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PAOLA ARDUINO and LUCIANO BULLINI
Department of Genetics and Molecular Biology, University of Rome
"La Sapienza", Via Lancisi, 29, I-00161 Rome, Italy.

A new species of Small Ermine moth (*Yponomeuta padellus* complex) from Japan, formerly assigned to *Y. malinellus* (Lepidoptera, Yponomeutidae)*.

INTRODUCTION

The taxonomic status of the Small Ermine moth *Yponomeuta malinellus* Zell. represented a puzzling problem since this species was described in 1838, up to few years ago (see refs. in Arduino and Bullini, 1985). The morphological differences found between *Y. malinellus* and *Y. padellus* (L.) are slight and often inconstant (Friese, 1960; Wiegand, 1962; Gershenson, 1970; Povel, 1984); the two taxa interbreed readily in the laboratory and fertile offspring is obtained (Thorpe, 1929). On the contrary, their larval trophic niche is markedly differentiated in the field: *Y. padellus* feeds on leaves of various Rosaceae (genera *Prunus*, *Crataegus*, *Sorbus*), whereas *Y. malinellus* feeds on leaves of apple (*Malus*) and occasionally pear (*Pyrus*) trees. The specific status of these two taxa was demonstrated by the use of allozyme markers that showed their reproductive isolation in the field by effective premating anti-hybridization barriers (Menken, 1980, 1989; Arduino et al., 1983; Arduino and Bullini, 1985, 1986). *Y. padellus* and *Y. malinellus* represent therefore a typical case of sibling species (i.e. reproductively isolated species, poorly or not differentiated at the morphological level), and not of "ecotypes" or "biological races", as supposed by various authors (e.g. Thorpe, 1929, 1930, 1931; Fiori, 1930; Servadei, 1930).

In the present paper, data are reported on the genetic and morphological differentiation between *Yponomeuta* populations from Japan, till now assigned to *Y. malinellus* (Moriuti, 1977), and various members of the *Yponomeuta padellus* complex from Europe.

MATERIALS AND METHODS

The following population samples of the Small Ermine moth from Japan, assigned to *Y. malinellus* according to Moriuti (1977), were studied:
- Morioka, Tohoku National Agricultural Experiment Station, Shimokuriyagawa,

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Iwate Prefecture, Honshu Island, 150 m a.s.l., collected as larvae on *Malus micromalus* Makino, May 1988-1992, Toshio Oku legit;

- Kuzakai, Kawai, south-western slope of Mt Kabutomyojin in the Kitakami mountain range, about 20 km east from Morioka, Iwate Prefecture, Honshu Island, between 700 and 900 m a.s.l., collected as larvae on *Malus sieboldii* (Regel) Rehder (= *M. tringo* Siebold), May 1990, Toshio Oku legit.

These two samples were compared genetically and morphologically with *Y. padellus* (*sensu stricto*) from Algeria, Sicily, Italy, The Netherlands and Finland, *Y. malinellus* from Sicily, Italy, Austria and The Netherlands, *Y. cagnagellus* (Hübner) from Italy (various locations) and *Y. mahalebellus* Guenée from central Italy.

Adults were deep frozen in toto at - 80° C, about 24 hours after their emergence. Standard horizontal starch gel electrophoresis was performed on moths after removing genitalia and wings; the latter were mounted on hard paper stripes by double scotch tape in order to compare wing pattern. A total of 35 enzyme loci were genetically analyzed; the electrophoretic procedures used are shown in Tables 1-3.

Isozymes were numbered in order of decreasing mobility from the most anodal, whereas allozymes were named with numbers indicating their mobility relative to the most common allele (designated as *100*) found in a reference population of *Y. padellus* from Cavalese (Trentino), Italy (*>100*=faster mobility; *<100*=slower mobility). Peptidase isozymes were designated following Harris and Hopkinson (1976). Deviations of genotype frequencies from the Hardy-Weinberg equilibrium were statistically analyzed by χ^2 and Fisher's exact tests (Sokal and Rohlf, 1981) as well as by *F* statistics (Wright, 1951, 1978). χ^2 and *G* heterogeneity tests were performed to detect significant differences in allele frequencies among samples (Workman and Niswander, 1970; Sokal and Rohlf, 1981). Genetic divergence between populations was estimated from allele frequencies with the formulae proposed by Nei (standard genetic identity, *I*, and distance, *D*, 1972) and Rogers (1972) modified by Wright, 1978 (genetic similarity, *S*, and distance, D_t).

Different statistical methods were used to show the genetic relationships between populations and taxa: two types of cluster analysis, one phenetic (UPGMA) from Nei's *D* matrix, and one cladistic, using Wagner procedure (Farris, 1972) from modified Rogers D_t matrix, as well as a principal component analysis (PC, using SYSTAT program, Wilkinson and Leland, 1989) using the frequencies of 47 alleles at 20 polymorphic loci as variables.

Results

Genetic data

Allozyme variation at the 35 loci analyzed was compared between the two populations from Japan and the European populations of the four considered members of the *Y. padellus* complex. As each of these taxa was found to be genetically rather homogeneous (Arduino and Bullini, 1985, and unpublished data), a single population from each species (from Abruzzi, central Italy) was chosen to illustrate allele frequencies (Table 4). Six loci (*Mdh-2*, *NADH-dh*, *Sod-1*, *Sod-3*, *Pk*,

Table 1 - Enzymes scored listed with their abbreviations, code number (E.C.), loci analyzed and electrophoretic migration conditions. When different, the enzyme abbreviation used in Arduino and Bullini (1985) is given in brackets.

Enzymes	Abbreviation	EC number	Loci	Migration	V/cm	Time (h)
Alcohol dehydrogenase	ADH	1.1.1.1	*Adh*	cathodal	7	3 3/4
α-Glycerophosphate dehydrogenase	α-GPDH	1.1.1.8	*α-Gpdh*	anodal	8	4
Hydroxybutyrate dehydrogenase	HBDH	1.1.1.30	*Hbdh*	cathodal	7	3 3/4
Malate dehydrogenase	MDH	1.1.1.37	*Mdh-1*	anodal	8	5 1/2
			Mdh-2	cathodal		
Malate dehydrogenase $NAPD^+$ dependent	MDHP (ME)	1.1.1.40	*Mdhp*	anodal	8	5 1/2
Isocitric dehydrogenase	ICDH (IDH)	1.1.1.42	*Icdh-1*	anodal	7	6 1/2
			Icdh-2	anodal		
Fucose dehydrogenase	FUDH	1.1.1.122	*Fudh*	anodal	7	3 1/2
Glyceraldheyde-3-phosphate dehydrogenase	GAPDH (G3PDH)	1.2.1.12	*Gapdh*	anodal	7	4
NADH-dehydrogenase	NADH-DH	1.6.99.3	*NADH-dh*	anodal	7	3 3/4
Superoxide dismutase	SOD	1.15.1.1	*Sod-1*	anodal	8,7	3 3/4
			Sod-2	anodal		
			Sod-3	cathodal		
Aspartate amino transaminase	AAT (GOT)	2.6.1.1	*Aat-1*	anodal	7	6 1/2
			Aat-2	anodal		
Hexokinase	HK	2.7.1.1	*Hk*	anodal	7	3 1/2
Phosphoenolpyruvate kinase	PK	2.7.1.40	*Pk*	anodal	7	4 1/2
Phosphogluco kinase	PGK	2.7.2.3	*Pgk*	anodal	7	4
Adenylate kinase	ADK	2.7.4.3	*Adk*	anodal	7	4 1/2
Phosphoglucomutase	PGM	2.7.5.1	*Pgm*	anodal	7	3 3/4
Esterase	EST	3.1.1.1	*Est-6*	anodal	8	4
β-Glucosidase	β-GLUC	3.2.1.31	*β-Gluc-3*	anodal	7	3 3/4
Peptidase C	PEP C	3.4.11 or 13	*Pep C-2*	anodal	7	3 1/2
Peptidase D	PEP D	3.4.13.9	*Pep D-1*	anodal	7	4 1/2
Peptidase S	PEP S	3.4.11 or 13	*Pep S-1*	anodal	7	4 1/2
			Pep S-3	anodal		
Aldolase	ALD	4.1.2.13	*Ald*	anodal	7	3 3/4
Carbonic anhydrase	CA	4.2.1.1	*Ca-1*	anodal	8	4 1/2
			Ca-2	anodal		
			Ca-3	anodal		
Aconitase	ACO	4.2.1.3	*Aco-1*	anodal	7	6
			Aco-2	cathodal		
Mannosephosphate isomerase	MPI	5.3.1.8	*Mpi*	anodal	8	3 3/4
Phosphoglucose isomerase	PGI	5.3.1.9	*Gpi*	anodal	8	5 1/2

Table 2 - Buffer systems for electrophoretic runs (1-6) and stainings (A-F).

Electrodes	*Gel*	*Staining*
1 0.03 M lithium hydroxyde / 0.19 M boric acid, pH 8.1	1 electrode buffer diluted 1:10 with 0.05 M Tris / 0.008 M citric acid, pH 8.3	A 0.05 M Tris / HCl, pH 8
2 0.678 M Tris / 0.157 M citric acid, pH 8	2 0.023 M Tris / 0.005 M citric acid, pH 8	B 0.125 M $Na_2HPO_4 \cdot 12 H_2O$; adj. to pH 7 with 0.125 M $NaH_2PO_4 \cdot H_2O$
3 0.21 M Tris / 0.15 M boric acid / 0.006 M EDTA, pH 8	3 0.021 M Tris / 0.02 M boric acid / 0.0007 M EDTA, pH 8	C 0.025 M KH_2PO_4 / 0.025 M K_2HPO_4, pH 7
4 0.15 M tri-sodium citrate / 0.24 M NaH_2PO_4, pH 6.3	4 electrode buffer diluted 1:40, adj. to pH 6.3 with 0.2 M citric acid	D 0.5 M Tris / HCl, pH 7.8
5 0.1 M Tris / 0.1 M maleic acid / 0.01 M EDTA / 0.015 M $MgCl_2$ / 0.125 M NaOH, pH 7.2	5 electrode buffer diluted 1:10, pH 7.4	E 0.1 M Na_2HPO_4 / 0.1 M KH_2PO_4, pH 6.5
6 0.125 M Tris, pH 7 with 1 M citric acid	6 0.05 M histidine-HCl / 1.40 mM EDTA, pH 7 with 1 M Tris. Stock diluted 4:1	F 0.02 M Tris / 0.02 M malic acid; adj. to pH 5.2 with 1 M NaOH

Adk) were found to be monomorphic for the same allele across all populations, whereas other nine (*Adh*, α-*Gpdh*, *Mdh-1*, *Gapdh*, *Sod-2*, *Aat-1*, *Pgk*, *Ald*, *Ca-3*) were polymorphic, with the same allele as the most common in all samples (frequency > 0.90); the remaining 20 loci showed marked differences in allele frequencies among two or more populations and taxa (Table 4). No significant discrepancies from Hardy-Weinberg expectations were observed within each sample at any of the polymorphic loci. The population samples from Japan proved to be genetically similar to each other, with differences in allele frequencies at only two loci (*Hbdh* and *Fudh*). All other pair comparisons showed larger differences, involving two or more loci with alternative alleles (diagnostic loci), summarized in Table 5. In particular, between the supposed *malinellus* from Japan and European *malinellus* six diagnostic (*Fudh*, *Hk*, β-*Gluc-3*, *Pep C-2*, *Pep D-1*, *Aco-2*) and five differentiated (*Hbdh*, *Pgm*, *Est-6*, *Pep S-3*, *Gpi*) loci were found. Japanese "*malinellus*", which is the only member of the *Y. padellus* complex recorded from this country (Moriuti, 1977), appears to be genetically equally differentiated from *Y. mahalebellus* and the true *Y. malinellus* (see below). As to the latter species, it is more related to *Y. padellus s. s.*, with only two diagnostic loci (*Est-6* and *Pep D-1*, Table 5) and two differentiated ones (*Pep S-3*, *Ca-2*), than to the Japanese taxon. Accordingly, the Japanese taxon appears to be a new, genetically distinct, member of the *Y. padellus* complex, sharing the same host plant with *Y. malinellus*; it will be designated below as *Yponomeuta okuellus* n. sp.

A quantitative estimate of the genetic divergence between populations and taxa of the *Y. padellus* complex, carried out with the indices of genetic distance by Nei

Table 3 - Electrophoretic procedures.

Enzyme	Buffer System	Staining buffer	Coenzymes	Linking enzymes	Substrates	Activators, inhibitors	Visualization methods
ADH	5	C 95 ml	NAD 15 mg		5 ml ethanol 5 ml propanol	$MgCl_2$ 10 mg	MTT 20 mg PMS 3 mg
α-GPDH	3	A 30 ml	NAD 15 mg		α-glycerophosphate 300 mg	EDTA 50 mg	MTT 10 mg PMS 3 mg agar 0.8 %
HBDH	5	A 30 ml	NAD 15 mg		hydroxybutyrate 300 mg	NaCl 200 mg	MTT 10 mg PMS 3 mg agar 0.8 %
MDH	4	A 30 ml	NAD 15 mg		L-malic acid 1 M pH 7 5 ml		MTT 10 mg PMS 3 mg agar 0.8 %
MDHP	4	A 30 ml	NADP 5 mg		L-malic acid 1 M pH 7 5 ml		MTT 10 mg PMS 3 mg agar 0.8 %
ICDH	2	A 30 ml	NADP 5 mg NAD 15 mg		DL-isocitrate 30 mg		MTT 10 mg PMS 3 mg agar 0.8 %
FUDH	2	A 30 ml	NAD 15 mg		L-fucose 100 mg	EDTA 30 mg	MTT 10 mg PMS 3 mg agar 0.8 %
GAPDH	2	A 100 ml	NAD 15 mg	EC 4.1.2.13 ALD 1 mg	F1,6DP 125 mg incubate with ALD for 30 min	Na arsenate 150 mg	MTT 10 mg PMS 3 mg
NADH-DH	5	B 100 ml	menadione 30 mg		NADH 60 mg		MTT 20 mg
SOD	3	A 30 ml	NAD 15 mg or NADP 5 mg			$MgCl_2$ 10 mg	MTT 20 mg PMS 3 mg agar 0.8 %
AAT	2	A 100 ml			aspartic acid 200 mg; α–keto-glucaric acid 100 mg; adj. to pH 7 with 1 M Tris	pyridossal-5'-phosphate 10 mg	incubate for 1 h, then add Fast Blue BB 150 mg
HK	2	A 30 ml	NADP 5 mg	EC 1.1.1.49 G6PDH 0.02 mg	ATP 10 mg glucose 45 mg	$MgCl_2$ 10 mg	MTT 10 mg PMS 3 mg agar 0.8 %

(continues)

Table 3 - (Continued).

Enzyme	Buffer System	Staining buffer	Coenzymes	Linking enzymes	Substrates	Activators, inhibitors	Visualization methods
PK	6	A 30 ml	NADP 5 mg	EC 1.1.1.49 G6PDH 0.02 mg EC 2.7.1.1 HK 0.02 mg	ADP 10 mg glucose 45 mg phosphoenol-pyruvic acid 5 mg	$MgCl_2$ 10 mg	MTT 10 mg PMS 3 mg agar 0.8 %
PGK	2	D 25 ml	NADH 10 mg	EC 1.2.1.12 GAPDH 1 mg EC 5.3.1.1 TPI 6 mg EC 1.1.1.8 αGPDH 1 mg	3-phosphoglyceric acid 15 mg ATP 30 mg	$MgCl_2$ 10 mg	under UV, with filter overlay
ADK	6	A 30 ml	NADP 5 mg	EC 1.1.1.49 G6PDH 0.02 mg EC 2.7.1.1 HK 0.02 mg	ADP 10 mg glucose 45 mg	$MgCl_2$ 10 mg	MTT 10 mg PMS 3 mg agar 0.8 %
PGM	5	A 30 ml	NADP 5 mg	EC 1.1.1.49 G6PDH 0.02 mg	G1P 80 mg	$MgCl_2$ 10 mg	MTT 10 mg PMS 3 mg agar 0.8 %
EST	3	E 100 ml			α-naphtylacetate 100 mg in 3 ml acetone		Fast Garnet GBC 100 mg
β-GLUC	5	F 25 ml			4-methyl-umbelli-feryl-β-glucoside 30 mg		under UV, with filter overlay
PEP	1	A 30 ml		EC 1.11.1.7 POD 300 U EC 1.4.3.2 L-AOD 0.06 mg	C: leu-ala 30 mg D: phe-pro30 mg S: leu-leu-leu 30 mg	o-dianisidine 15 mg $MnCl_2$ 50 mg	agar 0.8 %
ALD	5	A 30 ml	NAD 15 mg	EC 1.2.1.12 GAPDH 0.02 mg	F1,6DP 125 mg	Na arsenate 150 mg	MTT 10 mg PMS 3 mg agar 0.8 %
CA	3	E 25 ml			4-methyl-umbelli-feryl acetate 10 mg in 2 ml acetone		under UV, with filter overlay
ACO	6	A 30 ml	NADP 5 mg	EC 1.1.1.42 ICDH 1 mg	cis-aconitic acid 70 mg; adj. to pH 7.5	$MgCl_2$ 10 mg	MTT 10 mg PMS 3 mg agar 0.8 %
MPI	3	A 30 ml	NADP 5 mg	EC 1.1.1.49 G6PDH 0.02 mg EC 5.3.1.9 GPI 0.02 mg	mannose-6-phos-phate 25 mg	$MgCl_2$ 10 mg	MTT 10 mg PMS 3 mg agar 0.8 %
GPI	4	A 30 ml	NADP 5 mg	EC 1.1.1.49 G6PDH 0.02 mg	fructose-6-phos-phate 10 mg	$MgCl_2$ 10 mg	MTT 10 mg PMS 3 mg agar 0.8 %

Table 4 - Allele frequencies at 35 loci in populations of the *Yponomeuta padellus* complex: JMo: *Y.* sp. from Morioka, Japan; JKu, *Y.* sp. from Kuzakai, Japan; mal: *Y. malinellus* from Abruzzi (central Italy); pad: *Y. padellus* from Abruzzi (central Italy); cag: *Y. cagnagellus* from Abruzzi (central Italy); mah: *Y. mahalebellus* from Abruzzi (central Italy).

Loci	Alleles	JMo	JKu	mal	pad	cag	mah
Adh	*97*	—	—	0.03	0.03	0.06	0.02
	100	1.00	1.00	0.97	0.97	0.94	0.98
α-Gpdh	*94*	—	—	0.07	0.01	—	—
	100	1.00	1.00	0.93	0.99	1.00	1.00
Hbdh	*93*	—	—	0.01	0.01	0.05	—
	96	—	—	—	0.02	—	—
	100	0.64	0.41	0.98	0.78	0.95	1.00
	104	—	—	—	0.04	—	—
	106	0.36	0.58	—	0.12	—	—
	108	—	—	0.01	0.03	—	—
Mdh-1	*86*	—	—	0.02	0.01	—	—
	90	—	—	—	0.01	—	—
	93	0.01	—	—	—	—	—
	100	0.99	1.00	0.97	0.93	1.00	1.00
	111	—	—	0.01	—	—	—
	118	—	—	—	0.05	—	—
Mdh-2	*100*	1.00	1.00	1.00	1.00	1.00	1.00
Mdhp	*94*	—	—	—	—	0.03	—
	96	0.28	0.17	0.11	0.22	0.30	1.00
	100	0.72	0.83	0.88	0.77	0.67	—
	104	—	—	0.01	0.01	—	—
Icdh-1	*85*	—	—	0.01	0.01	—	—
	90	—	—	0.03	0.15	—	—
	97	—	—	0.01	0.01	—	—
	100	1.00	1.00	0.89	0.80	1.00	1.00
	104	—	—	0.02	0.01	—	—
	107	—	—	0.04	0.02	—	—
Icdh-2	*92*	—	—	0.01	0.01	—	—
	100	0.64	0.80	0.99	0.98	1.00	1.00
	106	0.36	0.20	—	0.01	—	—
Fudh	*87*	—	—	0.01	—	—	—
	92	—	0.29	—	—	—	1.00
	94	—	—	0.01	0.01	—	—
	96	—	—	—	—	0.36	—
	97	1.00	0.71	0.04	0.05	—	—
	100	—	—	0.93	0.92	—	—
	104	—	—	0.01	0.02	0.64	—
Gapdh	*96*	—	—	0.01	0.01	—	—
	100	1.00	1.00	0.99	0.99	1.00	1.00
NADHdh	*100*	1.00	1.00	1.00	1.00	1.00	1.00
Sod-1	*100*	1.00	1.00	1.00	1.00	1.00	1.00
Sod-2	*100*	1.00	1.00	1.00	0.96	1.00	1.00
	110	—	—	—	0.04	—	—

(continues)

Table 4- (Continued).

Loci	Alleles	JMo	JKu	mal	pad	cag	mah
Sod-3	*100*	1.00	1.00	1.00	1.00	1.00	1.00
Aat-1	*88*	—	0.07	0.02	0.02	—	—
	100	1.00	0.93	0.98	0.98	1.00	1.00
Aat-2	*88*	—	—	0.03	0.02	0.53	—
	90	—	—	—	—	—	1.00
	92	—	—	0.02	0.01	—	—
	100	0.94	1.00	0.95	0.97	0.47	—
	116	0.06	—	—	—	—	—
Hk	*94*	1.00	1.00	—	—	1.00	1.00
	96	—	—	0.04	0.02	—	—
	100	—	—	0.96	0.98	—	—
Pk	*100*	1.00	1.00	1.00	1.00	1.00	1.00
Pgk	*100*	1.00	1.00	0.95	0.98	1.00	1.00
	105	—	—	0.05	0.02	—	—
Adk	*100*	1.00	1.00	1.00	1.00	1.00	1.00
Pgm	*92*	—	—	0.01	—	0.16	—
	95	—	—	0.04	0.01	0.05	—
	97	—	—	0.26	0.08	—	—
	100	0.02	0.03	0.33	0.20	0.63	—
	102	—	—	0.22	0.19	—	—
	105	0.98	0.97	0.14	0.45	0.16	1.00
	107	—	—	0.01	0.05	—	—
	110	—	—	—	0.02	—	—
Est-6	*90*	—	—	—	0.01	—	—
	94	—	—	—	—	0.42	—
	96	—	—	0.01	0.02	—	—
	98	0.02	0.07	—	—	—	—
	100	—	—	0.01	0.94	—	1.00
	102	—	—	—	—	0.58	—
	104	0.98	0.93	0.18	—	—	—
	108	—	—	0.79	0.01	—	—
	113	—	—	0.01	0.02	—	—
ß-Gluc-3	*90*	0.05	—	—	—	—	1.00
	95	0.95	1.00	—	—	—	—
	100	—	—	1.00	1.00	1.00	—
Pep C-2	*88*	0.91	1.00	—	—	—	—
	90	—	—	—	—	0.08	—
	94	0.09	—	—	—	0.92	—
	100	—	—	1.00	1.00	—	1.00
Pep D-1	*94*	0.78	0.71	—	—	—	—
	97	0.22	0.29	—	0.04	—	—
	98	—	—	1.00	—	1.00	1.00
	100	—	—	—	0.96	—	—
Pep S-1	*100*	1.00	1.00	1.00	1.00	—	1.00
	110	—	—	—	—	1.00	—

(continues)

Table 4- (Continued).

Loci	Alleles	JMo	JKu	mal	pad	cag	mah
Pep S-3	*97*	0.25	0.21	—	0.05	0.36	—
	100	—	—	0.52	0.89	—	—
	103	0.12	—	0.48	0.06	0.05	—
	105	0.63	0.65	—	—	0.54	1.00
	107	—	0.14	—	—	0.05	—
Ald	*93*	—	—	0.01	0.02	—	—
	100	1.00	1.00	0.98	0.98	1.00	1.00
	102	—	—	0.01	—	—	—
Ca-1	*95*	0.05	0.04	0.03	0.05	0.27	—
	100	0.91	0.92	0.90	0.93	0.65	1.00
	107	0.04	0.04	0.07	0.02	0.08	—
Ca-2	*87*	0.05	0.04	0.01	0.01	—	0.01
	90	—	—	0.01	0.19	—	—
	93	0.85	0.79	0.64	0.02	0.02	0.99
	97	—	—	0.04	—	—	—
	100	0.10	0.17	0.28	0.76	0.78	—
	107	—	—	0.02	0.02	0.20	—
Ca-3	*95*	—	—	0.02	—	0.02	—
	100	1.00	1.00	0.97	0.99	0.98	0.96
	105	—	—	—	—	—	0.04
	110	—	—	0.01	0.01	—	—
Aco-1	*94*	0.02	0.03	0.02	0.01	—	0.30
	96	—	—	0.04	0.10	0.07	—
	98	0.06	0.07	0.09	0.12	—	—
	100	0.92	0.90	0.85	0.73	0.90	—
	104	—	—	—	0.03	—	—
	107	—	—	—	0.01	0.02	0.70
Aco-2	*90*	—	—	0.01	—	—	—
	92	1.00	1.00	—	—	—	—
	94	—	—	0.02	0.08	0.05	—
	98	—	—	0.01	—	—	—
	100	—	—	0.96	0.89	0.95	1.00
	102	—	—	—	0.01	—	—
	104	—	—	—	0.02	—	—
Mpi	*85*	—	—	0.01	—	—	—
	90	0.11	—	0.01	—	—	—
	95	0.87	0.90	0.94	0.11	0.29	0.98
	100	0.02	0.07	0.03	0.59	0.42	0.02
	105	—	0.03	0.01	0.08	0.29	—
	110	—	—	—	0.22	—	—
Gpi	*86*	0.02	—	—	—	—	—
	89	—	—	—	—	0.06	—
	93	0.91	0.83	0.27	0.33	0.44	1.00
	97	—	—	0.04	—	—	—
	98	0.07	0.17	—	—	—	—
	100	—	—	0.68	0.67	0.50	—
	104	—	—	0.01	—	—	—

Table 5 - Loci found diagnostic at the 0.99 level between members of the *Yponomeuta padellus* complex.

	Y. malinellus	*Y. padellus*	*Y. cagnagellus*	*Y. mahalebellus*
Y. sp. (Japan)	*Fudh, Hk, β-Gluc-3, Pep C-2, Pep D-1, Aco-2*	*Fudh, Hk, Est-6, β-Gluc-3, Pep C-2, Pep D-1, Aco-2*	*Fudh, Est-6, β-Gluc-3, Pep D-1, Pep S-1, Aco-2*	*Aat-2, Est-6, β-Gluc-3, Pep C-2, Pep D-1, Aco-1, Aco-2*
Y. malinellus		*Est-6, Pep D-1*	*Fudh, Hk, Est-6, Pep C-2, Pep S-1, Pep S-3*	*Fudh, Aat-2, Hk, Est-6, β-Gluc-3, Pep S-3, Ca-2*
Y. padellus			*Fudh, Hk, Est-6, Pep C-2, Pep D-1, Pep S-1*	*Fudh, Aat-2, Hk, β-Gluc-3, Pep D-1, Pep S-3, Ca-2, Aco-1*
Y. cagnagellus				*Fudh, Aat-2, Est-6, β-Gluc-3, Pep C-2, Pep S-1, Ca-2, Aco-1*

Table 6 - Average values of genetic distance between members of the *Yponomeuta padellus* complex obtained with the formulae by Nei, 1972 (below the diagonal) and Rogers, 1972, modified by Wright, 1978 (above the diagonal).

Species	1	2	3	4	5
1 ***Y. okuellus* n. sp.**		0.48	0.50	0.48	0.48
2 ***Y. malinellus***	0.30		0.31	0.42	0.48
3 ***Y. padellus***	0.33	0.12		0.42	0.51
4 ***Y. cagnagellus***	0.32	0.22	0.24		0.50
5 ***Y. mahalebellus***	0.29	0.29	0.32	0.31	

(1972) and Rogers (1972, modified by Wright, 1978), is summarized in the matrix in Table 6. At the interspecific level, the values of Nei's genetic distance (D) range from 0.12 (between *Y. padellus s.s.* and *Y. malinellus s.s.*) to 0.33 (between *Y. padellus* and *Y. okuellus*). In particular, average Nei's D between European *Y. malinellus* and *Y. okuellus* is 0.30, similar to that found between both these species and *Y. mahalebellus*. At the intraspecific level, the genetic divergence found between the two Japanese populations of *Y. okuellus* is D=0.008, as among *Y. malinellus* populations, whereas a wider range of variation was observed within *Y. padellus* (Arduino and Bullini, 1985).

The genetic relationships among the considered species of the *Y. padellus* complex are summarized by the UPGMA dendrogram from average Nei's D values, given in Fig. 1. The closest species are *Y. padellus* and *Y. malinellus*; the following node links these two species to *Y. cagnagellus*. The node at D=0.29,

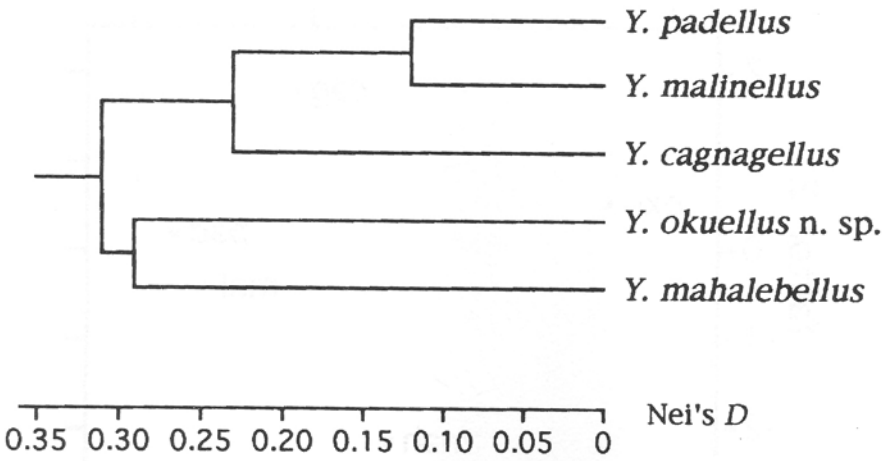


Fig. I - UPGMA dendrogram based on Nei's *D* (1972) showing the genetic relationships between *Y. okuellus* n. sp. and the considered members of the *Y. padellus* complex.

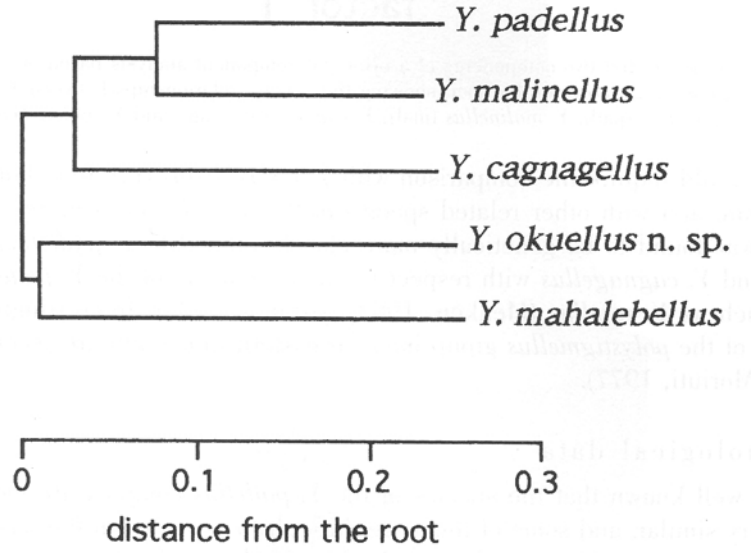


Fig. II - Wagner cladogram based on Rogers D_t (1972, modified by Wright, 1978) showing the genetic relationships between *Y. okuellus* n. sp. and the considered members of the *Y. padellus* complex.

linking *Y. okuellus* to *Y. mahalebellus*, is very close to that joining these two species to the former three (average D=0.31). Consistent patterns of genetic relationships were obtained with the phylogenetic tree using Wagner procedure (Farris, 1972) shown in Fig. 2, and by the plot of the first two components of a principal component analysis performed using the frequencies of 47 alleles at 20 polymorphic loci as variables (Fig. 3).

A more comprehensive picture of the genetic relationships of the considered

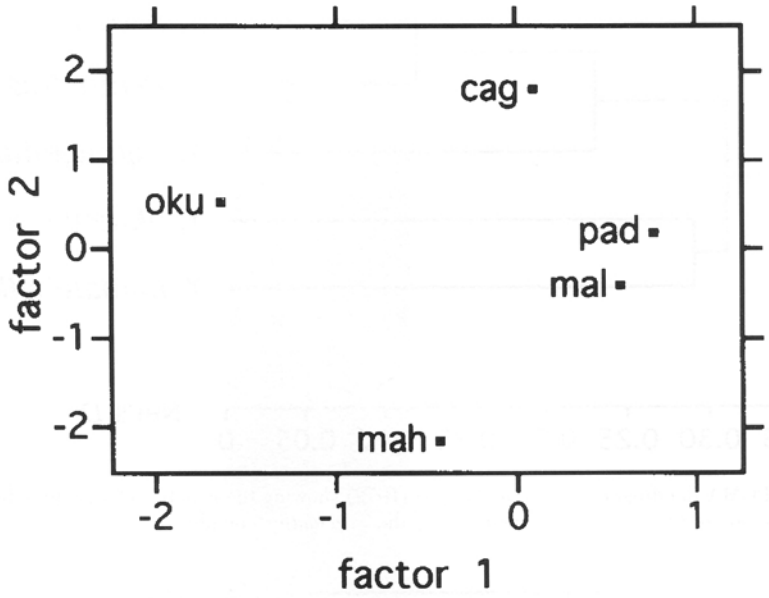


Fig. III - Plot of the first two components of a principal component analysis based on the frequencies of 47 alleles at 20 polymorphic loci, showing the genetic relationships between *Y. okuellus* n. sp. (oku), *Y. padellus* (pad), *Y. malinellus* (mal), *Y. cagnagellus* (cag), and *Y. mahalebellus* (mah).

species would require the comparison with *Y. evonymellus* (L.), from both Europe and Japan, and with other related species of the *polystigmellus* group. *Y. evonymellus* was found to be genetically more closely related to *Y. padellus*, *Y. malinellus* and *Y. cagnagellus* with respect to other members of the *Y. padellus* complex, such as *Y. rorellus* (Menken, 1982; Arduino, 1983). Interestingly, various species of the *polystigmellus* group have an eastern range and are recorded from Japan (Moriuti, 1977).

Morphological data

It is well known that the species of the *Y. padellus* complex are morphologically very similar, and some of them are difficult to separate on the basis of both external characters (Fig. 4) and genitalia (Fig. 6); this is also the case of *Y. okuellus* n. sp. (Figs. 5, 6a, b). We have compared a number of morphological characters between this species and the following members of the *Y. padellus* complex: *Y. padellus*, *Y. malinellus*, *Y. cagnagellus*, *Y. mahalebellus*. A useful character to recognize *Y. okuellus* at the adult stage was found to be the number and pattern of distribution of black dots on forewing. All Small Ermine moths show longitudinal series of black dots on the veins of forewing. Moriuti (1972) named these series, beginning with the one nearest to the upper margin: subcostal, radial, subradial, supramedian, submedian, and subdorsal, as shown in Fig. 7. In *Y. okuellus* the number of dots ranges from 28 to 62 (average 38) in a sample of 175 specimens (Fig. 9), most with the following distribution: 4-9 subcostal, 4-8

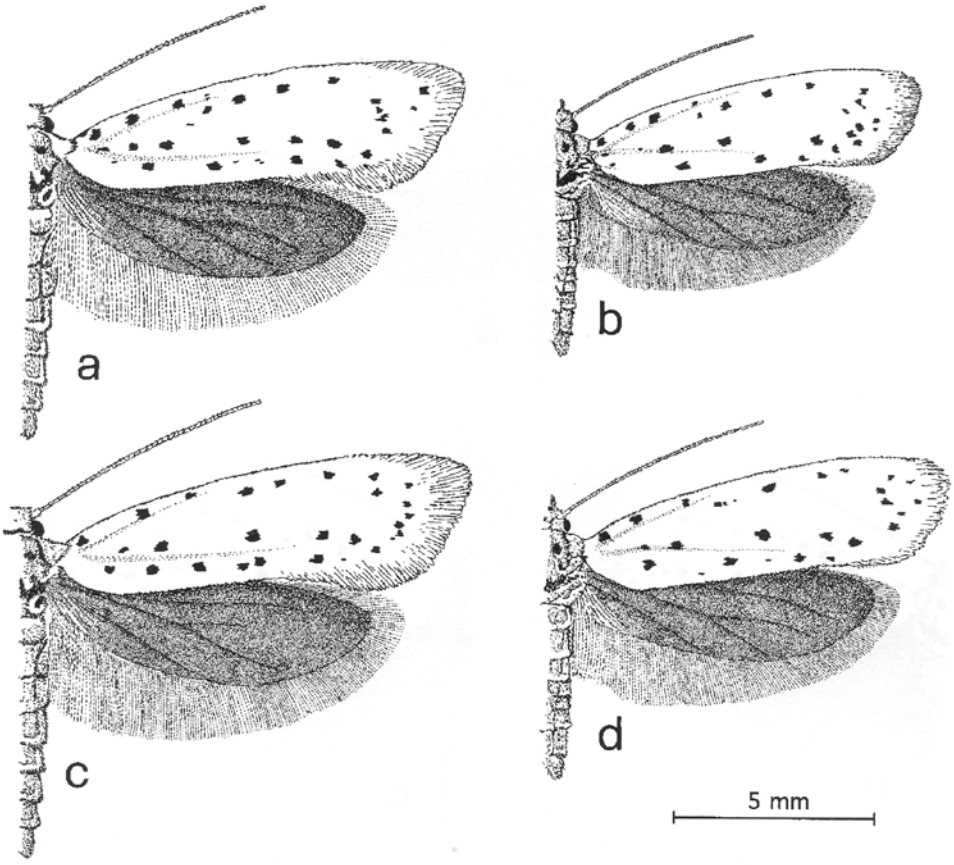


Fig. IV - Drawings of adult specimens of *Y. malinellus* (a, male; b, female) and of *Y. padellus* (c, male; d, female) from central Italy.

radial, 5-11 submedian, 5-12 subdorsal, and 5-17 on distal 1/4 of the forewing, between radial and submedian. Some plusvariant specimens showed two further series of black dots, on the subradial (2-6) and supramedian (2-5) veins, respectively, resembling *Y. evonymellus* for this character. The other members of the *Y. padellus* complex generally miss the supramedian and subradial series of black dots; moreover, in these species, the dot number is on average lower. In *Y. padellus* and *Y. malinellus* from Europe (Fig. 8), the average number are respectively 25 (range 18-33) and 23 (range 16-31). The differences of the mean number of dots between *Y. okuellus* and either *Y. padellus* or *Y. malinellus* is highly significant ($P<0.001$ in both cases, using Student's t).

Other characters concern pupae, which are generally darker in *Y. okuellus* (from dark brown to blackish brown) than in the considered *Yponomeuta* species, and cocoons, which are more loosely woven and transparent in *Y. okuellus* than in *Y. padellus*, while they are densely woven, silver white and opaque in *Y. malinellus*. Male and female genitalia of *Y. okuellus* are illustrated in Fig. 6 together with

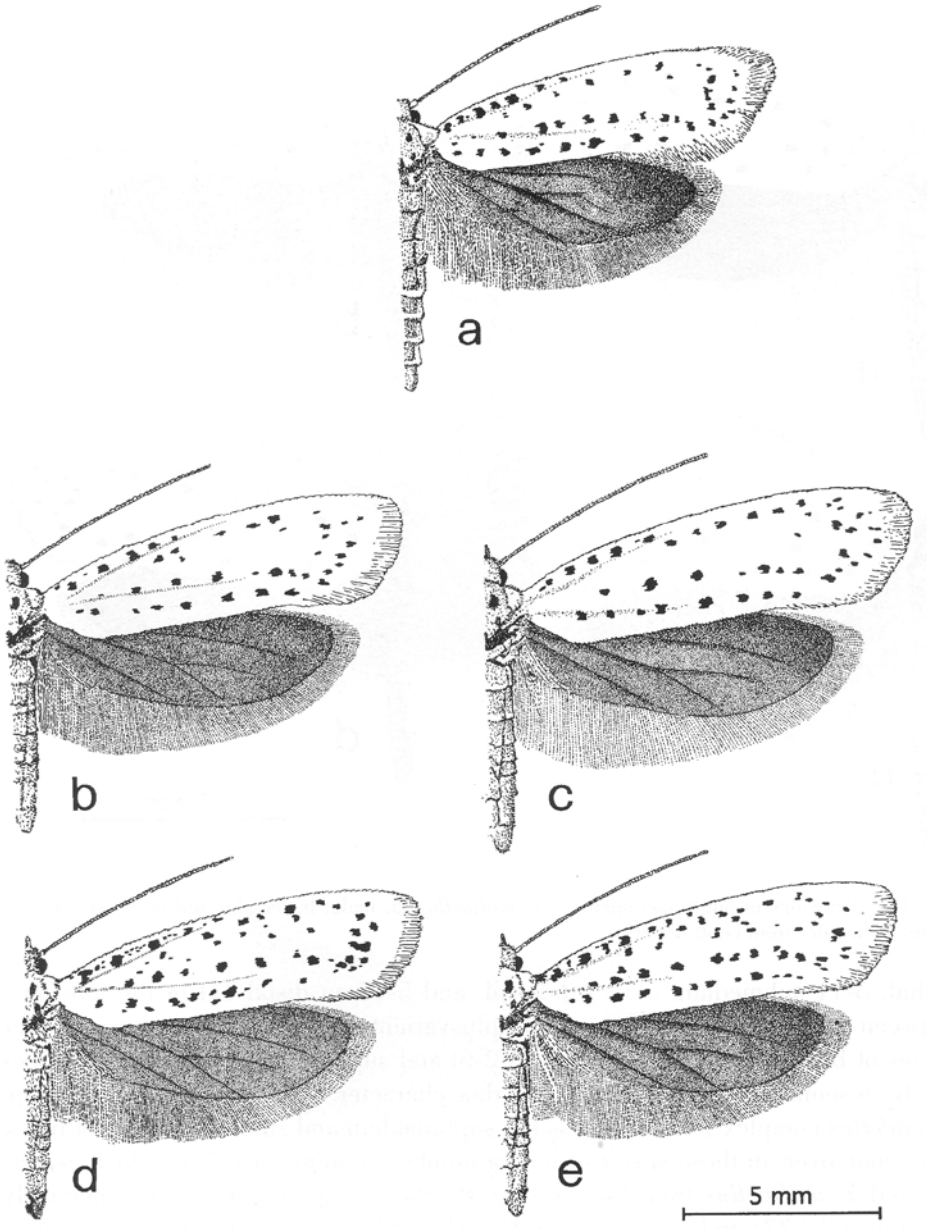


Fig. V - Drawing of the holotype (a) and paratypes (b,c,d,e) of *Yponomeuta okuellus* n. sp. from Morioka, Honshu Island, Japan.

those of *Y. padellus* and *Y. malinellus*. They are similar to one another and difficult to separate specifically.

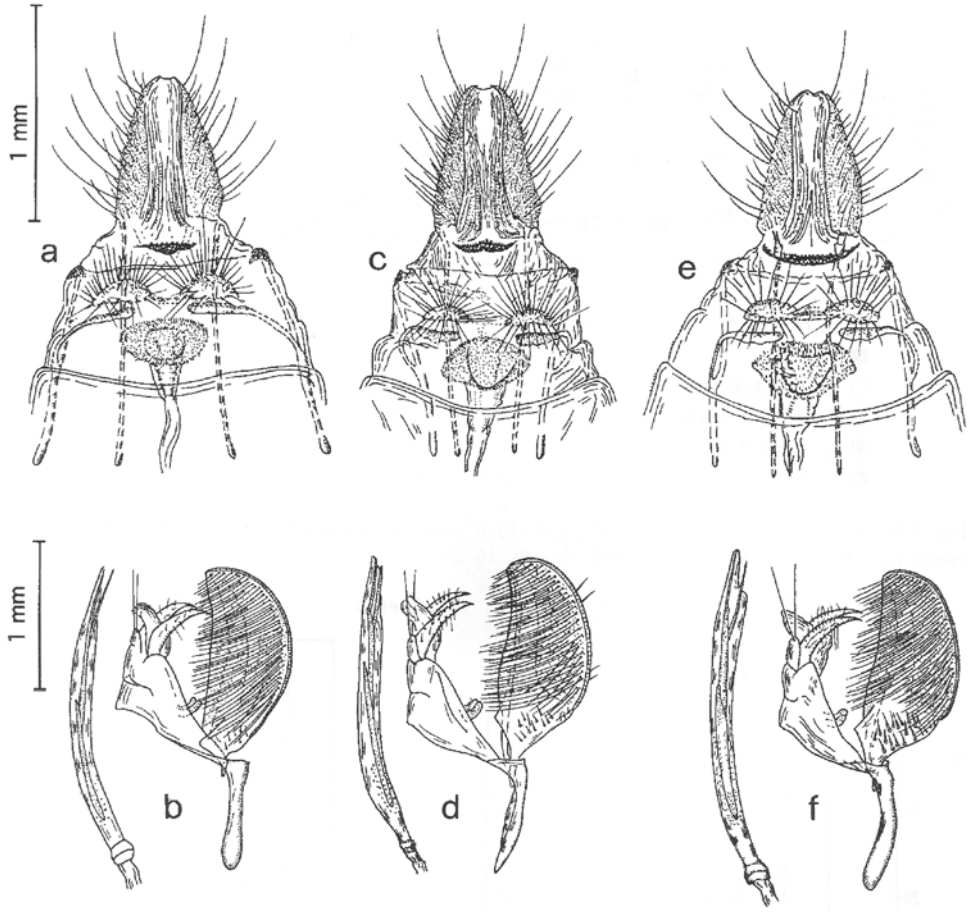


Fig. VI - Drawings of genitalia of females (above) and males (below) of *Y. okuellus* n. sp. (a,b), *Y. malinellus* (c,d), and *Y. padellus* (e,f).

YPONOMEUTA OKUELLUS N. SP.

Type Material — *Holotype* - Adult male (Fig. 5a), Morioka, Honshu Island, 150 m a.s.l. (Japan), June 1988, Toshio Oku legit as larva. Preserved in coll. Bullini, Rome.

Paratypes - 1 ♂ , 3 ♀♀ , same locality and data (Fig. 5b-e). 1 ♂ , 1 ♀ in coll. Brit. Mus. (Nat. Hist.) London; 1 ♀ in coll. Civic Mus. Nat. Hist. Milan; 1 ♀ in coll. Civic Mus. Nat. Hist. Verona.

Derivatio Nominis - The species is named in honour of the distiguished lepidopterologist Dr Toshio Oku, who since 1988 has been kindly collecting and sending us the specimens of Small Ermine moth from Japan studied in the present paper.

Diagnosis - A *Yponomeuta* belonging to the *Y. padellus* complex, characterized by the number and pattern of distribution of the black dots on forewing, the darker colour of the pupa and the very loosely woven and transparent cocoon, as

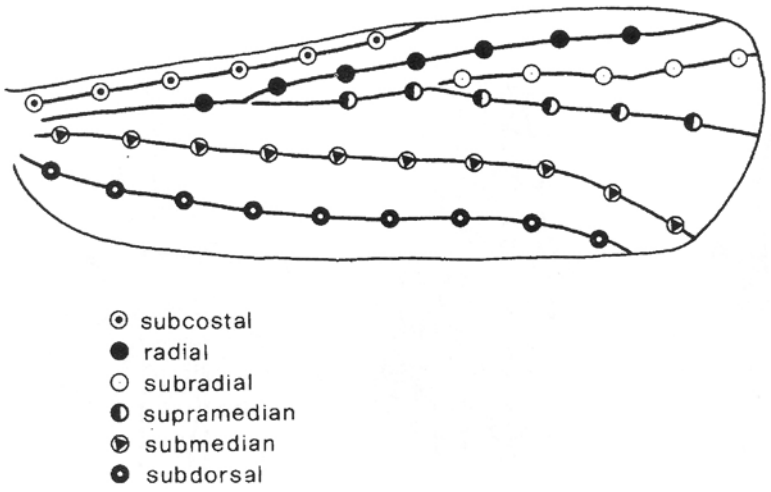


Fig. VII - Schematic representation of longitudinal series of black dots on forewing in the *Yponomeuta padellus* complex (modified from Moriuti, 1972).

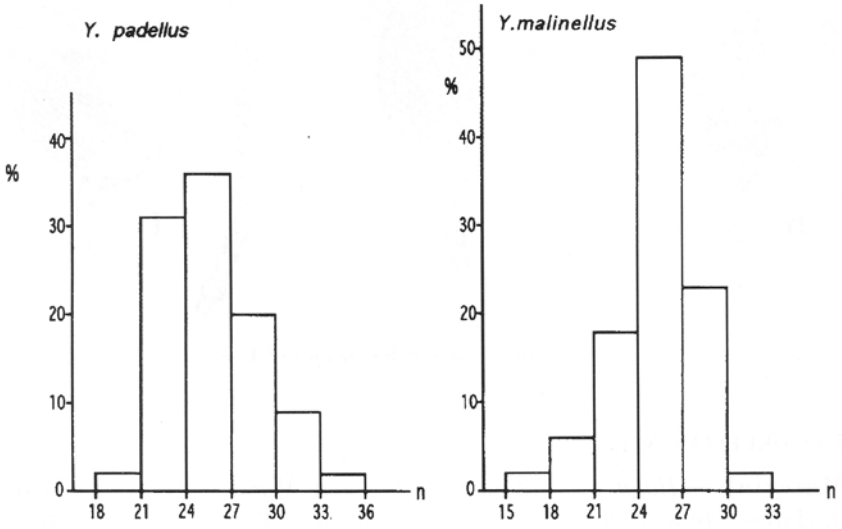


Fig. VIII - Number of black dots on forewing (n) in a sample of 107 *Y. padellus* (left) and 105 *Y. malinellus* (right) from western Europe.

well as by the presence of diagnostic allozymes at the loci *Fudh*, *Hk*, β-*Gluc-3*, *Pep C-2*, *Pep D-1*, *Aco-2* (Fig. 10).

Description of the Holotype - Adult male. External features (Fig. 5a): total body length: 7.5 mm; wing span: 19.2 mm; length of forewing: 9.0 mm. Head, antenna and palpus white; thorax white, with 5 black dots; forewing white with 44 small black dots, distributed as follows: 7 subcostal, 6 radial, 11 submedian, 8 subdorsal, and 12 on distal 1/4 of the forewing, between radial and submedian; cilia white. Hindwing dark grey; cilia pale grey, whitish round the apex. Genitą-

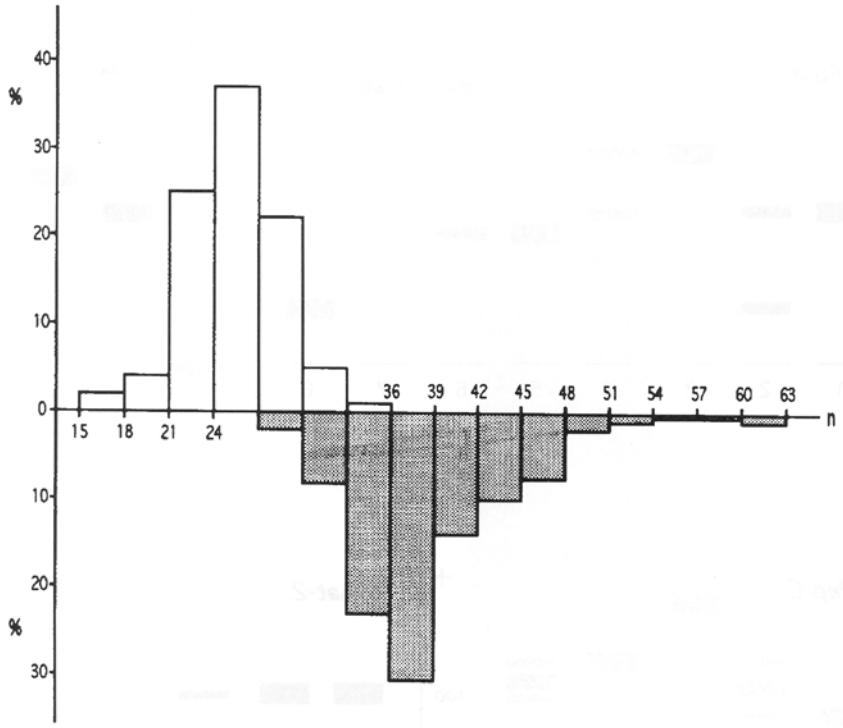
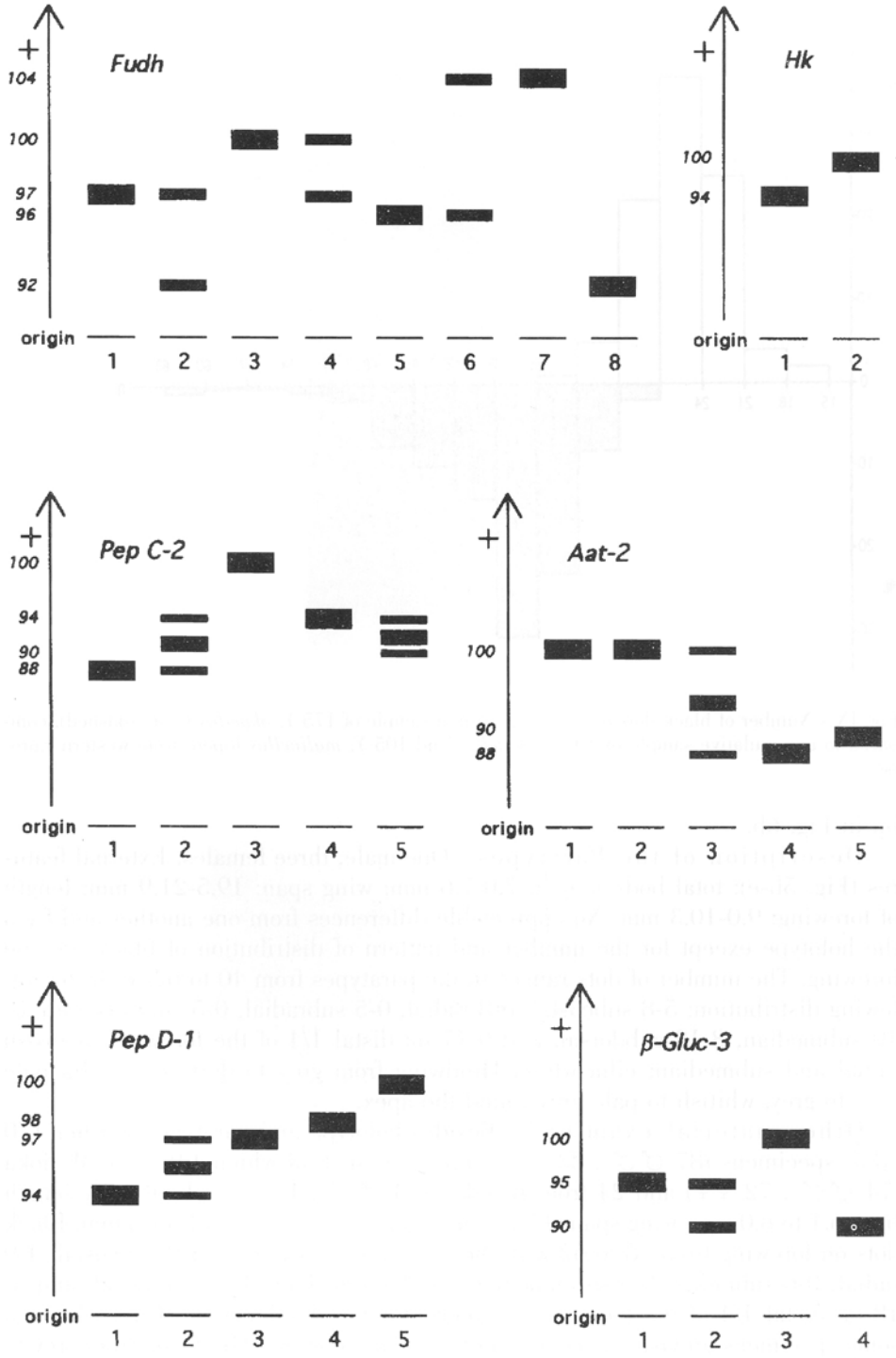


Fig. IX - Number of black dots on forewing (n) in a sample of 175 *Y. okuellus* n. sp. (dashed), compared to a cumulative sample of 107 *Y. padellus* and 105 *Y. malinellus* (open) from western Europe.

lia in Fig. 6b.

Description of the Paratypes - One male, three females. External features (Fig. 5b-e): total body length: 7.0-7.6 mm; wing span: 19.5-21.9 mm; length of forewing: 9.0-10.3 mm. No appreciable differences from one another and from the holotype except for the number and pattern of distribution of black dots on forewing. The number of dots ranges in the paratypes from 40 to 62, with the following distribution: 5-8 subcostal, 6-8 radial, 0-5 subradial, 0-5 supramedian, 8-10 submedian, 8-12 subdorsal, and 9-17 on distal 1/4 of the forewing, between radial and submedian; cilia white. Hindwing from grey to dark grey; cilia pale grey to grey, whitish to pale grey round the apex.

Other material examined - Besides holotype and paratypes, further 170 adult specimens (87 ♂♂ , 83 ♀♀) were examined, of which 146 from Morioka (74 ♂♂ , 72 ♀♀) and 24 from Kuzakai (13 ♂♂ , 11 ♀♀). Total body length from 6.1 to 8.0 mm; wing span: 17-24 mm; length of forewing: 8.1-11.6 mm. Black dots on forewing from 28 to 62 with the following distribution: 4-9 subcostal, 4-9 radial, 0-6 subradial, 0-5 supramedian, 5-12 submedian, 5-12 subdorsal, and 5-18 on distal 1/4 of the forewing, between radial and submedian. Cilia white, in some specimens greyish, especially on tornus and dorsum. Hindwing from grey to dark grey; cilia pale grey to grey, whitish or pale grey round the apex.


Fudh
104
100
97
96
92
origin
1 2 3 4 5 6 7 8
Hk
100
94
origin
1 2
Pep C-2
100
94
90
88
origin
1 2 3 4 5
Aat-2
100
90
88
origin
1 2 3 4 5
Pep D-1
100
98
97
94
origin
1 2 3 4 5
β-Gluc-3
100
95
90
origin
1 2 3 4

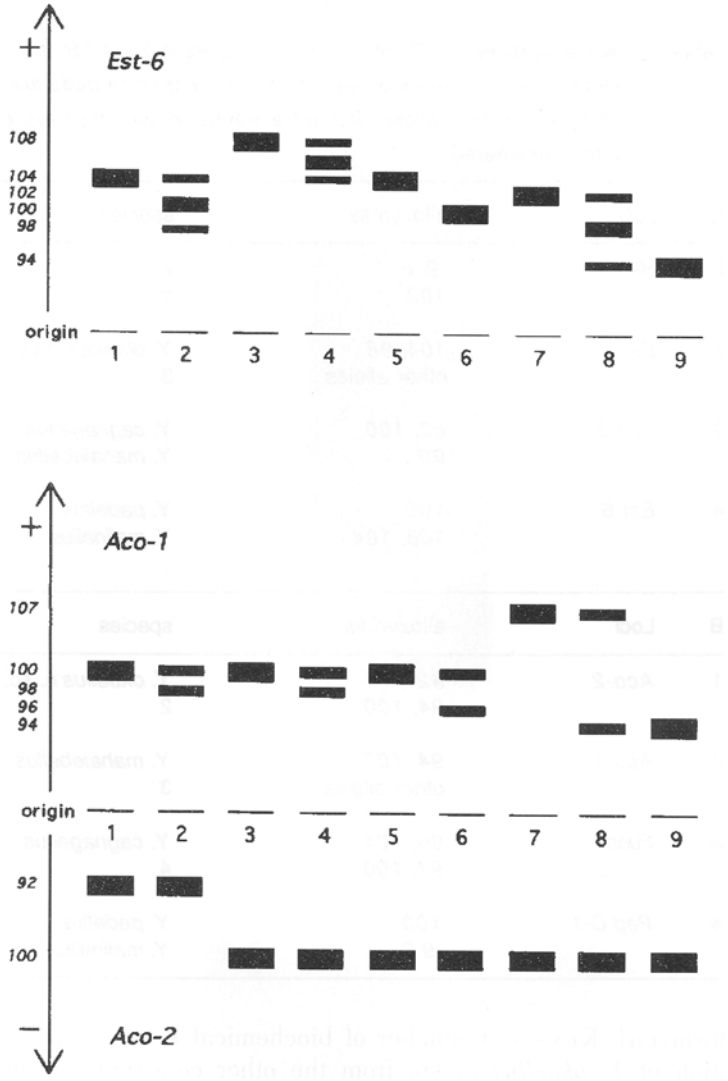


Fig. X - Zymograms observed in the *Yponomeuta padellus* complex at a number of differentiated and diagnostic loci. *Fudh*: 1,2: *Y. okuellus* n. sp., 3,4: *Y. padellus* and *Y. malinellus*, 5,6,7: *Y. cagnagellus*, 8: *Y. mahalebellus*; 1=*97/97*, 2=*92/97*, 3= *100/100*, 4=*97/100*, 5=*96/96*, 6=*96/104*, 7=*104/104*, 8=92/92. *Hk*: 1: *Y. okuellus* n. sp., *Y. cagnagellus*, *Y. mahalebellus*; 2: *Y. padellus* and *Y. malinellus*; 1=*94/94*; 2=*100/100*. *Pep C-2*: 1,2: *Y. okuellus* n. sp., 3: *Y. padellus, Y. malinellus*, *Y. mahalebellus*, 4,5: *Y. cagnagellus*; 1=*88/88*, 2=*88/94*, 3=*100/100*, 4=*94/94*, 5=*90/94*. *Aat-2*: 1: *Y. okuellus* n. sp., *Y. padellus* and *Y. malinellus*, 2,3,4: *Y. cagnagellus*, 5: *Y. mahalebellus*; 1, 2=*100/100*; 3=*88/100*, 4=*88/88*, 5=*90/90*. *Pep D-1*: 1,2,3: *Y. okuellus* n. sp., 4: *Y. malinellus*, *Y. cagnagellus*, *Y. mahalebellus, 5=Y. padellus*; 1=*94/94*, 2=*94/97*, 3=*97/97*, 4=*98/98*, 5=*100/100*. β-*Gluc-3*: 1,2: *Y. okuellus* n. sp., 3: *Y. padellus, Y. malinellus*, *Y. cagnagellus*, 4: *Y. mahalebellus*; 1=*95/95*, 2=*90/95*, 3=*100/100*, 4=*90/90*. *Est-6*: 1,2: *Y. okuellus* n. sp., 3,4,5: *Y. malinellus, 6: Y. padellus* and *Y. mahalebellus*, 7,8,9: *Y. cagnagellus*; 1,5=*104/104*, 2=*98/104*, 3=*108/108*, 4=*104/108*, 6=*100/100*, 7=*102/102*, 8=*94/102*, 9=*94/94*. *Aco-1*: 1,2: *Y. okuellus* n. sp., 3,4=*Y. malinellus, Y. padellus*, 5,6: *Y. cagnagellus*, 7,8,9: *Y. mahalebellus*; 1,3,5=*100/100*, 2,4=*98/100*, 6=*96/100*, 7=*107/107*, 8=*94/107*, 9=*94/94*. *Aco-2*: 1,2: *Y. okuellus* n. sp., 3-9: *Y. padellus, Y. malinellus*, *Y. cagnagellus*, *Y. mahalebellus*. 1,2=*92/92*, 3-9=*100/100*.

Table 7 - Two examples (A, B) of allozyme diagnostic keys for the identification of the members of the *Yponomeuta padellus* complex studied; alleles with a frequency below 0.05 have not been considered.

A	Loci	allozymes	species
1	*Hk*	*9 4*	2
		100	4
2	*Est-6*	*104, 98*	*Y. okuellus* n.sp.
		other alleles	3
3	*Aat-2*	*88, 100*	*Y. cagnagellus*
		90	*Y. mahalebellus*
4	*Est-6*	*100*	*Y. padellus*
		108, 104	*Y. malinellus*

B	Loci	allozymes	species
1	*Aco-2*	*92*	*Y. okuellus* n. sp.
		94, 100	2
2	*Aco-1*	*94, 107*	*Y. mahalebellus*
		other alleles	3
3	*Fudh*	*96, 104*	*Y. cagnagellus*
		97, 100	4
4	*Pep D-1*	*100*	*Y. padellus*
		9 8	*Y. malinellus*

Biochemical Keys - A number of biochemical keys can be made for the identification of *Y. okuellus* n. sp. from the other considered members of the *Y. padellus* complex using allozymes at the loci found diagnostic (Tables 4,5; Fig. 10). Two examples of such allozyme diagnostic keys are given in Table 7. Each key gives a probability of correct identification of above 99%. These two keys, and similar others, can be used either independently or together; in the latter case the probability of misidentification becomes virtually null. Diagnostic keys can be used for the identification not only of the adult specimens, but also of larvae and pupae.

Ecological Observations - The habitats in which *Y. okuellus* n. sp. larvae were found can be described as follows (Toshio Oku, personal communication):

- Morioka: a garden for wild bee culture, surrounded by crop fields, where crab apple trees (*Malus micromalus* Makino, and its hybrids with European *Malus* taxa) are heavily infested by Small Ermine moth larvae, together with the Hall's crab *Malus halliana* Koene (less infested than the former). Other shrubs and

trees present are: Chinese plum (*Prunus salicina* Lindley), *Cercis chinensis* Bunge, *Chaenomeles* spp., *Salix integra* Thunberg. and *Robinia pseudo-acacia* L.

- Kuzakai: southwestern slope of Mt Kabutomyojin in the Kitakami mountain range, where the host plant of *Y. okuellus* n. sp. is the native *Malus sieboldii* (Regel) Rehder (= *M. tringo* Siebold); this crab apple is usually found in thick forests and sporadically grows as shrub or lower tree under higher trees such as *Quercus mongolica* Fisher, *Prunus sargentii* Rehder, *P. grayana* Maximowicz, *Sorbus alnifolia* (Sieb. et Zucc.) C. Kock, *Acer* spp., *Tilia japonica* (Miq.) Simonkai. Near the border of the area *M. sieboldii* intermingles with *Prunus* spp., *Betula platyphylla* Sukatchev, *Corylus* spp., *Salix* spp. *M. sieboldii* is found also in pastures, where it grows as bush at the foot of isolated *Betula* trees, usually together with *Rosa multiflora* Thumb., *Salix* spp., and *Corylus heterophylla* Fisch.

Concluding remarks

In various groups of phytophagous insects, distinct host plant preferences have been found among populations morphologically very similar or even identical. Such populations are frequently designated as "ecotypes" or "biological races" or, when evidence of reproductive isolation in the field becomes available, as sibling species (Mayr, 1970). The latter case is proving to be much more frequent than previously supposed, since the use of genetic methods in systematic biology has allowed an easy detection of distinct gene pools (Coluzzi and Bullini, 1971; Bullini and Sbordoni, 1980; Ferguson, 1980; Bullini and Cianchi, 1984; Paggi and Bullini, 1994). The lack of gene flow between populations can be either directly evidenced by diagnostic markers, when such populations are at least partially sympatric, or, if fully allopatric, indirectly inferred by the finding of levels of genetic divergence similar to, or higher than those found between related, reproductively isolated taxa. An example of the first case is given by the largely sympatric *Y. padellus* (*s. s.*) and *Y. malinellus*, whose specific status was genetically proved by the absence of F_1 hybrids, recombinant and introgressed individuals in the field. As to the presumably allopatric *Y. okuellus* n. sp., its specific status is inferred by the pattern of its genetic relationships with the other considered members of the *Y. padellus* complex , with lack of a direct link between this species and the cluster formed by *Y. malinellus-Y. padellus-Y. cagnagellus* (Figs. 1,2). On the other hand, the eventual coexistence of *Y. okuellus* n. sp. and *Y. malinellus* (e.g. owing to passive transport phenomena) is expected to enhance interspecific competition, as they share the same host plants, hence leading to exclusion (in agreement with Gause principle) or to character displacement.

A Japanese origin for *Y. malinellus*, suggested by various authors (e.g., Matsamura, 1931; Inoue, 1954; Issiki, 1957), is apparently ruled out by genetic data.

ACKNOWLEDGMENTS.- We wish to thank most sincerely Dr Toshio Oku, who since 1988 has been kindly collecting and sending us the Japanese specimens of Small Ermine moth studied in the present paper; Professor Steph B. J. Menken for helpful discussions; Dr Rossella Cianchi for her critical comments to the manuscript; Professor Luigi Masutti for his kind advises; Professor Egidio

Mellini for his friendly and constant encouragment; Dr Niccolo' Falchi for drawing the figures.

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SUMMARY

Data are reported on the genetic and morphological differentiation between *Yponomeuta* populations from Japan, till now assigned to *Y. malinellus*, and various members of the *Y. padellus* complex from Europe (*Y. padellus sensu stricto*, *Y. malinellus*, *Y. cagnagellus* and *Y. mahalebellus*). On the basis of allozyme variation at 35 loci, the Japanese taxon is shown to be a new, genetically distinct, member of the *Y. padellus* complex, sharing the same host plants (genus *Malus*) with *Y. malinellus*. The new Small Ermine moth from Japan, named *Y. okuellus* n. sp. from its collector Dr Toshio Oku, is genetically similarly differentiated (Nei's *D* about 0.30) from European *Y. malinellus* and *Y. mahalebellus*. No direct genetic link is apparent between *Y. okuellus* and the cluster formed by Y. *padellus-Y. malinellus-Y. cagnagellus*. The suggested Japanese origin for *Y. malinellus* is ruled out by genetic evidence. Allozyme diagnostic keys are provided for the identification of *Y. okuellus* n. sp. from the other considered members of the *Y. padellus* complex, both adults, larvae and pupae. At the morphological level, *Y. okuellus* n. sp. shows a significantly different number and pattern of distribution of black dots on forewing (from 28 to 62), which in some specimens are present also on the subradial (2-6) and supramedian (2-5) veins, resembling *Y. evonymellus* in this respect. Other distinctive characters are the darker colour of the pupa and the very loosely woven cocoon. A description of the new species is provided, together with some ecological data (e.g. habitat, food plants).

Una nuova specie del complesso *Yponomeuta padellus* in Giappone, finora attribuita a *Y. malinellus* (Lepidoptera, Yponomeutidae).

RIASSUNTO

Viene analizzato il differenziamento genetico e morfologico tra popolazioni di *Yponomeuta* del Giappone, finora attribuite a *Y. malinellus*, e vari membri europei del complesso *Y. padellus* (*Y. padellus sensu stricto*, *Y. malinellus*, *Y. cagnagellus* e *Y. mahalebellus*). La struttura genetica delle varie entita' e' stata analizzata mediante elettroforesi di 35 loci enzimatici. Viene dimostrato che il taxon giapponese e' un nuovo membro del complesso *Y. padellus*, geneticamente distinto, che condivide con *Y. malinellus* le stesse piante ospiti (genere *Malus*). La nuova specie, chiamata *Y. okuellus* n. sp. dal nome del suo raccoglitore Dr Toshio Oku, risulta altrettanto differenziata geneticamente (*D* secondo Nei circa 0.30) da *Y. malinellus* e *Y. mahalebellus*. Non e' stato evidenziato un legame genetico diretto tra *Y. okuellus* n. sp. e il cluster *Y. padellus-Y. malinellus-Y. cagnagellus*. L'ipotizzata origine di *Y. malinellus* dal Giappone e' esclusa dai dati genetici. Vengono fornite chiavi diagnostiche allozimiche per l'identificazione di *Y. okuellus* n. sp. dagli altri membri del complesso considerati, utilizzabili sia allo stadio adulto che a quelli preimaginali. A livello morfologico, *Y. okuellus* n. sp. risulta significativamente differenziato per il numero e la disposizione delle macchiette nere sull'ala anteriore (da 28 a 62); in alcuni esemplari tali macchiette sono presenti anche in posizione subradiale (2-6) e sopramediana (2-5), come in *Y. evonymellus*. Altri caratteri che distinguono *Y. okuellus* n. sp. sono la colorazione piu' scura della pupa e il bozzolo estremamente rado. Viene fornita una descrizione della nuova specie ed alcune osservazioni ecologiche sul suo habitat e sulle sue piante alimentari.

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