

Leptidea sinapis and *Leptidea reali* (Lepidoptera: Pieridae) in The Netherlands

In 1988 the wood white (*Leptidea sinapis* s.l.) was split up into two largely sympatric sibling species, now known as *Leptidea sinapis* Linnaeus and *L. reali* Reissinger. An attempt has been made to trace and identify as many collected Dutch wood white specimens as possible. In this material both species are present. *Leptidea sinapis* has always been an irregular immigrant. Since 1992, however, a strong population has gained foothold near Maastricht. *Leptidea sinapis* therefore meets the criteria of a resident species. Prior to 1950, *L. reali* has only been caught incidentally. In the 1950's a population persisted for a number of years near Bemelen, Limburg. Consequently, *L. reali* has to be regarded an irregular resident. Dutch specimens belong to the subspecies *L. r. jonvillei* Mazel, 2000.

Recently it has been demonstrated that aedeagus length is a reliable diagnostic character, provided that captures from the same geographic area are being compared. The frequency distribution of aedeagus length, resulting from this study, can serve as a reference for identification of future *Leptidea* captures in The Netherlands and the adjacent area.

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Key words: faunistics, diagnosis

Introduction

The European butterflies are among the best explored insect groups in the world. Nonetheless, new species are described occasionally. In a minority of cases the new species has long been overlooked because of its isolated/restricted area. Mostly, however, it concerns two very similar species which were not recognized previously. The latter is the case with the common wood white (figure 1). The two species we know now as *Leptidea sinapis* (Linnaeus, 1758) and *L. reali* Reissinger, 1989 were distinguished by Réal (1988) on the basis of female genital features. He described the species that was new to him as *Leptidea lorkovicii* Réal, 1988. Reissinger (1989) replaced this name, which was preoccupied, by

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reali. Lorković (1993) completed the morphological diagnosis, describing differences in the male genitalia and wing pattern. It was not before 2001 that dissection of Linnaeus' holotype (conserved in the British Museum) revealed that Réal was correct in deciding which of the two is *L. sinapis* (Kudrna 1991).

The two taxa show slightly different habitat preferences (Embacher 1996, Mazel & Leestmans 1996, Gianti & Gallo 2002) and the females exhibit different food plant preferences for oviposition (Freese & Fiedler 2002). Martin *et al.* (1997) found a genetic distance compatible with a pair of sibling species. In addition, allozyme studies did not reveal any sign of gene flow between sympatric populations of the two taxa (Martin *et al.* 1997). This is consistent with the conclusions of Lorković (1993) and Freese & Fiedler (2002), who provided experimental evidence for reproductive isolation by pre-copulatory behavioural barriers. Obviously the taxa *L. sinapis* and *L. reali* represent two biological species. The most reliable diagnostic feature is the length of the ductus bursae in females. In males, the length of aedeagus and sacculus are traditionally used for identification, aedeagus length being the most reliable of the two (Freese & Fiedler 2004). Both *L. sinapis* and *L. reali* are Palearctic species with a Euro-siberian distribution; they are believed to have their origins in the Turkestanian (=Mongolian sensu de Lattin 1967) expansion center (Mazel & Eitschberger 2003, Mazel & Leestmans 2003).

Since the early 1990's the newly described species has been reported from most European countries (Laštuvka *et al.* 1995, Neumayr & Segerer 1995, Embacher 1996, Kristal & Nässig 1996, Ábrahám & Uherkovich 1998, Bělin 1999, Karsholt 1999, Nelson *et al.* 2001, in connection with Corke 2001, Gianti & Gallo 2002, Mazel & Eitschberger 2003) and from Asia (Mazel & Leestmans 2003), in some cases living syntopically with *L. sinapis* (Mazel & Leestmans 1996, 1999b). This paper aims to establish the present and past distribution, as well as the faunistical status, of each species in The Netherlands.

Table 1. Aedeagus length (x 10 micron), frequency table. In brackets: bad preparation allows no reliable measurement. Origin of the the samples: 1. Bemelen, 1950's, 2. Maastricht, 1990's, 3. Meerssen, 2000's, 4. Cadier en Keer, 1999, 5. individual captures in The Netherlands in the 1877-1994 period. See text for more information.

Frequentieverdeling van de aedeaguslengte (x 10 micron). Tussen haakjes: meting onmogelijk door slechte preparatie. Het materiaal per steekproef is afkomstig van 1. Bemelen, jaren 1950, 2. Maastricht, jaren 1990, 3. Meerssen, jaren 2000, 4. Cadier en Keer, 1999, 5. individuele vangsten in Nederland in de periode 1877-1994. Zie de tekst voor aanvullingen.

sample	131-135	-140	-145	-150	-155	-160	-165	-170	-175	-180	-185	-190	-195	-200	-205
1	-	-	-	-	-	-	-	-	-	3	1	-	4	-	-
2	-	-	2	10	18	24	19	6	2	-	-	-	-	-	-
3	-	-	-	-	1	2	1	-	-	-	-	-	-	-	-
4	(5)	1	1	5	8	5	6	1	1	-	-	-	-	-	-
5	-	-	1	5	8	8	2	2	2	-	1	3	2	-	1

Table 2 Saccus length (x 10 micron), frequency table. In brackets: bad preparation allows no reliable measurement. See also table 1. *Frequentieverdeling van saccuslengte (x 10 micron). Tussen haakjes: meting onmogelijk door slechte preparatie. Zie verder tabel 1.*

sample	36-40	41-45	46-50	51-55	56-60	61-65	66-70	71-75	76-80	81-85	86-90	91-95	96-100
1	-	-	-	-	-	-	1	2	2	1	1	-	-
2	(4)	1	-	13	31	18	11	3	-	-	-	-	-
3	(1)	-	1	1	-	1	-	-	-	-	-	-	-
4	(4)	-	2	5	9	10	3	-	-	-	-	-	-
5	-	-	-	6	8	9	4	2	5	1	-	-	1

(part of the results have been published in Hoen (2004))

Until 1990 *L. sinapis* s.l. had been considered a very rare, irregular resident species in The Netherlands (Lempke 1953b, 1956, Geraedts 1986, Tax 1989). It was not observed annually and always in very small numbers. Since 1990 however it has been observed more regularly. There are three documented cases of populations that persisted for several years in the same place:

1. Bemelen 1950's: *L. sinapis* s.l. was present on the Bemerberg near Bemelen, some 15 km north-east of Maastricht (Lempke 1951, 1952, 1953a, b, 1954, Tax 1989, Sogeler, pers. comm.),

2. Maastricht 1990's: since 1992 a strong population of *L. sinapis* s.l. has persisted on the St.-Pietersberg near Maastricht (Peeters 1996),

3. Meerssen 2000's: since 2001 *L. sinapis* s.l. has been recorded each year in the Curfs-quarry, Meerssen (G. Smeets, pers. comm.).

A remarkable observation was made in 1999: a few dozen males of *L. sinapis* were retrieved from a light trap in an abandoned chalk quarry at Cadier en Keer (G. Smeets, pers. comm.). It was a unique event, and none have been observed since.

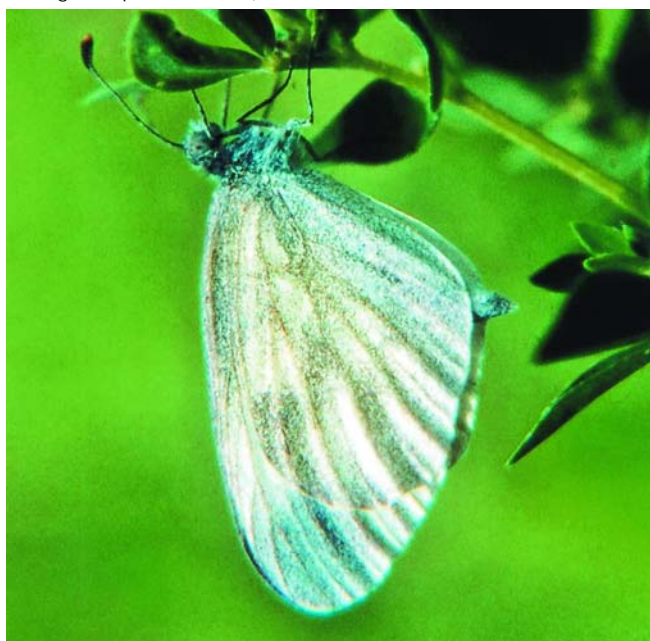


Figure 1. *Leptidea sinapis*, male, Sint-Pietersberg, Maastricht, 27 May 2000. Photo: Olaf op den Kamp
Leptidea sinapis, mannetje, Sint-Pietersberg, Maastricht, 27 mei 2000.

Material and methods

All *Leptidea* in four Dutch museum collections and in three private collections have been studied: Zoölogisch Museum Amsterdam (ZMAN), Nationaal Natuurhistorisch Museum Naturalis Leiden (RMNH), Natuurhistorisch Museum Maastricht (NHMM), Natuurmuseum Rotterdam (NMR), and the private collections of P.J. Sogeler (PJS), G. Smeets (GS) and M. van Stiphout (MS). The material (201 specimens) is grouped into five samples:

1. Bemelen 1950's (8 ♂ and 1 ♀, all in PJS),
2. Maastricht 1990's (81 ♂ and 26 ♀),
3. Meerssen 2000's (4 ♂ and 4 ♀),
4. Cadier en Keer 1999 (33 ♂ from the light trap), and
5. individual captures in The Netherlands in the 1877-1994 period (36 ♂ and 8 ♀).

Genitalia were prepared and mounted in euparal (F.C.) or glycerin-gelatin (FW.H.). Lengths of ductus bursae, aedeagus and saccus were measured with a toolmaker's microscope Mitutoyo TM-100, magnification 30x, reading accuracy 0.01 mm.

Results

Males

Samples 2-4 show corresponding frequency distributions for both aedeagus and saccus lengths (tables 1 and 2), which are in agreement with published data for *L. sinapis*. The fre-

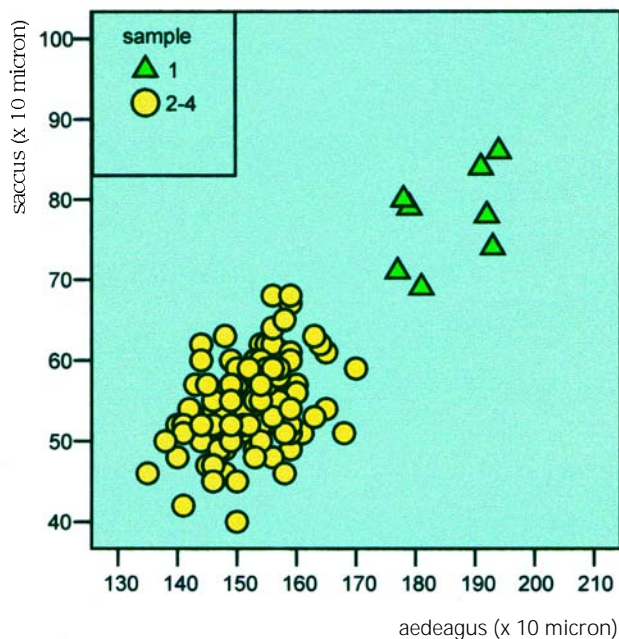


Figure 2. Scatterplot of samples 1 (Bemelen) and 2, 3 and 4 (Maastricht, Meerssen, Cadier en Keer).
Strooidiagram van de monsters 1 (Bemelen) en 2, 3 en 4 (Maastricht, Meerssen, Cadier en Keer).

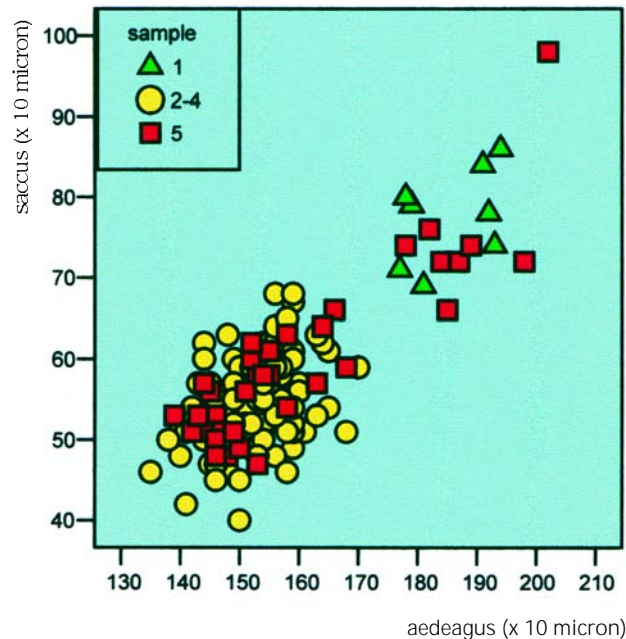


Figure 3. Sample 5 (occasional captures 1870-1994) superimposed on figure 2.
Groep 5 (incidentele vangsten 1870-1994) gesuperponeerd op figuur 2.

quency distribution of sample 1 is strikingly different and matches published data of *L. reali*. The spread in sample 5 covers the whole range of both *L. sinapis* and *L. reali*, suggesting that both species are represented. In a scatterplot of samples 2-4 (pooled for this purpose) and sample 1 the areas of both species are sufficiently separated (figure 2). Projection of the data of sample 5 into the same scatterplot enables a reliable separation of both species in this mixed sample (figure 3). It obviously contains 28 males of *L. sinapis* and eight males of *L. reali*.

Females

Only 39 females were available for dissection. The majority belongs to *L. sinapis* (table 3). The single female from sample 1 and two from sample 5 turn out to be *L. reali*.

situated in the southern half of the country, in the province of Limburg and the eastern part of the province of Gelderland, and all within 20 km from the German or Belgian border (figure 5). *Leptidea reali* thus seems to be less expansive than *L. sinapis*. There is a striking difference in the temporal distribution of the two species (figure 6): *L. reali* had its maximum occurrence in the 1950's and none has been identified since, whereas *L. sinapis* has been recorded in increasing numbers since the beginning of the 1990's. Capture dates of *L. reali* (table 4) fit perfectly into the phenological bar charts of *L. sinapis* (s.l.) by Tax (1989) and Smeets & van Swaay (2002).

Faunistical status

The species which gained the faunistical status of resident species after ten years of uninterrupted presence in The Netherlands (Smeets & van Swaay 2002, van Swaay & Smeets 2003) is now definitely identified as *L. sinapis*. In establishing the faunistical status of *L. reali*, the Bemelen population is of special interest. Most *Leptidea* observations there are accounted for by P. Sogeler, who visited the Bemelerberg almost annually during the flight period of the wood whites, in most years repeatedly. Moreover, he kept daily notes of his field work. Documented *Leptidea* observations at the Bemelerberg exist for the following years:

Table 3. *Ductus bursae*-length (x 10 micron), frequency table. See also table 1.

Frequentieverdeling van de lengte van de ductus bursae (x10 micron). Zie verder tabel 1.

sample	51-55	56-60	61-65	66-70	71-75	76-80	81-85	86-90
1	-	-	-	-	-	-	-	1
2	8	12	6	-	-	-	-	-
3	3	-	1	-	-	-	-	-
4	-	-	-	-	-	-	-	-
5	1	3	2	-	-	-	-	2

Discussion

Occurrence

Both species are present in Dutch collection material (figure 4). Consequently, *L. reali* is definitely to be entered on the list of indigenous species. Obviously, it is the less common of the two species in The Netherlands. The known localities are

- 1949 notes P. Sogeler
- 1950 Lempke 1951
- 1951 notes P. Sogeler; specimens in collection; Lempke 1952
- 1953 notes P. Sogeler; Lempke 1954
- 1954 notes P. Sogeler; specimen in collection
- 1955 notes P. Sogeler
- 1956 notes P. Sogeler; Lempke 1957
- 1958 notes P. Sogeler; specimens in collection.



Figure 4. The oldest specimens of *Leptidea reali* in Dutch collections. Nijmegen, 2 August 1902 (top) and Slangenburg, 4 August 1901 (bottom). G.J. Klokman leg., collection ZMAN. Photo: Frans Cupedo
De oudste exemplaren van Leptidea reali in Nederlandse collecties. Nijmegen, 2 augustus 1902 (boven) en Slangenburg, 4 augustus 1901 (onder). G.J. Klokman leg., collectie ZMAN.

In 1952, 1957 and 1959 Sogeler did not visit the Bemelerberg. In 1960 he did so on 18 May, 10 August and 15 August, without observing any *Leptidea*. As all conserved specimens have been identified as *L. reali*, it is plausible that between 1949 and 1958 a population of *L. reali* resided on the Bemelerberg. An uninterrupted ten year presence (in which case it has to be considered a disappeared resident species) may well have been the case, but is insufficiently documented. Consequently, *L. reali* in The Netherlands is to be qualified as an irregular resident species.

Table 4. All specimens of *Leptidea reali* identified in Dutch collections. *Alle gedetermineerde Leptidea reali in Nederlandse collecties.*

locality	n	sex	date	legit	collection
Venlo	1	♂	14 June ??	Oudemans	ZMAN
Slangenburg	1	♂	4 August 1901	Klokman	ZMAN
Nijmegen	1	♂	2 August 1902	Klokman	ZMAN
Valkenburg	1	♂	8 July 1913	Kranendonk	ZMAN
Houthem	1	♂	18 July 1919	Kranendonk	ZMAN
St-Pietersberg	2	♂	28 May 1931	?	RMNH
Arnhem	1	♂	12 June 1931	?	RMNH
Epen	1	♀	16 July 1950	van Wisselingh	ZMAN
Bemelen	3	♂	?? May 1951	Sogeler	PJS
Bemelen	1	♀	30 August 1954	Sogeler	PJS
Montfort	1	♀	12 August 1956	Maassen	NHMM
Bemelen	4	♂	15 August 1958	Sogeler	PJS
Bemelen	1	♂	16 August 1958	Sogeler	PJS

Taxonomy and nomenclature

Three subspecies of *L. reali* have been described. The nominal subspecies is resident in southern Europe. Subspecies *jonvillei* Mazel, 2000 inhabits northern Europe and Central Europe north of the Alps and the Pyrenees. The Asian populations have been described as ssp. *yakovlevi* Mazel, 2001 (Mazel 2000, 2001b, Mazel & Eitschberger 2003). Dutch specimens belong to ssp. *jonvillei*.

Certainly the present nomenclature is not yet definitive. Prior to 1989, some dozen synonyms and subspecific names were published in connection with *L. sinapis* s.l. (Reissinger 1989, Leraut 1997). Each of these names would replace Reissinger's *reali* as the valid name at the moment that its holotype would turn out to belong to the newly discovered species. Mazel (2001a) already proved that *juvernica* Williams, 1946 has priority over *reali* Reissinger, 1989. However, as long as the *oldest* available name has not been established, replacing the name *reali* would be senseless.

Reliable diagnosis

Leptidea sinapis and *L. reali* show slightly different habitat preferences (Embacher 1996, Mazel & Leestmans 1996, Gianti & Gallo 2002) and the females exhibit different food plant preferences for oviposition (Freese & Fiedler 2002). It is to be expected that for the purposes of inventory and habitat management there will be an increasing need for a simple and reliable identification – preferably based on males – without killing too many individuals. For identification purposes, wing characters are quite useless (Lorković 1993). In females, shape and length of the ductus bursae is an excellent diagnostic feature: it permits each single specimen to be identified without doubt (Lorković 1993). As to the males, Freese & Fiedler (2004) convincingly demonstrated that aedeagus length enables unequivocal identification of male

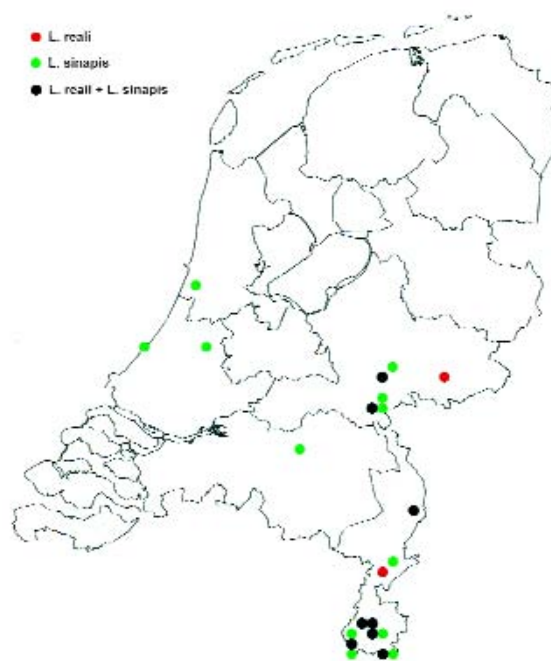


Figure 5. Locations of identified *Leptidea reali* and *L. sinapis* in The Netherlands.

Vindplaatsen van gedetermineerde Leptidea reali en L. sinapis in Nederland.

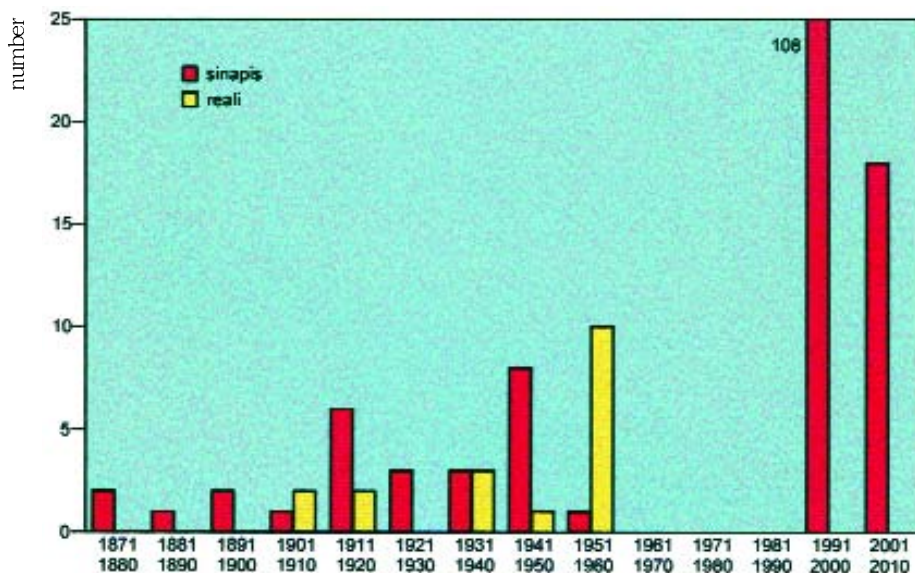


Figure 6. Number of identified *Leptidea sinapis* and *L. reali* since 1870. Note: the *L. sinapis* bar for 1991-2000 has been truncated.

Aantal gedetermineerde *Leptidea sinapis* en *L. reali* sinds 1870. Nota bene: de staaf van *L. sinapis* voor 1991-2000 is ingekort.

specimens. Its frequency distributions for *L. sinapis* and *L. reali* are separated, provided that free-living populations from the same region are compared. (In Friese & Fiedler's (2004) laboratory-reared specimens the spread increased for unknown reasons). All publications in which basic population statistics (mean, maximum and minimum value) have been listed for a defined region confirm Freese & Fiedler's conclusions (Lorković 1993, Neumayr & Segeler 1995, Embacher 1996, Ábrahám 1998, Karsholt 1999, Mazel & Leestmans 1999a, Nelson *et al.* 2001, and the present paper). This means that also individual males can be identified unambiguously. A necessary condition is that sufficient measurements of aedeagus length from the same geographical area are available as a reference. The relatively high number of preserved *L. sinapis* males from recent Dutch populations (118 specimens) provides a reliable frequency distribution of the aedeagus length of *L. sinapis* in this region (figure 7). This can serve as a reference for the identification of *Leptidea* captures in the future. As The Netherlands (and adjacent parts of Belgium and Germany) are situated at the outer limits of both species' range, isolated populations will be established, as a rule, by one or a few migrants, and the occurrence of mixed populations will be highly unlikely. Under these circumstances, even identification of isolated populations (in this region) will require the dissection of a single male or female.

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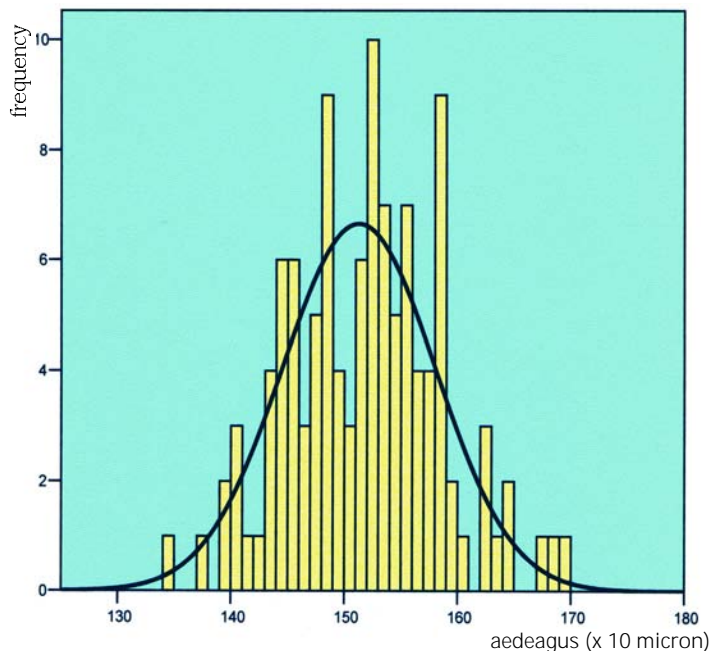


Figure 7. Frequency distribution of aedeagus lengths of Dutch *Leptidea sinapis*, samples 2, 3 and 4. Mean 152.3, standard deviation 6.8, range 136-170.

Frequentieverdeling van de aedeaguslengte van Nederlandse Leptidea sinapis, monsters 2, 3 en 4. Gemiddelde 152,3, standaarddeviatie 6,8, spreiding 136-170.

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Summary

Leptidea sinapis and *Leptidea reali* (Lepidoptera: Pieridae) in Nederland

Het gebeurt nog maar zelden dat in Europa een nieuwe vlindersoort ontdekt wordt en als het al gebeurt, dan is er meestal sprake van twee sterk op elkaar lijkende soorten die tot dan toe voor een soort gehouden waren. Dit is ook het geval bij het boswitje (*Leptidea sinapis*) en zijn zustersoort het verborgen boswitje (*L. reali*). Deze soorten zijn in 1989 onderscheiden op grond van genitaal-kenmerken. Inmiddels staat vast dat ze zowel in de natuur als onder experimentele omstandigheden niet met elkaar paren, een bewijs dat het inderdaad twee biologische soorten zijn. Beide komen voor in een groot deel van Europa en Azië. In Nederland waren boswitjes tot 1990 zeldzaam, maar sinds 1992 bestaat er een permanente populatie op de Sint-Pietersberg bij Maastricht. In 2003 verwerfde het boswitje daarom de status van standvlinder, al stond op dat moment niet vast welke soort het betrof. Om te bepalen of beide soorten in Nederland zijn aangetroffen, en zo ja in welke mate, zijn zoveel mogelijk exemplaren uit museumcollecties en particuliere collecties onderworpen aan een genitaalonderzoek. Dat leverde de volgende resultaten op:

- 1 zowel *L. sinapis* als *L. reali* zijn in Nederland gevangen,
- 2 *L. sinapis* was in de periode vóór 1990 een zeldzame zwerver. Sinds 1992 handhaaft de soort zich op de Sint-Pietersberg en wordt derhalve beschouwd als standvlinder,
- 3 *L. reali* was in de periode vóór 1950 een zeldzame zwerver. Van 1949 tot 1958 heeft *L. reali* zich op zijn minst een aantal jaren weten te handhaven op de Bemelerberg, ten oosten van Maastricht. Daarmee verwerft deze soort de status van onregelmatige standvlinder. Uit de periode na 1960 is in de onderzochte collecties geen *L. reali* aangetroffen,
- 4 vangsten van *L. reali* zijn in de minderheid en beperken zich tot het uiterste oosten van Zuid-Nederland. Dat suggereert dat de soort niet alleen zeldzamer is, maar ook minder zwerfneiging heeft dan *L. sinapis*,
- 5 de Nederlandse exemplaren van *L. reali* behoren tot de ssp. *jonvillei* Mazel, 2000,
- 6 uit het grote aantal mannetjes dat in Nederlandse collecties te vinden is kan een betrouwbare frequentieverdeling van de aedeaguslengte van *L. sinapis* afgeleid worden. Deze kan dienen als referentie bij het determineren van boswitjes uit Nederland en het aangrenzende Duitse en Belgische grensgebied. In Nederland, waar populaties zeldzaam zijn en gesticht worden door een of hooguit enkele immigranten, is het ontstaan van gemengde populaties erg onwaarschijnlijk. Identificatie van een ongemengde populatie is daardoor mogelijk op basis van een enkel mannetje of vrouwtje.