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A CLARIFICATION OF THE STATUS OF FOUR TAXA IN THE *ECTOEDEMIA ANGULIFASCIELLA* GROUP (NEPTICULIDAE: LEPIDOPTERA)

by

CHRISTOPHER WILKINSON, GEORGINA BRYAN, STEPH B. J. MENKEN and ERIK J. VAN NIEUKERKEN

(Department of Animal Systematics and Zoogeography, Vrije Universiteit, P.O. Box 7161, 1007 MC Amsterdam, The Netherlands)

SUMMARY

The status of the four taxa *Ectoedemia angulifasciella*, *E. atricollis*, *E. rubivora* and *E. arcuatella*, has always been in debate. They have now been studied using several different systematic methods, relating to morphology, gel electrophoresis, host plant differentiation, life histories and distribution. Evidence is given here which demonstrates that they should be regarded as two pairs of sibling species.

INTRODUCTION

In the genus *Ectoedemia* Busck in Europe there is a group of uniform and similar moths whose larvae are exclusively leaf miners of Rosaceae. As they are so similar, not only in external features of the adult and larva, but also in genitalia structure, the status of these taxa has long been in dispute. Two species pairs, one comprising *Ectoedemia angulifasciella* (Stainton) and *E. atricollis* (Stainton), the other comprising *E. rubivora* (Wocke) and *E. arcuatella* (Herrich-Schäffer), are the subject of this study.

When in the 19th century it seemed that most species of Nepticulidae were host specific many similar taxa were discriminated on the basis of food plant only. However, in the first half of this century, the recognition of genitalia as providing important diagnostic features often brought taxa together which had hitherto been separated or, conversely, divided taxa whose separate identity had not previously been appreciated. Thus biological features often suggested a different interpretation from anatomical ones.

In 1945 BEIRNE made the first serious analysis of the male genitalia which resulted in his synonymising the *Rosa* feeding species *E. angulifasciella* with the *Crataegus* feeding species *E. atricollis* because he could find no differences in the genitalia. In addition JOHANSSON (1971) and BORKOWSKI (1975) regarded *E. arcuatella* on *Fragaria* as the same species as *E. rubivora* on *Rubus* for a similar reason. However,

these views were not shared by those who preferred to place more stress on biological detail and life cycles (VÁRI, 1951; EMMET, 1973, 1976) than on genital structure.

Thus a situation of uncertainty was presented similar to those in North America as shown in our revisions of nearctic *Ectoedemia* (WILKINSON & SCOBLE, 1979; WILKINSON & NEWTON, 1981). Today we realise that it may be necessary to consider many branches of biology where difficult taxa are concerned and it may be only by recourse to hitherto unusual attributes (*e.g.* behaviour) that a sound biosystematic assessment can be made. We were provided, therefore, with an ideal topic for co-operative working, which forms a part of our overall research programme—to study the biosystematics of leaf mining microlepidoptera and their co-evolution with plants (see WILKINSON, 1982).

METHODS

1. *Collecting and Rearing*

Field work has been carried out mainly in the Netherlands and mines were collected from as many localities as possible. They were placed on a thin layer of sterilised potting compost in small glass jars with plastic lids or in specially designed stainless steel tins. These tins are 20-60 cms in diameter with gauze top and bottom. When fully grown a proportion of larvae was transferred to the freezer for the biochemical experiments. Once the mines had been vacated the leaves were removed from the jars but not from the tins. The jars were stored during the winter in unheated observation rooms and the tins were buried to their rims in soil, outside.

From June until the end of the emergence period the jars and tins were checked regularly for adults. The tins are fitted with glass specimen tubes in the sides into which the moths collect, attracted by the light.

2. *Morphology*

Morphological studies of adults are based on material collected and reared by us. In addition, specimens from many parts of Europe have been borrowed from several museums. The larvae studied were collected on our field trips.

The genitalia were dissected, macerated and left for 20 minutes in potassium hydroxide (10%) at 85°C. After washing in distilled water, they were transferred to ethanol for cleaning (70%), staining and dehydration (100%). This was followed by embedding in euparal.

Larvae were also macerated in potassium hydroxide (10%) and after rinsing, cleaning and staining they were mounted in polyvinyllactophenol.

The stain used for the male genitalia was haemaluin whilst for the female genitalia and larvae chlorazol black E was used.

3. *Biochemistry*

The localities sampled, together with numbers of larvae studied biochemically are listed in table I.

TABLE I
Sampling localities, number of individuals analysed electrophoretically (N_L) and mean heterozygosity level (H) for four species of Nepticulidae.

	locality	N_L	H
E. angulifasciella	Winterswijk (Netherlands)	90	.132
E. atricollis	New Forest (Gr. Britain)	26	.065
E. arcuatella	Goblin Combe (Gr. Britain)	32	.114
E. rubivora	Rockanje (Netherlands)	40	.145

Larvae stored at -30°C were used for electrophoresis. Specimen preparation, electrophoretic and staining techniques are essentially as previously described (MENKEN, 1982). The following 10 enzymes and one general protein were analysed (locus abbreviations in parentheses): malate dehydrogenase (*Mdh*), malic enzyme (*Me*), NADH dehydrogenase (*Nadh. dh-2*), catalase (*Cat-1* and *-2*), glutamic oxaloacetic transaminase (*Got*), phosphoglucomutase (*Pgm*), esterase (*Est*α*-2*), phosphoglucose isomerase (*Pgi*), tetrazolium oxidase (*To-2*) and the general protein (*Pt-2*). At each locus the most common allele of *E. angulifasciella* (reference species) is arbitrarily designated as 100. All other alleles are numbered according to their differences in migration distance from this standard allozyme in millimeters. Only those enzymes were used that were well resolved and easily interpretable in all four species. The resolving power of the gels allowed discrimination in migration between differences as small as 0.5 mm in side-by-side comparisons.

4. *Distribution Records*

In accordance with the recommendation of the European Invertebrate Survey (EIS) Mapping Schemes, distribution maps using the UTM grid have been constructed, based for the most part on material collected and reared within our department. A few extra records were provided by material in the collections of the Rijksmuseum van Natuurlijke Historie Leiden (RMNH) and the Zoological Museum Amsterdam (ZMA).

RESULTS

General Description

The following description covers all four taxa and therefore indicates how similar the moths and larvae are: Very small moths, with a wingspan of about 5-6 mm. Head with a variably coloured frontal tuft, and a blackish collar. Forewings blackish, with a silvery fascia in the middle of the wing, fringe silvery white, demarcated from wing by a border of dark scales. Hindwing in male with a short yellowish brush of hairscales near the socket of the frenulum, in female costal bristles present. The male genitalia are typical for *Ectoedemia* (*cf.* WILKINSON & NEWTON, 1981; SCOBLE, 1978), as can be seen from figs 1-8. The female genitalia are characterised by a blunt, wide ovipositor and a large bursa with a pair of reticulate signa (figs 9-12).

The larvae are yellowish white or greyish, and feed with their venter upwards. During the first three instars there is a ventral chain of, at most, 12 blackish plates, each associated with a thoracic or an abdominal segment. In *E. rubivora* there is also a similar chain of smaller plates on the dorsal side. In the fourth instar, the plates are lost and the ganglia become visible ventrally.

The egg is deposited on the underside of a leaf, from where the larva usually makes a much contorted gallery mine, which is filled with reddish to brown coiled or dispersed frass. Later, when the larva is in its fourth and last instar, the mine changes into a large blotch with irregularly scattered frass. The larva leaves its mine through a curved slit in the upper epidermis and makes a cocoon in the soil. The larva hibernates in its cocoon, and does not pupate until the spring.

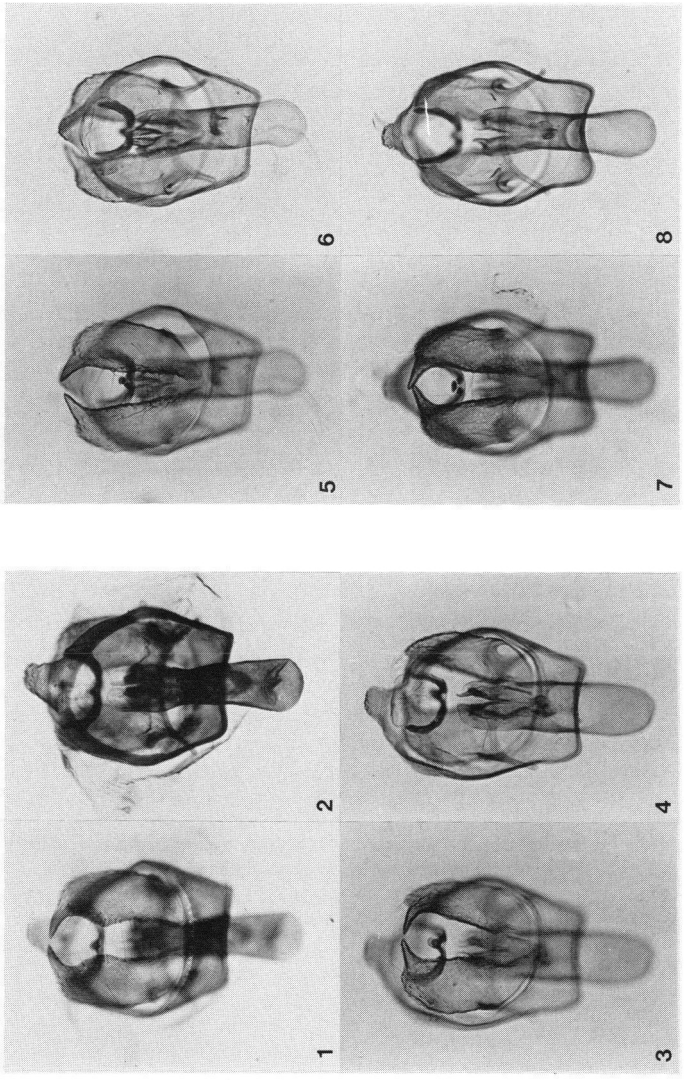
All these species are univoltine, with the moths on the wing in late spring or early summer; the larvae feed for about 2-3 weeks during the autumn.

Morphological Diagnosis

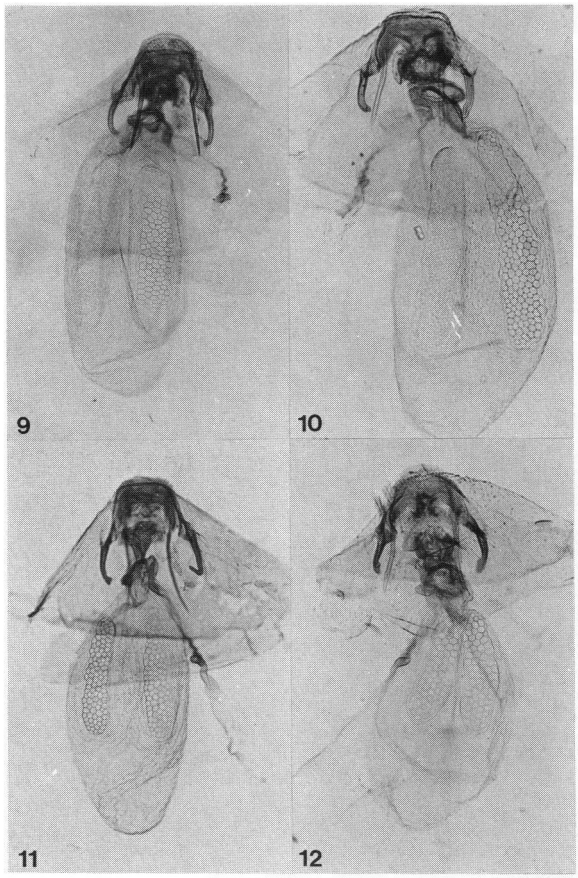
The head of *E. angulifasciella* is pale ochreous to yellowish orange, in general slightly lighter than *E. atricollis* and on balance is the lightest of the four species discussed here. In *E. atricollis* the head may be described as ferruginous or pale orange but still somewhat lighter than the yellow head of *E. arcuatella* which has been darkened by the presence of fuscous scales. *E. rubivora* is much darker than the other three and so its almost black head is a clear distinguishing feature.

The male genitalia have few important diagnostic features, but in *E. angulifasciella* (figs 1 and 2) the tip of the valve is pointed and curved inwards, whilst the inner margin is apically slightly concave and also has a small protuberance more basally. This contrasts with *E. atricollis* (figs 3 and 4) where the inner margin is almost straight. It may have a slight protuberance in the middle. The male genitalia of *E. arcuatella* (figs 5 and 6) cannot yet be reliably separated from either *E. atricollis* or *rubivora* (figs 7 and 8), but small differences in proportion have been observed. For example the transtilla in *E. arcuatella* appears to be slightly shorter and there are small differences in the length/width ratios of the genital capsules—usually smaller in *E. angulifasciella* than *E. atricollis* for example.

In females the length of the signa in the bursa can vary. In *E. angulifasciella* the length is 278-376 μ ($\bar{x} = 329.6 \pm 33.1$, $n = 12$) whilst in *E. atricollis* it is 363-449 μ ($\bar{x} = 406.2 \pm 31.7$, $n = 10$; see figs 9 and 10). This means that those of *E. atricollis* are the longest of the four taxa and only one measurement falls within the range of *E. angulifasciella*. The



Figs 1-8. Male genitalia of *Ectoedemia* spp., ventral aspect. Figs 1, 3, 5, 7 focussed at ventral surface; 2, 4, 6, 8 more dorsally. Figs 1, 2 *E. angulifasciella* (Stainton), Austria, slide VU 913. Figs 3, 4 *E. atricollis* (Stainton), France, slide VU 1152.



Figs 9-12. Female genitalia of *Ectoedemia* spp. Fig. 9 *E. angulifasciella* (Stainton), Austria, slide VU 1155. Fig. 10 *E. atricollis* (Stainton), Austria, slide VU 1149. Fig. 11 *E. arcuatella* (Herrich-Schäffer), Austria, slide VU 1102. Fig. 12 *E. rubivora* (Wocke), Netherlands, slide VU 964.

length in *E. rubivora*, 205-269 μ ($\bar{x} = 235.9 \pm 21.1$, $n = 12$; fig. 12), falls within the limits of *E. arcuatella*, 201-321 μ ($\bar{x} = 253.7 \pm 43.5$, $n = 6$; fig. 11), and both tend to be smaller than *E. angulifasciella* and much smaller than *E. atricollis*.

The larvae of *E. angulifasciella* and *E. arcuatella* are a similar yellowish green but *E. atricollis* can be diagnosed by the extremely dark colour of the head and prosternal plate—hence the name *E. atricollis*. *E. rubivora* also exhibits a curious feature in the presence of a dorsal chain of black plates in addition to the usual ventral plates of the penultimate stages.

Biochemical Inferences

Data are given (table II) on allele (electromorph) frequencies of nine variable genetic loci. Two other loci (*Nadh. dh-2* and *Pt-2*) are monomorphically fixed for the same allele in the four species under

TABLE II

Allozyme frequencies at 9 enzyme loci with numbers of individuals analysed (*N*) and single locus heterozygosity (*h*) per species.

		ang	*arc*	*atr*	*rub*			*ang*	*arc*	*atr*	*rub*
Pgi	*N*	90	32	20	40	*Pgm*	*N*	90	29	24	40
	90	.02	—	—	.03		78	—	—	.02	—
	94	—	.11	—	—		82	—	—	.58	—
	94.5	—	—	—	.72		86	—	—	.15	—
	95	.03	—	1.00	—		87	—	—	—	.01
	96	—	.89	—	—		89	—	—	—	.09
	100	.57	—	—	.01		90	—	.02	.25	—
	101	.01	—	—	.09		92	—	.59	—	.15
	104	—	—	—	.15		93	—	—	—	.01
	105	.37	—	—	—		96	—	.29	—	.11
	h	.537	.196	.000	.450		99	—	—	—	.11
							100	1.00	—	—	.30
Mdh	*N*	74	32	22	40		101	—	—	—	.01
	96	—	—	.05	—		105	—	.10	—	.20
	100	1.00	1.00	.95	1.00		*h*	.000	.557	.578	.815
	h	.000	.000	.095	.000						
						Cat-1	*N*	90	32	26	40
Got	*N*	87	31	25	40		90	—	.02	—	—
	97	.10	—	—	—		97	.02	—	.02	—
	98.5	—	—	—	.90		100	.97	.98	.98	1.00
	100	.89	—	—	—		104	.01	—	—	—
	102	—	—	—	.10		*h*	.059	.039	.039	.000
	103	.01	—	—	—						
	104	—	.61	—	—	*Cat-2*	*N*	75	32	26	40
	105	—	—	1.00	—		97	.30	—	—	—
	106	—	.39	—	—		98	—	—	1.00	—
	h	.198	.476	.000	.180		99	—	1.00	—	—
							100	.53	—	—	—
Estα-2	*N*	90	32	26	40		101	—	—	—	1.00
	97	—	—	—	.03		103	.17	—	—	—
	98	—	—	—	.01		*h*	.600	.000	.000	.000
	99	—	1.00	—	.96						
	100	.99	—	1.00	—	*Me*	*N*	89	32	26	40
	102	.01	—	—	—		97	—	1.00	—	—
	h	.020	.000	.000	.077		98	.02	—	1.00	—
							98.5	—	—	—	1.00
To-2	*N*	90	32	26	40		100	.98	—	—	—
	97	—	—	—	.04		*h*	.039	.000	.000	.000
	100	1.00	1.00	1.00	.96						
	h	.000	.000	.000	.077						

consideration. All four are almost identical for *To-2*, *Mdh* and *Cat-1* but a small number of heterozygotes do occur in some species. The *Est*α*-2* locus is interesting in that it divides the species into two pairs. It is almost identical in *E. angulifasciella* and *E. atricollis* (allele 100) and again in *E. arcuatella* and *E. rubivora* (allele 99). The remaining five loci are polymorphic varying considerably between all combinations of species pairs and there is little (*Pgm*, *Pgi* and *Me*) or no (*Cat-2*, *Got*) overlap between any of the pairs. Consequently the last two loci provide an absolute diagnosis for these species. These taxa also differ much in their single locus heterzygosities (h; table II). For example in *Pgi*, h ranges from .000 in *E. atricollis* to .537 in *E. angulifasciella* whilst the reverse occurs at the *Pgm* locus where $h = .000$ in *E. angulifasciella* and .578 in *E. atricollis*. At the same locus (*Pgm*) over 80% of the individuals in *E. rubivora* are heterozygotes.

Although this pattern of variability is species specific (MENKEN, 1981, 1982) no clear pattern can be observed in related taxa within their species group.

In *E. atricollis* the mean heterozygosity level (H) over all the 11 loci is only half that of the other three species (table I). The H level averaged over the four species ($\bar{H} = .114 \pm .035$) is relatively high when compared with an analysis of 23 insect species compiled by NEVO (1978) ($\bar{H} = .074 \pm .081$). Genetic identity (I) and distance values (D) (NEI, 1972) were computed for each of the six pairwise comparisons (table III). The four species divided into two pairs with the pair *E. angulifasciella* and *E. atricollis* somewhat less similar ($I = .599$ and 5 diagnostic loci) than the other pair *E. rubivora* and *E. arcuatella* ($I = .631$ and 4 diagnostic loci), resulting in the dendrogram (fig. 13).

TABLE III

Genetic identity (above diagonal) and genetic distance (below diagonal) based on 11 loci. Standard error of genetic distance calculated according to NEI (1971).

	1.	*2.*	*3.*	*4.*
1. *E. angulifasciella*	—	.599	.513	.553
2. *E. atricollis*	.512 ± .246	—	.419	.497
3. *E. arcuatella*	.667 ± .294	.711 ± .306	—	.631
4. *E. rubivora*	.593 ± .271	.699 ± .303	.460 ± .230	—

Host Plant Differences

E. angulifasciella is recorded from *Rosa* spp. There are also records from *Sanguisorba* spp., but this probably refers to a Central European form, described under several names, such as *schleichiella* Frey, the status of which is still under study. Hence it is here excluded from the treatment of *E. angulifasciella*.

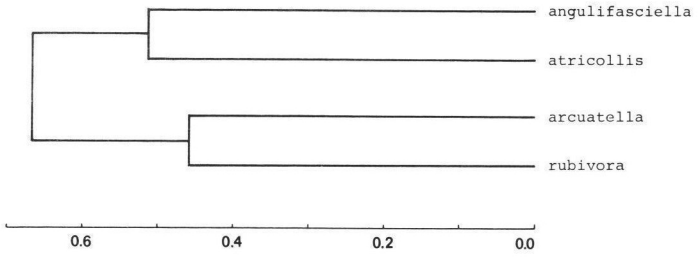


Fig. 13. Dendogram based on nine enzyme loci between four species of *Ectoedemia*; scale represents genetic distance.

The host plants of *E. atricollis* are *Crataegus* spp., on which it is very common; *Malus* spp., where it is also common; and less common on *Pyrus communis* L. and *Prunus avium* L. A few mines have been found on *Mespilus germanica* L.

E. arcuatella is normally found on *Fragaria vesca* L. and *F. moschata* Duchesne, but it has also been recorded from *Potentilla sterilis* (L.) Garcke and *P. erecta* (L.) Räuschel.

Rubus species provide the food plants of *E. rubivora*. In the Netherlands it is found on all kinds of the *R. fruticosa* complex. In northern Europe it is also found on *R. chamaemorus* L., but apparently does not occur on *R. idaeus* L. According to EMMET (1976) *E. rubivora* has a preference for *R. caesius* L. in Britain.

Notes on Life Histories

All four species produce only one generation per year with larvae being found in late summer and autumn and moths appearing the following June and July. Our collecting results in the Netherlands are demonstrated in fig. 14.

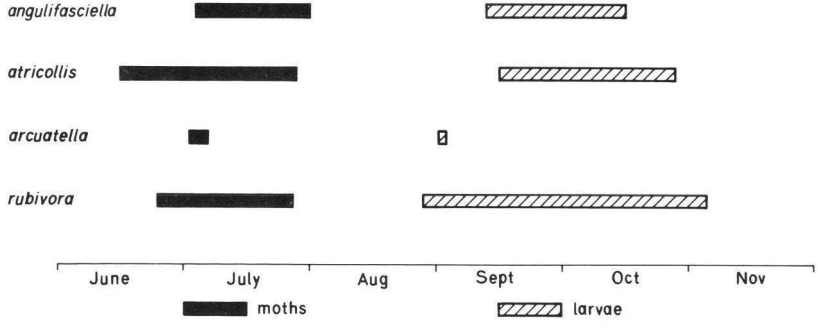


Fig. 14. Phenology of four *Ectoedemia* species in the Netherlands in the period 1978-1981. Larval data based on field collection, emergence data based on out-door rearing.

Although tenanted mines of *E. angulifasciella* have been found here from mid-September to mid-October, EMMET (1976) indicates that larvae may be found until early November in southern Britain. The adults are seen to emerge in July.

Larvae of *E. atricollis* have been found from mid-September to late October, irrespective of the host plant. In Britain EMMET (1976) speaks of an earlier appearance of larvae in late August, and moths appear in June. Here moths from *Crataegus* emerge from mid-June to mid-July; from *Malus* and *Prunus* somewhat later and from *Pyrus* from late June to late July. Further tests will be carried out to see if these observations continue consistently.

Several mines with young larvae of *E. arcuatella* were found in the Netherlands for the first time at the beginning of September 1981 (VAN NIEUKERKEN, 1982). The adults appeared in early July 1982. The periodicity is the same in England.

In the Netherlands first and second instar larvae of *E. rubivora* have been found as early as late August and larvae continue feeding until early November, with peak numbers in October. In England EMMET (1976) records them only in October. Fig. 15 shows that more females have been reared than males (87♀♀, 63♂♂; sex ratio ♀: ♂ 1.38). Another point of interest also demonstrated in fig. 15 is that males tend to emerge before females, although the day of maximum emergence was the same for both sexes. The phenomenon of male precedence is common in insects and there is evidence that it occurs widely in the Nepticulidae.

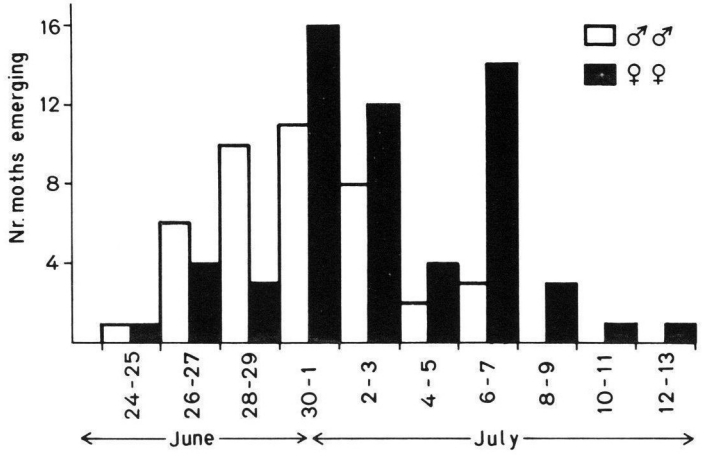


Fig. 15. Emergence of adult *Ectoedemia rubivora* (Wocke) in 1979, from material collected in the Netherlands and reared out of doors.

Distribution

All four species are widely distributed at least in central Europe. *E. rubivora* extends further north than the other three, being recorded up to the Arctic Circle in Finland. It is also common in southern Sweden and Norway, as are *E. angulifasciella* (including Åland) and *E. atricollis*. Although in southern Sweden and Finland, *E. arcuatella* has not been found in Norway. The four are known from Britain but *E. rubivora* is recorded only in the south-eastern counties, which is perhaps surprising, since it is the only one recorded from Ireland and the west at that.

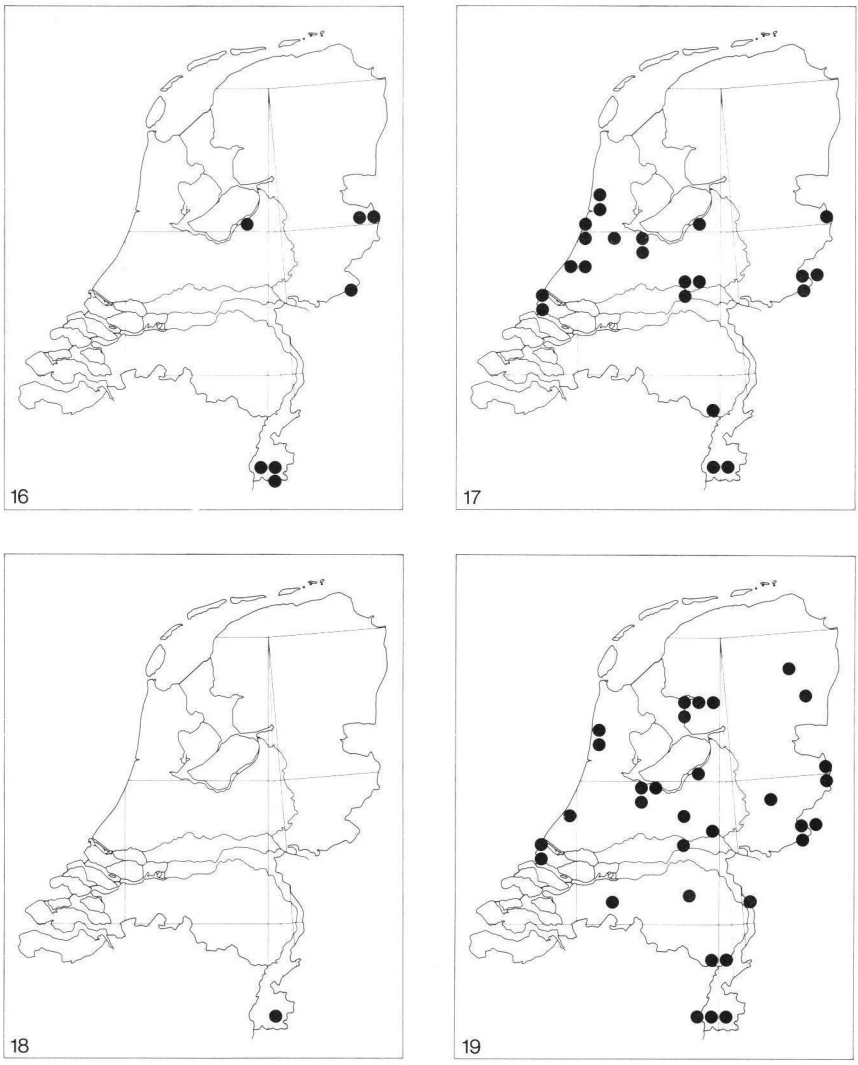
In the south of Europe the distribution is not so well investigated but probably all species occur in Hungary and northern Italy, and apart from *E. rubivora*, all are known to occur in Rumania.

During a field trip in central Greece we could only find *E. arcuatella* and *E. angulifasciella*, although seemingly suitable sites and food plants of the other two species were plentyful.

The distribution in the Netherlands is shown on the figs 16-19. Two species, *E. atricollis* and *E. rubivora*, appear to be widespread and occur throughout the country. Another, *E. angulifasciella*, appears to have a limited distribution in the south and east where it can be locally common. It probably does occur in more westerly localities as is indicated by a series reared in 1946 by Vári from Nunspeet (coll. ZMA). The fourth species, *E. arcuatella*, has only been found in one locality near Wijlre and although it may occur in other localities in Limburg, it is unlikely to be found further north since it has a preference for chalk downland. Although *E. atricollis* occurs throughout the Netherlands on *Crataegus*, it has only been found on *Prunus* in the south (Limburg) and the east (Achterhoek). On the host plant *Mespilus* the species is only recorded from Winterswijk. The absence of records of *E. rubivora* in some localities such as Zeeland and the Waddenzee islands is probably due to lack of collecting rather than reflecting gaps in its distribution.

DISCUSSION

The species treated here are extremely similar morphologically. When adult specimens are examined without knowledge of the food plants it is difficult to identify them with any degree of certainty. However, we can regard the four taxa as forming two species pairs with at least one character diagnostic. Between *E. angulifasciella* and *E. atricollis* there is at least one difference in both the male and female genitalia in spite of comments to the contrary (Beirne, 1945; Borkowski, 1975). The other pair, *E. rubivora* and *E. arcuatella*, is less easily separable on the



Figs 16-19. Distribution of *Ectoedemia* spp. in the Netherlands plotted on the 10 x 10 km UTM-grid. 16: *E. angulifasciella* (Stainton). 17: *E. atricollis* (Stainton). 18: *E. arcuatella* (Herrich-Schäffer). 19: *E. rubivora* (Wocke).

genitalia but differences in adult head colour and larval plates separate the two species satisfactorily.

One approach which is missing from this account is an analysis of measurements. It is felt that were this to be undertaken for a large

number of morphological features, that it might produce four distinct clusters and thus the taxa would be separable.

When the food plant is known, identification is straightforward for it was on this basis that the species were first described.

With regard to life histories and distribution, *E. angulifasciella* and *E. atricollis* can be separated on their dissimilar life-styles even when found in the same locality. However, *E. atricollis* is more widespread in the Netherlands and its distribution overall differs sufficiently to show that its populations are distinct from those of *E. angulifasciella*. It has also been demonstrated that the second species pair vary in their distribution and ecology to the extent that their existence as separate populations is clearly seen. So, with morphological and biological data only it seems already justified to regard the four taxa treated as forming distinct 'clusters'. However, it is impossible with these methods to give an indication of the degree of gene flow between the four species.

Electrophoretic techniques can measure gene flow unambiguously in cases where diagnostic characters occur. In nepticulids it has a high sensitivity for identifying sibling species. When compared they show low genetic identities ($\bar{I} = .547 \pm .058$; table III, fig. 13), as with the values found for *Drosophila* ($\bar{I} = .563 \pm .023$; AYALA *et al.*, 1974) and remarkably lower than those in, for example, *Rhagoletis* and *Anastrepha* ($\bar{I} = .741 \pm .088$ and $.838 \pm .005$ resp.; MORGANTE *et al.*, 1980) and *Yponomeuta* ($\bar{I} = .821 \pm .090$; MENKEN, 1981). Because in sympatric situations no hybrids have been found at any diagnostic enzyme locus, it can be stated with certainty that no gene flow takes place. The *Est*α*-2* locus is the only character that discriminates the two species pairs. As conspecific populations normally have the same allele fixed at monomorphic loci and similar frequency distributions at polymorphic loci, a single population can be used as a good representative for interspecific comparisons. Low genetic identities between sibling species seems to be a general phenomenon in Nepticulidae, which may indicate that no recent speciation has taken place and most, if not all, species are relatively old.

Thus we conclude that the weight of evidence favours the existence of four species divided into two pairs of sibling species. None of the evidence suggests that this is not so. Apart from the food plants, the electrophoresis most clearly shows that there are four taxa and that these can be grouped into two similar pairs.

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