the Sarajevo population of *L. sinapis* L. occurs in two variants:

1 tegumen concave (crenellate),
2 tegumen convex, oval (Figs. 39–40).

It was found that the variant of genital capsule with a crenellate tegumen occurred significantly more frequently than the variant with an oval, convex form. It was established that in the first generation 6.60% of all males have a more convex shape of genital capsule. The relative frequency of this characteristic falls still further in the second generation, where it was found that only 1.71% of males have this variant of genital capsule. In the third generation this characteristic was not found, i.e. all third generation males have a genital capsule with a crenellate tegumen.





Figs. 39–40. Variation in the shape of the genital capsule in males of the species *L. sinapis* L. from the area around Sarajevo (tegumen concave – 1. tegumen convex – 2)

2.1.2. Length of the a*edeagus*

Measurement of the length of the aedeagus (Fig. 41) of males of *L. sinapis* L. showed that this characteristic varies from 1.373 mm to 1.802 mm and that there exists a trend for the value to decrease in the later, second and third, generations (I – 1.487–1.802. II – 1.373–1.745. III – 1.430–1.659).

On the basis of the range of variation three length categories were formed: short aedeagus (1.373–1.516), medium long aedeagus (1.517–1.659) and long aedeagus (1.660–1.802). Then an analysis of relative frequency was carried out by length category within each generation.

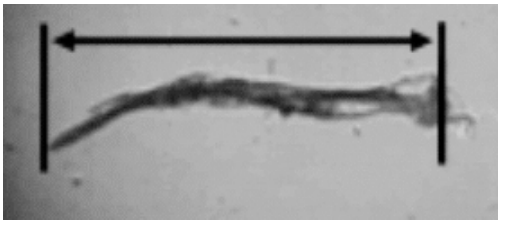


Fig. 41. Line of measurement for length of aedeagus in the butterfly *L. sinapis* L.

This analysis showed that among first generation males of the Sarajevo population of *L. sinapis* L. the long aedeagus is dominant (50.00%), while individuals with a short aedeagusa are quite rare (7.55%). However, in the second and third genera-

tions the medium long aedeagus is dominant (II – 67.08%, III – 62.50%), while individuals with a short aedeagus are rare or non-existent (II – 5.59%, III – 0.00%).

Comparison of the values obtained (1.373 – **1.606** – 1.802) with those given by Lorković (LORKOVIĆ, 1993) shows that there is a significantly larger range of variation in this characteristic among individuals in the Sarajevo population than exists in Croatia (1.33 – **1.50** – 1.60) or Spain (1.37 – **1.56** – 1.70).

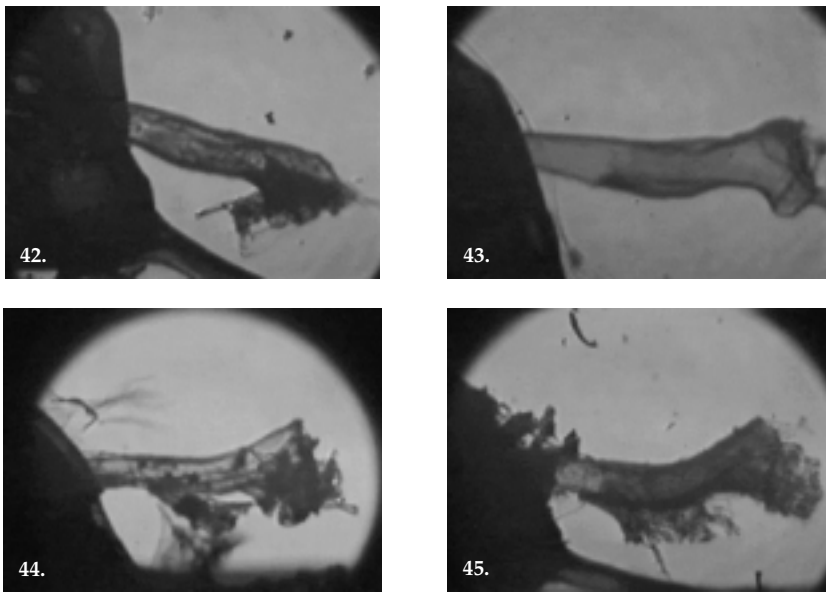
2.1.3. Concavity in front of spatula

Careful analysis of preparations of the genitalia of males, and much published work, has shown significant variability in this characteristic. This feature was measured with the aid of a micrometer eyepiece so that the non-numbered line is aligned with the dorsal side of the aedeagus, and then the number of subintervals is counted, starting from the aligned line. It was concluded that the measured concavity varies from 0 to 0.1375 mm.

After this, from the observed variation, categories were designated as follows:

1 no concavity,
2 concavity faintly defined (1 – 4 x 0.01375),
3 concavity clearly defined (5 – 8; 0.01375),
4 deep concavity (9. or more, x 0.01375) (Figs. 42–45).

Analysis of frequencies of each variant was then carried out. It was established that the most frequent variant of this feature in all three generations is variant 3, i.e. a clearly defined concavity in front of the spatula on the dorsal side of the aedeagus



Figs. 42–45. Degrees of variation in the concavity in front of the spatula on the dorsal side of the aedeagus in males of the species *L. sinapis* L. from the area around Sarajevo

(I – 74.76%, II – 67.05%, III – 87.50%). The next most frequent variant to appear is variant 2, i.e. a faintly defined concavity in front of the spatula on the dorsal side of the aedeagus in the first and second generations (I – 21.36%, II – 27.75%) while in the third generation this feature did not appear. In this third generation besides variant 3 there also appeared variant 4, a deep concavity in front of the spatula on the dorsal side of the aedeagus, which otherwise rarely occurred (I – 3.88%, II – 4.62%, III –12.50%). It is interesting to note that variant 1 of this feature (aedeagus without a concavity in front of the spatula) occurred only once, and that was in the second generation (II – 0.58%, or, out of all specimens collected: 0.36%).

2.1.4. Length of the *saccus*

The length of the saccus in this study was measured from the base, that is from the edge of the genital capsule to the farthest part of the saccus (Fig. 46). Thus it was found that the length of this organ varies from 0.529 mm to 0.825 mm and that there exists a trend towards decreasing length of this feature in the second and third generations (I – 0.529–0.825. II – 0.529–0.825. III – 0.564–0.688). On the basis of this range of variation, three length categories for this feature were differentiated, and then the relative frequencies of individuals of each variant of the feature were calculated.

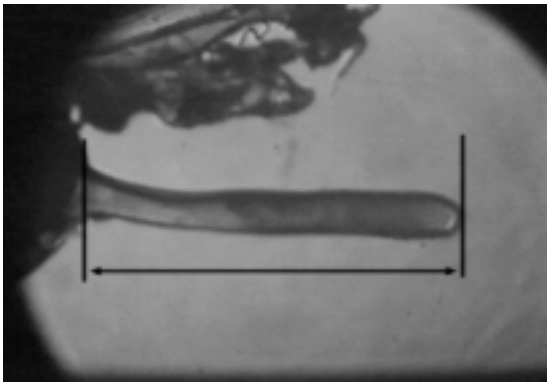


Fig. 46. Line of measurement for length of saccus of the butterfly *L. sinapis* L.

It was found that in all three generations the individuals predominated that had the medium length of saccus (I – 55.24%, II – 65.14%, III – 62.50%). In the first generation the next most frequent were individuals with a long saccus, but these were rare in the second generation, and in the third generation did not appear at all (I – 36.19%, II – 6.29%, III – 0.00%). However, individuals with a short saccus were rare in the first generation, but were the second most frequent variant in the second and third generations (I – 8.57%, II – 28.57%, III – 37.50%).

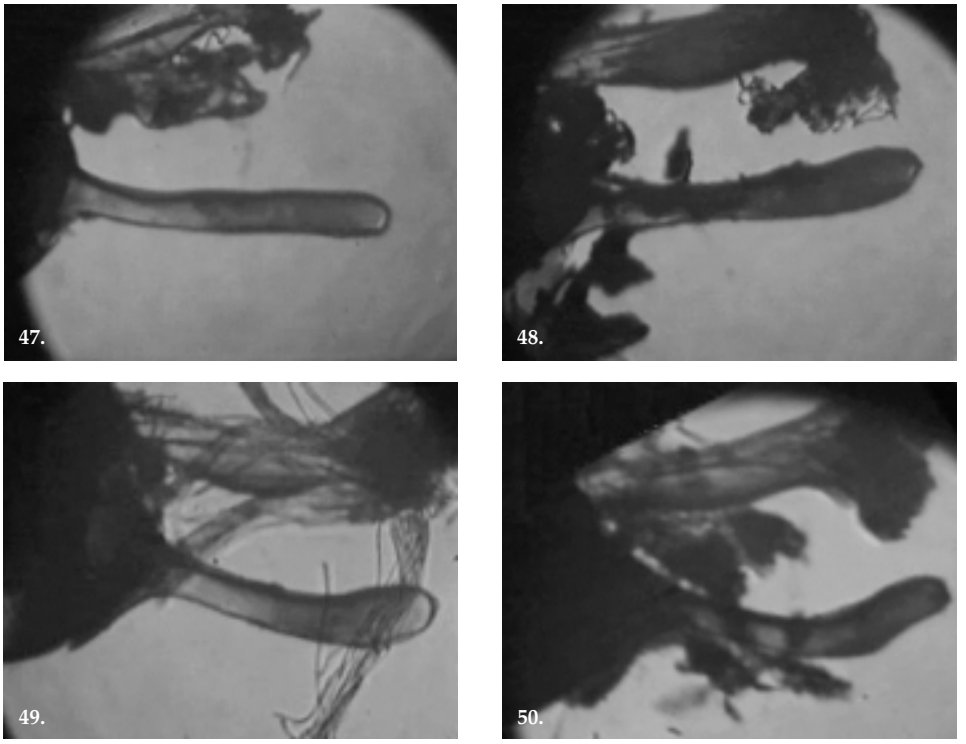
Comparing the observed range of variation and average values of this feature (0.529 – **0.670** – 0.825) with similar data for populations in Croatia (0.50 – **0.60** – 0.72) and Spain (0.58 – **0.65** – 0.77) (LORKOVIĆ, 1993) shows larger values.

2.1.5. Shape of the *saccus*

The shape of the saccus represents a very interesting characteristic which is, according to (HIGGINS, 1975), a basic systematic character for recognition of species of this genus and in this work was given special attention. Categorization was carried out partly according to descriptions and drawings of this organ in European species of the genus *Leptidea* Billberg, taken from various sources (HIGGINS, 1975; HIGGINS & RILEY, 1980, 1993; LORKOVIĆ, 1927, 1930), and partly from study of the author's own specimens. Thus it was found that the shape of the saccus in males of *L. sinapis* L. in the Sarajevo population varies in four general variants (Figs. 47–50):

1 straight without widening (generally thin),
2 straight without widening,
3 sabre-shaped (typical),
4 clearly curved and somewhat thickened.

Analysis of the percentage frequency of individuals by variant of this characteristic showed that in the first and third generations the most frequent variant is the sabre-shaped saccus, variant 3, and that this variant is the second most frequent in the second generation, while the straight, slightly widened saccus is the most fre-



Figs. 47–50. Variants in the shape of the saccus in males of the species *L. sinapis* L. from the area around Sarajevo.

quent in the second generation, and the second most frequent in the first and third generation (variant 3: I – 54.46%, II – 40.94%, III – 62.50%; variant 2: I – 28.71%, II – 54.39%, III – 37.50%). The straight saccus, and also the clearly curved saccus among individuals of this species are quite rare or rare. It was found that among the first generation were found rather more individuals with a curved saccus than with a straight saccus, while in the other generations the ratios were different (I generation: variant 4 – 11.88%, variant 1 – 4.95%; II generation: variant 1 – 3.51%, variant 4 – 1.16%). However, in the third generation not a single individual was found with either of these variants.

2.1.6. Height of the saccus

In a very detailed examination of the genitalia of the male it was impossible to ignore this characteristic. Research has shown that this characteristic also varies much more than was expected. The height of the saccus was measured at its widest part, usually in the first third of the proximal region, using a micrometer eyepiece (Fig. 51).

It was found that the height of the saccus varies from 0.062 mm to 0.158 mm and that these exists a general trend towards decreasing height in the summer and autumn generations of this species in the area around Sarajevo (I – 0.076–0.158. II – 0.062–0.144. III – 0.062–0.124).

Based on the range of variation, three height categories were formed: low saccus (0.062–0.090), medium-high saccus (0.091–0.131) and high saccus (0.132–0.158). The relative frequencies of individuals per category were then analysed for each generation.

Analysis of the relative frequencies of males by height category showed that in all generations the second variant, medium-high saccus, predominates (I – 80.40%, II – 79.43%, 62.50%), while the first variant, low saccus, in all generations except the first (where it has the same frequency as variant 3) is the second most frequent (I – 9.80%, II – 16.00%, 37.50%). It is interesting that the frequency of variant 1 tends to increase through the successive generations, while the frequency of variant 3 drops off through the seasons (I – 9.80%, II – 4.57%, 0.00%).

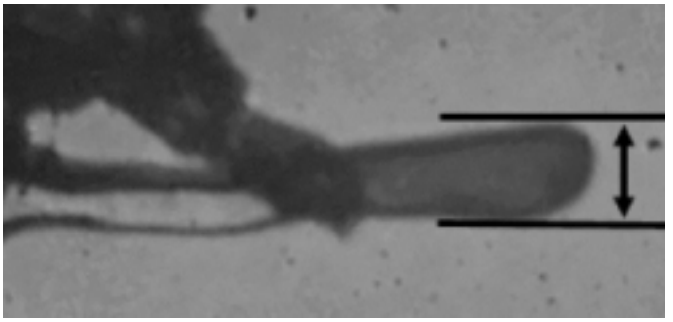


Fig. 51. Line of measurement for height of *saccus* of the butterfly *L. sinapis* L.

2.2. Observations of characteristics of the genitalia of the female

2.2.1. Length of the *ductus bursae*

The length of the ductus bursae is a basic measurement of the female sexual organs. The characteristic is measured between the two farthest vertical points on the organ (Fig. 52).

It was found that the length of the ductus bursae varies from 0.550 mm to 0.701 mm and that there is a general trend for the values to become smaller in the summer and autumn generations of this species in the area around Sarajevo. However, it was found that there is little difference in the average values between spring and summer generations ($\bar{x}$ I – 0.632. $\bar{x}$ II – 0.622).

Based on the range of variation three length categories were formed: short ductus bursae (0.550–0.607), medium long ductus bursae (0.608–0.664) and long ductus bursae (0.665–0.701). Then the relative frequencies of individuals were analysed by category within each generation.

Analysis of relative frequencies showed that the most common variant is variant 2, medium long ductus bursae, among females of both generations examined (I – 50.00%, II – 55.56%). The second most frequent is variant 1, short ductus bursae (I – 32.14%, II – 40.74%), while the variant with long ductus bursae occurs rarely, especially in the second generation (I – 17.86%, II – 3.70%).

The range of variation of length of ductus bursae among the Sarajevo specimens (0.550 – **0.627** – 0.701) compared with the published data for populations from Croatia (0.48 – **0.56** – 0.64) and Spain (0.63 – **0.64** – 0.65) shows higher values (the somewhat higher average for the Spanish population comes from a very small sample – 3).

2.2.2. Width of the *ductus bursae*

In the available literature there is no evidence that this characteristic has been analysed, although it is logically the next measurable characteristic. The feature

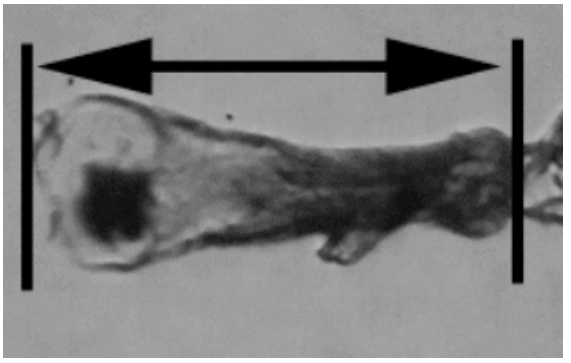


Fig. 52. Line of measurement of length of the ductus bursae of butterflies of the species *L. sinapis* L.

Fig 53. Line of measurement of the width of the ductus bursae in butterflies of the species *L. sinapis* L.

is measured between the two furthest horizontal points on the organ in question (sl. 53).

It was found that the width of the ductus bursae in females from the Sarajevo population of *L. sinapis* L. varies from 0.172 mm to 0.315 mm. Further, it was found that there is a significantly larger range of variation in the first generation (0.172 – **0.241** – 0.315) than in the second (0.186 – **0.216** – 0.241) and we can conclude that there is a falling tendency in the value of this characteristic among the second generation females.

Based on the range of variation, three length categories were formed: narrow ductus bursae (0.172–0.220), medium wide ductus bursae (0.221–0.268) and wide ductus bursus (0.269–0.315). Then the relative frequencies were analysed by category within the two generations examined.

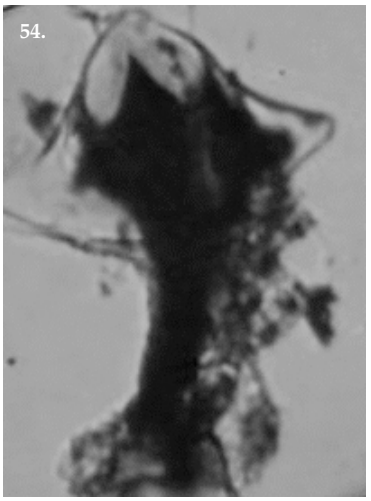
Analysis of the relative frequencies showed that in both generations the most frequent variant was the narrow ductus bursae, variant 1 (I – 39.12%, II – 69.56%). The proportion of individuals with a medium wide ductus bursae is the same in both generations (I – 30.44%, II – 30.44%), and the high percentage of individuals with a wide ductus bursae in the first generation fell to zero in the second generation (I – 30.44%, II – 0.00%).

2.2.3. Shape of the *ductus bursae*

Again for this feature no data were found in the available literature. However its variability can clearly be observed in preparations of the genitalia. It was found that the shape of the ductus bursae in females of *L. sinapis* L. from the area around Sarajevo has two variants (Figs. 54–55.):

1 regular form;
2 irregular form.

Analysis of the relative frequencies showed that among females of both generations the more frequent variant was variant 1, the regular form of ductus bursae (I – 52.17%, II – 88.46%). However it can also clearly be seen that variant 2 of this characteristic becomes still rarer in the second generation.



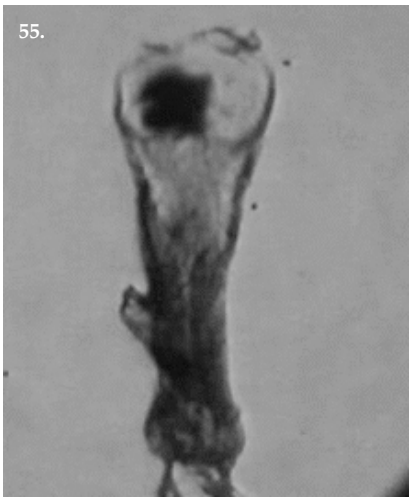


Fig. 54–55. Variants in the shape of the ductus bursae among females of the species *L. sinapis* L. from the area around Sarajevo

CONCLUSIONS

- ✔ Following careful analysis of 11 exogenous characteristics in 353 specimens of the species *L. sinapis* L. it was established that there exists greater variability than expected on the basis of the published data.
- ✔ It was unexpectedly found that a »ragged« outer edge of the forewing was present in some specimens of this species, which contradicts the published data asserting that this characteristic exists only in the species *L. morsei major* Grund within this region.
- ✔ It was found that there is a greater range of variation in the length of the right forewing than reported in the literature.
- ✔ Specimens were found showing an intense yellow shade on the upper side of the wing, which is more typical of *L. morsei major* Grund and *L. duponcheli* Staudinger. It is necessary to add that these specimens did not show the other phenotypic characteristics of these related species, so that there was no possibility of wrong identification.
- ✔ Apical marks varied within the boundaries of the described phenotypes of the spring and summer generations.
- ✔ Particularly interesting is the variation of pigmentation on the whole lower surface of the forewing, where the last variant, variant 4, could be identified with f. *subapicata* Lorković.

- ✔ Pigmentation and drawing on the lower side of the hind wing also varied within the limits of the described phenotypes of the spring and summer generations (f. *diniensis* Boisduval, f. *erysimi* Borkenhausen), but specimens were also found with a darker phenotype.
- ✔ It was found that the genital capsule of the male has two variants, as was expected on the basis of the published data, but with a very rare occurrence of the »convex« tegumen.
- ✔ It was found that the range of variation of the length of the aedeagus from 1.373 mm to 1.802 mm among individuals from the Sarajevo population of this species was greater than that found among the Croatian and Spanish populations.
- ✔ It was also found that the range of variation of the saccus from 0.529 mm to 0.825 mm is greater than the data given for specimens from Croatia and Spain.
- ✔ Observation of the concavity in front of the spatula of the aedeagus showed great variability in this part of the organ, from 0 to 0.1375 mm.
- ✔ It was established that the shape of the saccus has four variants, so that the accepted view, especially of Higgins (HIGGINS, 1975) does not match the real variation found in nature.
- ✔ It was found that there are great differences in the height of the saccus, which varies from 0.062 mm to 0.158 mm.
- ✔ The variation in the length of the ductus bursae from 0.550 mm to 0.701 mm is comparable with the data for specimens from Croatia and Spain, confirming the significantly greater values found among males.
- ✔ It was found that the width of the ductus bursae varies from 0.172 mm to 0.315 mm.
- ✔ It was found that the shape of the ductus bursae in females of the Sarajevo population has two variants.

Received May 15, 2001

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SAŽETAK

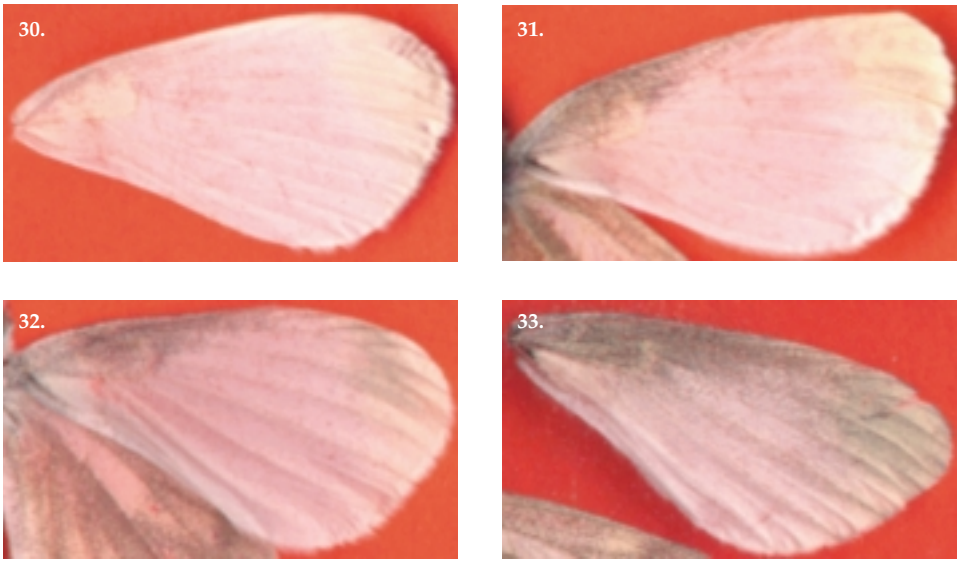
Varijacija egzofenotipskih i endofenotipskih (genitalni aparat) osobina leptira vrste *Leptidea sinapis* Linnaeus, 1758 (*Lepidoptera, Pieridae*) unutar populacije iz šire okoline Sarajeva

S. Lelo

Precizna analiza niza egzomorfoloških svojstava na jedinkama vrste *L. sinapis* L. iz područja šire okolice Sarajeva pokazala je znatno veći stupanj promjenjivosti nego što je to zabilježeno u dostupnoj literaturi. Krajnje neočekivano je konstatirano da pojedine jedinke »sinapisa« imaju »neravan vanjski rub prednjeg krila« što je, inače, jedna od najkarakterističnijih determinanti vrste *L. morsei major* Grund. Osim toga, evidentirane su jedinke sa intenzivnije žućkastom nijansom gornje strane krila što je svojstvenije jedinkama *L. duponcheli* Staudinger. Uočeno je da jedinke ove

populacije imaju i znatno veći raspon variranja dužine desnog krila u odnosu na postojeće monografske podatke. Raspon variranja apikalne oznake ostao je u okvirima opisanih formi: f. *diniensis* Boisduval, f. *erysimi* Borkenhausen. Zapaženo je vrlo interesantno variranje pigmenta na cijeloj površini donje strane prednjeg krila gdje se posljednja, varijanta (4), može poistovjetiti sa f. *subapicata* Lorković. Pigmentacija, crtež, donje strane stražnjih krila je također varirala u okviru opisanih fenotipova proljetne i ljetne generacije (f. *diniensis* Boisduval, f. *erysimi* Borkenhausen), ali su uočene i jedinke tamnijeg fenotipa: f. *pseudoduponchely* Verity. Ustanovljeno je da genitalna kapsula mužjaka ima dva alternativna fenotipa od kojih varijanta »konkavnog« tegumena dominira u svim generacijama. Nadalje je pokazano da stupanj variranja dužine edeagusa te srednja vrijednost za ovu osobinu premašuje podatke za populaciju okolice Zagreba i uzorka iz Španjolske koje je dao Lorković (LORKOVIĆ, 1993). Novopromatrana osobina, udubljenje dorzalnog dijela edeagusa ispred spatule, enormno varira, i to od ravnog pa do fenotipa s udubljenjem od 0.138 mm. Uočeno je da oblik sakusa varira u četiri fenotipske varijante, a da njegova visina-debljina, također, ima značajno veliki stupanj variranja. Također je ustanovljeno da vrijednosti dužine duktusa burze ženki sarajevske okolice premašuju vrijednosti za Hrvatsku, tj. zagrebačku i španjolsku populaciju (prema LORKOVIĆ, 1993). Na kraju, konstatirano je da i duktus burze sarajevskih ženki varira u dva fenotipa od kojih uobičajena forma apsolutno dominira.

Figs. 34–38. Variation in drawing on the underside of the hind wing of representatives of *L. sinapis* L. from the area around Sarajevo



Figs. 30–33. Variation in pigmentation on the underside of the forewing of representatives of *L. sinapis* L. from around Sarajevo