Sex pheromones and their potential role in the evolution of reproductive isolation in small ermine moths (Yponomeutidae)

Christer Löfstedt[1], Wim M. Herrebout[2], and Steph B. J. Menken[3]

[1] Department of Ecology, Lund University, S-22362 Lund, Sweden
[2] Division of Systematics and Evolutionary Biology, University of Leiden, P.O. Box 9516, NL-2300 RA Leiden, The Netherlands
[3] Institute of Taxonomic Zoology, University of Amsterdam, P.O. Box 4766, NL-1009 AT Amsterdam, The Netherlands

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Summary

Sex pheromone communication in the nine European species of small ermine moths (*Yponomeuta*) is reviewed in regard to the potential role of pheromones in the speciation process. Six of the nine species studied (viz., *Y. evonymellus, Y. cagnagellus, Y. padellus, Y. irrorellus, Y. plumbellus,* and *Y. vigintipunctatus*) use a mixture of (*E*)-11- and (*Z*)-11-tetradecenyl acetate in different ratios as primary pheromone components, with combinations of tetradecyl acetate, (*Z*)-9-tetradecenyl acetate, (*Z*)-11-hexadecenyl acetate and the corresponding alcohols of the acetates as additional pheromone components. Analysis of (*Z*)- to (*E*)-11-tetradecenyl acetate ratios produced by individual females of these species demonstrated significant variation among females of all species. However, the ranges of ratios produced by *Y. cagnagellus, Y. irrorellus,* and *Y. plumbellus*, sharing the same host-plant species, spindle tree, did not overlap. Niche separation of all six species mentioned required consideration of at least one additional pheromone component or of temporal aspects. The remaining three species, *i.e. Y. malinellus, Y. mahalebellus* and *Y. rorellus*, have pheromones that differ qualitatively.

Biosynthetic routes to the pheromone components identified are proposed on the basis of fatty acid pheromone precursors found in the pheromone glands. A phylogenetic tree for the genus is constructed based on allozyme frequency data and changes in pheromone composition are superimposed on this tree. We suggest that the ancestral ermine moth pheromone is a mixture of (*Z*)-11- and (*E*)-11-tetradecenyl acetate and the corresponding alcohols, and a scenario of how present-day patterns evolved is outlined. The pheromone differences among the three species using spindle tree as their host-plant might have evolved through *reproductive character displacement* upon secondary contact between populations that had already diverged genetically in allopatry. Pheromone differences within the so-called *padellus*-complex (including *Y. cagnagellus, Y. mahalebellus, Y. malinellus, Y. padellus*, and *Y. rorellus*) in which species might have originated sympatrically, may have evolved by *reinforcing selection* as these species still hybridise and produce viable offspring when confined in cages. The role of pheromones in reproductive isolation among *Yponomeuta* species is emphasised by (1) the function of pheromone components of some of the species as behavioural antagonists to other species, (2) the cross-attraction under experimental conditions between allochronic species with similar pheromones, and (3) the formation of hybrids in the laboratory between species that are isolated in nature by pheromone differences.

Key words

speciation, reinforcement, character displacement, biosynthesis, phylogeny, sex pheromones, reproductive isolation (*Z*)-11-tetradecenyl acetate, (*E*)-11-tetradecenyl acetate, Lepidoptera, Yponomeutidae, *Yponomeuta*

Introduction

In order to coexist, biological species need to be reproductively isolated. Consequently, the evolution of reproductive isolation is generally regarded as a key step in speciation. The generally accepted mode of speciation in sexually reproducing animals requires that populations initially are geographically isolated from each other for many generations (Mayr 1963). During this period of time different selection pressures together with random changes will result in significant genetic differences between the populations with reproductive isolation as a possible by-product. If the potential hybrids formed upon secondary contact are inferior, then effective *premating* reproductive isolation may evolve provided that the populations are ecologically differentiated (Littlejohn 1981). This is the model of *speciation by reinforcement.* However, recent theoretical studies suggest that the efficacy of reinforcing selection is low and furthermore there is a lack of well-substantiated empirical examples (Butlin 1987, 1989).

In moths the primary means of premating reproductive isolation are species-specific sex pheromones, emitted by females to attract males for mating. Paterson (1978,

Tab. 1 European small ermine moths of the genus *Yponomeuta* and their host plant affiliations

Yponomeuta species	Host-plant	Plant family
Y. evonymellus (L.)	*Prunus padus* L.	Rosaceae
Y. cagnagellus (Hübner)	*Euonymus europaeus* L.	Celastraceae
Y. mahalebellus Guenée	*Prunus mahaleb* L.	Rosaceae
Y. malinellus Zeller	*Malus* sp.	Rosaceae
Y. padellus (L.)	*Crataegus* sp. *Prunus spinosa* L. *Prunus domestica* L. *Sorbus aucuparia* L.	Rosaceae
Y. rorellus (Hübner)	*Salix* sp.	Salicaceae
Y. irrorellus (Hübner)	*Euonymus europaeus* L.	Celastraceae
Y. plumbellus (Denis & Schiffermüller)	*Euonymus europaeus* L.	Celastraceae
Y. vigintipunctatus (Retzius)	*Sedum telephium* L.	Crassulaceae

1985) has stressed that divergence in specific mate recognition systems (SMRS) presents a problem as SMRS function primarily to achieve efficient mating within species and are therefore expected to be under stabilising selection. Challenging the model of speciation by reinforcement Paterson claims that reproductive isolation mechanisms do not evolve because of selection for reproductive isolation, but are mere by-products of selection for SMRS. In the present paper we discuss the potential role of sex pheromones in the evolution of reproductive isolation within a group of moths.

European small ermine moths of the genus *Yponomeuta* (Lepidoptera, Yponomeutidae) have been studied for more than a decade in regard to their evolution and their host relations in particular (Wiebes 1976; Menken *et al.* 1991). Nine species of small ermine moths occur in the western Palearctic region (Table 1). Traditionally some forms presented great difficulties to the taxonomist, and in the laboratory members of this so-called *"padellus*-complex" (*viz., Y. cagnagellus, mahalebellus, malinellus, padellus,* and *rorellus*) will hybridise and produce offspring, the fertility and fecundity of which differs by combination (Menken 1980; Van Drongelen & Van Loon 1980; Hendrikse 1988).

The sex pheromones of 7 of the European small ermine moths have been identified (Löfstedt & Van Der Pers 1985; Löfstedt et al. 1986a; Löfstedt & Herrebout 1988). In the present paper pheromone communication in *Yponomeuta* is reviewed and new information on pheromone precursors and individual variation in female pheromone production is presented. A phylogenetic tree is constructed based on allozyme variation patterns and biosynthetic routes to the identified pheromone components are proposed based on analysis of pheromone precursors from 9 species. Together with available information on morphology and host-plant selection this allows us to discuss the potential role of sex pheromones in speciation and the evolution of reproductive isolation in the ermine moths.

Pheromone communication in the small ermine moths

Our analysis of sex pheromones in the small ermine moths has been guided by a simple model of niche separation in the imaginary *sex communication channel* (terminology of Greenfield & Karandinos 1979). Moths that are dependent on this "resource" for their mate finding are separated in one or more chemical, temporal or spatial niche dimensions. If the species do not differ in the chemical composition of their pheromones, they are selected to be sexually active at different times of the day or the year, or they are restricted to different geographical areas. The European small ermine moths are sympatric and synchronic, *i.e.* fly at the same time of year/day, over much of their distribution area. Females of the small ermine moths display their pheromone gland in a typical calling posture, indicative of pheromone release, around the onset of the photophase (with the exception of *Y. plumbellus* and *Y. vigintipunctatus* that call preferentially at night). Thus, periods of calling overlap more or less in all of the species and so do periods of male receptivity (Hendrikse 1978, 1979).

Gas chromatographic analyses of pheromone glands from calling female moths of *Y. evonymellus, Y. cagnagellus, Y. padellus, Y. irrorellus, Y. plumbellus,* and *Y. vigintipunctatus* showed that all have (*Z*)-11-tetradecenyl acetate (Z11-14:OAc) as a primary pheromone component (Fig. 1) (Löfstedt & Van Der Pers 1985; Löfstedt & Herrebout 1988). A graphical model of niche overlap in the sex communication channel between these species was obtained by construction of a niche diagram from analysis of pheromone production by individual females (Löfstedt 1986). The species differ more or less with respect to the amount of complementary (*E*)-11-tetradecenyl acetate (E11-14:OAc) (Fig. 2), but to separate *Y. padellus* from *Y. vigintipunctatus* and *Y. evonymellus* it is necessary to consider additional pheromone components. (*Z*)-11-hexadecenyl acetate (Z11-16:OAc) is not only a necessary pheromone component for the attraction of *Y. padellus*, but it has a negative influence on the attraction of other sympatric ermine moths (Löfstedt & Van Der Pers 1985; Löfstedt 1987; C. Löfstedt unpubl.). However, the remaining species pair, *Y. evonymellus* and *Y. vigintipunctatus*, cannot be

separated on the basis of differences in their female-produced sex pheromones (Figs 1 and 2).

All species have additional acetates in their glands. For instance (Z)-9-tetradecenyl acetate (Z9-14:OAc) is an important fourth pheromone component in *Y. padellus* (C. Löfstedt unpubl.). All of the species seem to store the saturated 14:OAc and 16:OAc,and in at least *Y. evonymellus, Y. cagnagellus, Y. irrorellus,* and *Y. vigintipunctatus* 14:OAc has a clear behavioural effect (Fig. 3 and Löfstedt & Herrebout 1988). This might also be the case in *Y. plumbellus,* whereas 14:OAc seems to be behaviourally neutral in *Y. padellus* (C. Löfstedt unpubl.). The activity of 16:OAc is obscure in most of the species, except for *Y. irrorellus* where it seems to be a behaviourally active pheromone component (Löfstedt & Herrebout 1988). A small amount of all of the alcohols corresponding to the acetates are usually found in gland extracts, and they do have a clear synergistic effect on attraction of male *Y. evonymellus, Y. irrorellus,* and *Y. vigintipunctatus* (Löfstedt & Van Der Pers 1985; Löfstedt & Herrebout 1988), and possibly also *Y. cagnagellus* (de Jong 1987). Interestingly, subtraction of any of the five components of the synthetic pheromones of *Y. evonymellus* and *Y. vigintipunctatus* seems to reduce their attractivity (Fig. 3) and thus confirms their status as pheromone components in the respective species. At the same time it may be noted that subtraction of one component from the synthetic pheromones in several cases causes significant atraction of other moth species. For instance, subtraction of Z11-14:OH from the *Y. vigintipunctatus* pheromone results in significant attraction of a tortricid moth, *Aphelia paleana* (Hübner), whereas subtraction of Z11-14:OAc causes attraction of *Dichrorampha petiverella* (L.). Subtraction of E11-14:OAc from the *Y. evonymellus* pheromone elicits attraction of another tortricid, *Tortrix viridana* (L.).

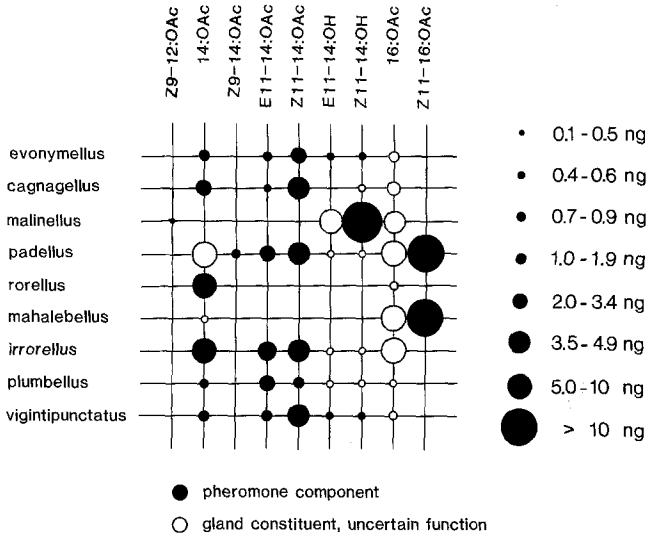

Fig. 1 Pheromone components of nine European small ermine moths. The diameter of a dot is approximately proportional to the gland titre of an individual pheromone component. Filled circles indicate components with confirmed behavioural activity in the respective species

Y. cagnagellus, Y. irrorellus, and *Y. plumbellus* coexist on the same host-plant, the European spindle tree *Euonymus europaeus* (Celastraceae), and share the same pheromone components. Their pheromones differ in the ratio of E11-14:OAc to Z11-14:OAc (2, 60 and 150% resp., Fig. 2). Moreover, the ten times lower pheromone release rate in *Y. plumbellus* seems to contribute to pheromone specificity (Löfstedt & Herrebout 1988). Hardly any cross-attraction is observed with synthetic pheromones.

The last three species, *Y. rorellus, Y. mahalebellus,* and *Y. malinellus,* differ in their pheromones from the six species already described in a number of ways. In general, the pheromone gland secretions of these species contain a reduced number of acetates and alcohols of potential relevance

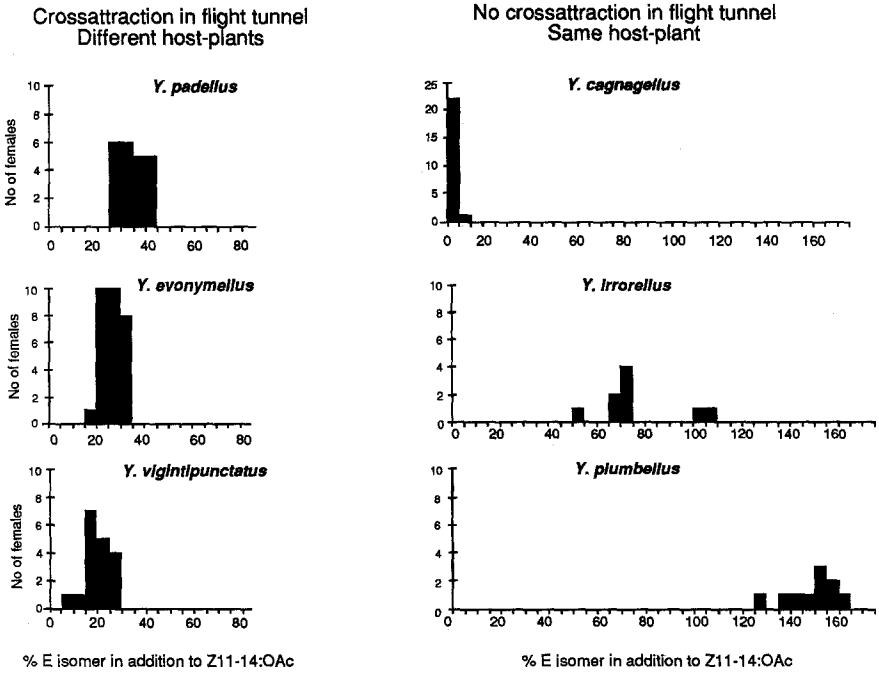

Fig. 2 Frequency diagrams of (*E*)- to (*Z*)-11-tetradecenyl acetate ratios produced by individual females of six species of small ermine moths (*Yponomeuta*). Individual female gland extracts were analysed by gas chromatography on capillary columns to determine the ratio of the geometric isomers

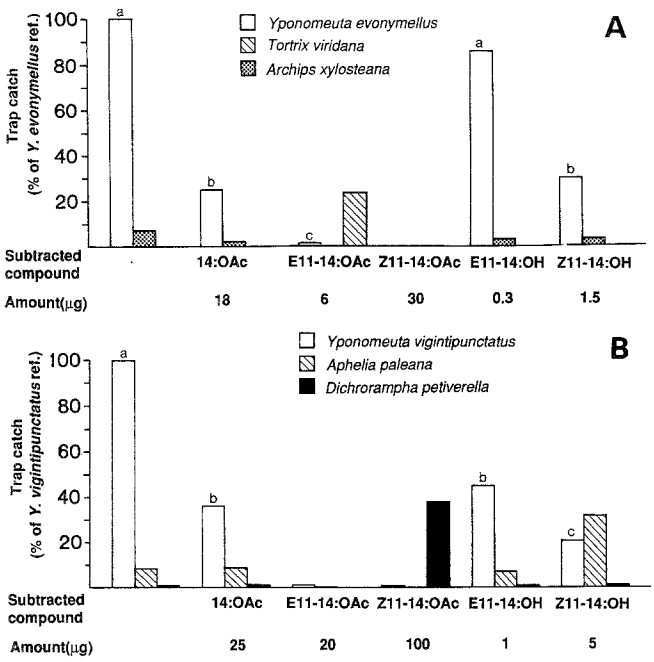

Fig. 3 Mean relative trap catches with synthetic pheromones for *Y. evonymellus* (A) and *Y. vigintipunctatus* (B), and corresponding blends with individual pheromone components subtracted. Catches of four tortricid moths, *Tortrix viridana* and *Archips xylosteana* in (A) and *Aphelia paleana* and *Dichrorampha petiverella* in (B), are also shown. The trap catch of the respective *Yponomeuta* species with the full synthetic mixture is 100 by definition in each series. Bars accompanied by the same letter are not significantly different based on ANOVA of log (catch + 1) followed by Fisher's protected LSD method for multiple comparisons ($P<0.05$). The experiment with *Y. evonymellus* was carried out at Dalby Norreskog, Lund, Sweden, July 11 to August 7, 1985 (n=5). Average trap catch of *Y. evonymellus* with the reference bait was 70 males. The experiment with *Y. vigintipunctatus* was carried out at Hagesta, Ystad, Sweden, August 8 to 29, 1985 (n=6). Average trap catch of *Y. vigintipunctatus* with the reference bait was 82 males

to sexual attraction. *Y. rorellus* has the most simple pheromone bouqet. Calling females of this species release a mixture of 14:OAc, 14:OH, 16:OAc, and 16:OH, but 14:OAc on its own suffices to evoke complete pheromone response in males (Löfstedt *et al.* 1986a, 1990). In this species addition of a small amount Z11-14:OAc, the primary pheromone component of most other sympatric *Yponomeuta*, reduces attraction dramatically. The pheromone glands of *Y. mahalebellus* produce three 16-carbon acetates and 14:OAc, but no clear evidence of any unsaturated 14-acetates was found (C. Löfstedt unpubl.). Analysing *Y. malinellus* females, we found only alcohols in their pheromone glands and a mixture of 14:OH, E11-14:OH, and Z11-14:OH attracts some males in the field (W. M. Herrebout & C. Löfstedt unpubl.). However, McDonough *et al.* (1990) recently reported (*Z*)-9-dodecenyl acetate (Z9-12:OAc) to be present in trace amounts in pheromone gland extracts of *Y. malinellus,* which were introduced into North America some years ago (Herrebout & Menken 1990), and a mixture of Z11-14:OH and Z9-12:OAc was a more attractive lure than virgin females in field trapping experiments. The occurrence of Z9-12:OAc as a pheromone component in *Y. malinellus* is remarkable as no 14 carbon acetates have been found in this species although the potential precursors (corresponding alchohols) are present in large amounts. Generally, acetylation of alcohols in moth pheromone glands is believed to be a non-selective reaction (Bestmann *et al.* 1987; Jurenka & Roelofs 1989), whereas acetylation in *Y. malinellus* has to be highly chain-length specific to produce Z9-12:OAc only. Alternatively Z9-12:OAc may be a substitute for some other alcohol or aldehyde pheromone component in *Y. malinellus.*

The role of E11-14:OAc, Z11-14:OAc, and Z11-16:OAc as primary pheromone components in the small ermine moths could be predicted from the electrophysiological investigations of Van Der Pers (1982). He found receptor cells specialised to E11- and Z11-14:OAc in 8 out of the 9 species studied. In *Y. malinellus* one receptor cell responded to E11-14:OAc but responded even more strongly to Z11-14:OH. Cells responsive to Z11-16:OAc occurred in *Y. cagnagellus, Y. padellus, Y. rorellus,* and *Y. mahalebellus* (Fig. 4).

The results of our chemical analyses and behavioural experiments with synthetic pheromones explain almost every aspect of the observations reported by Hendrikse (1986). She used a flight tunnel assay to study intra- and interspecific attraction of male ermine moths to calling females. Between 50 and 90% of the males were able to locate their conspecific females (Fig. 5). The highest level of mutual cross-attraction was observed between *Y. evonymellus* and *Y. vigintipunctatus* (50–60%), the species with the closest similarity of pheromones. However, it is interesting to note that the synthetic pheromones of these species, in spite of their almost identical composition, in our field experiments were species-specific in the natural habitat of the respective species, *i.e.* no *Y. vigintipunctatus* males were trapped in the *Y. evonymellus* experiments and *vice versa* (Fig. 3). Hendrikse (1986) also found significant (mutual) cross-attraction (*i.e.,* more than 15%) between *Y. evonymellus* and *Y. irrorellus, Y. evonymellus* and *Y. padellus,* and *Y. vigintipunctatus* and *Y. irrorellus.* In the remaining interactions there was no cross-attraction.

The cross-attraction between *Y. padellus* females and *Y. evonymellus* and *Y. vigintipunctatus* males does not follow logically from our our experiments with synthetic pheromones. When synthetics were tested in the field the large amount of Z11-16:OAc in the *Y. padellus* pheromone seemingly reduced the attraction of *Y. evonymellus* and *Y. vigintipunctatus* males to zero (Löfstedt & Van Der Pers 1985; Löfstedt 1987).

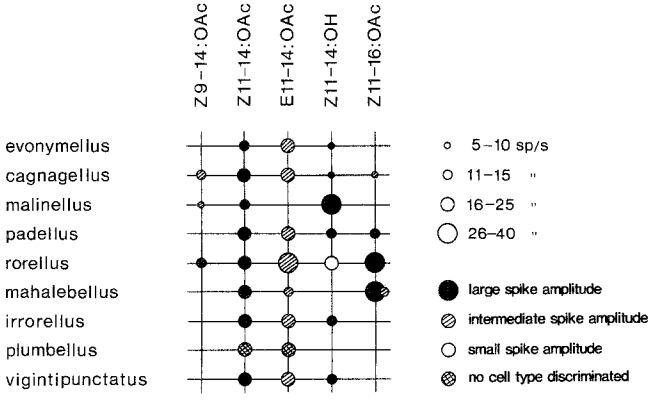

Fig. 4 Electrophysiological single cell response of antennal sensilla trichodea with low spontaneous activity to pheromone components in males of nine *Yponomeuta* species (modified from Van Der Pers 1982)

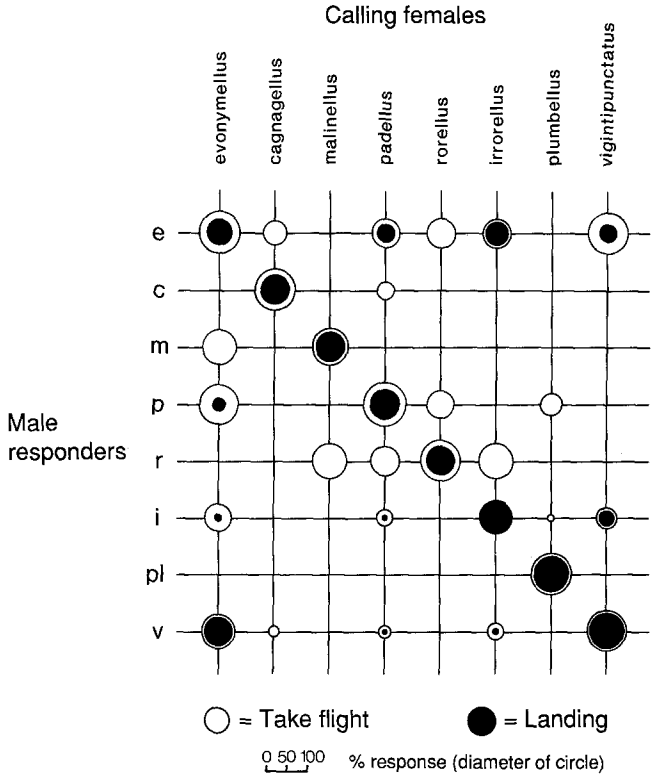

Fig. 5 Conspecific and interspecific behavioural response of males of 8 *Yponomeuta* species to calling females (constructed from Hendrikse 1986)

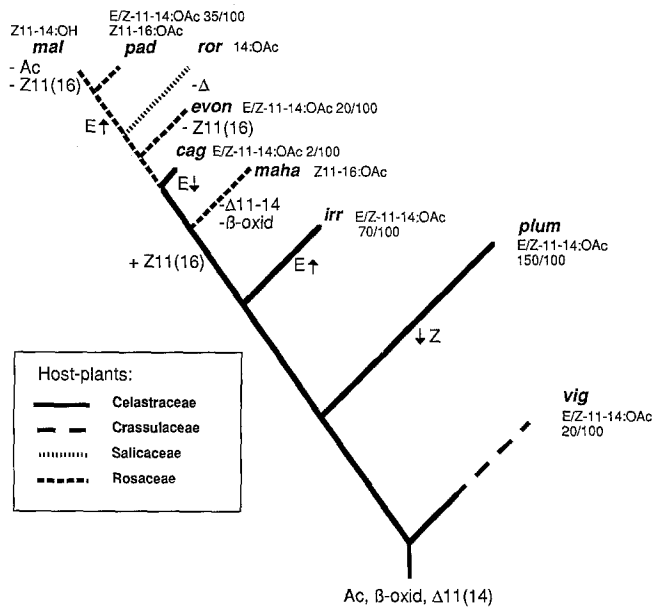

Fig. 6 Proposed shifts in pheromone composition and host plants superimposed on a phylogenetic tree constructed using "Jelly" (courtesy of W. N. Ellis, University of Amsterdam), a modified version of Rogers' (1984) algorithm, portraying the evolution of reproductive isolation in the European small ermine moths. The cophenetic correlation coefficient is 0.984, its F value 30.9 %. −denotes lost character, +denotes acquired character, E ↑/↓ denotes increased/decreased amount of E isomer, Δ11 (14C) denotes delta 11 desaturation of 14 carbon acyl moiety, Ac denotes acetylation, β-oxid stands for β-oxidation (chain-shortening), *etc.*

The taxonomic status of small ermine moths and their phylogeny

All nine species have been studied by allozyme analysis at more than 50 larval and adult loci (Menken 1982). From the absence of heterozygotes at diagnostic loci in samples taken from sympatry and the species-specific allozyme patterns, it was concluded that all nine are genuine biological species (Menken 1980, 1989). Locally, however, a very low amount of gene flow between *Y. padellus* and *Y. malinellus* might occur (Arduino & Bullini 1985). The specific status of the nine forms of *Yponomeuta* is also corroborated by recent work on morphological characters (Povel 1984, 1986). Numerical taxonomic studies, based on over a hundred continuous characters, showed that seven of nine species could be distinguished as separate clusters. After removing redundant characters, the remaining two species, *Y. padellus* and *Y. malinellus*, also tend to cluster separately (Povel 1986). From the original allozyme data (Menken 1982) a phylogenetic tree was constructed (Fig. 6) by means of a modified version of Rogers' algorithm (Rogers 1984), using *Y. vigintipunctatus* as the outgroup (Menken *et al.* 1991). The position of *Y. evonymellus* within the group of species that was called the *padellus*-complex by Friese (1960) underscores the fact that this group of species, in spite of morphological similarities among its members, is an artificial taxonomic entity.

All European *Yponomeuta* forms should also be regarded as good biological species by virtue of the differentiation among their sex communication systems. The differences in female-produced sex pheromones make it likely that all are reproductively isolated from one another at the premating level with the possible exception of *Y. evonymellus* and *Y. vigintipunctatus*. However, these two species are fixed for different alleles at some 90 % of their allozyme loci, so postmating reproductive isolation should be complete even if premating isolation fails.

Pheromone differences and similarities *per se* are not necessarily useful for the analysis of phylogenetic relationships. Large differences in pheromone attractivity might be due to small differences in, for instance, ratios between the same compounds, and such ratios could change back and forth due to selection for premating reproductive isolation. As Roelofs & Bjostad (1984) postulated, biosynthetic routes to pheromones should be a more useful criterion for elucidation of evolutionary relationships (see also Löfstedt 1991). We therefore analysed the occurrence of fatty acid pheromone precursors in the pheromone glands of the small ermine moths.

Biosynthesis of the ermine moth pheromones

All of the pheromone components identified from the small ermine moths are monounsaturated or saturated, 12-carbon, 14-carbon or 16-carbon acetates or alcohols. The great similarity between the pheromone gland constituents indicates that they are all of a common biosynthetic origin. It has been shown in several other moths that pheromone components are biosynthesized from corresponding fatty acids (Roelofs & Bjostad 1984), which are derived from pal-

Fig. 7 Fatty acids (potential pheromone precursors) identified from 9 species of European small ermine moths. The size of a dot is approximately proportional to the relative amount of the respective acyl moiety in the gland

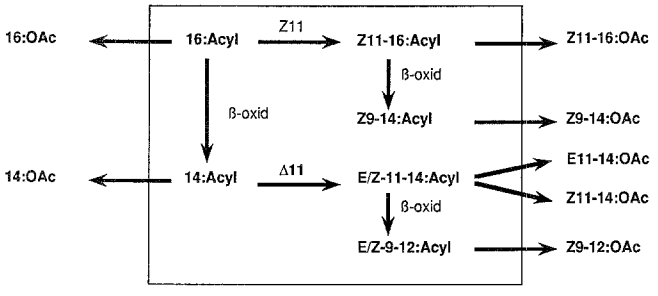

Fig. 8 Proposed biosynthetic routes to pheromone components identified from European small ermine moths. The pheromone biosynthesis starts from the ubiquitous palmitic acid (16:Acyl). Unusual fatty acids are synthesised by Δ11-desaturation and chain-shortening (within the frame). The fatty acids are selectively reduced and acetylated to give alcohols and acetates, active as pheromone components

mitic acid by a combination of Δ11-desaturation and chain shortening (beta-oxidation). We analysed the fatty acid composition of pheromone gland extracts of all nine small ermine moths to obtain information on what kind of pheromone compounds could potentially be biosynthesized by these species. Fatty acyl moieties in the extracts were converted to methyl esters, which were analysed by gas chromatography and identified by correspondence of their retention times with those of synthetic references (Fig. 7; see, *e.g.*, Löfstedt *et al.* 1986b for a detailed description of methods).

All of the species contain a number of common 16- and 18-carbon fatty acyl moieties; hexadecanoate, (*Z*)-7-hexadecenoate, (*Z*)-9-hexadecenoate, octadecanoate, (*Z*)-9-octadecenoate, (*Z,Z*)-9,12-octadecadienoate, and (*Z,Z,Z*)-9,12,15-octadecatrienoate. In addition, all species seem to store tetradecanoate (14:acyl) and (*Z*)-9-tetradecenoate (Z9-14:acyl). The Δ9-unsaturated monoenes could be produced by the ubiquitous Δ9-desaturase. More interesting then is the occurrence of Δ11-unsaturated acyl groups in most of the ermine moth pheromone glands (Fig. 7). Δ11-desaturases have been suggested to be unique to pheromone biosynthesis in Lepidoptera (Roelofs & Bjostad 1984). (*Z*)-11-hexadecenoate (Z11-16:acyl) occurs in *Y. cagnagellus, Y. padellus,* and *Y. mahalebellus*. (*E*)-11- and (*Z*)-11-tetradecenoate (E11- and Z11-14:acyl) occur in all species with the exception of *Y. mahalebellus* and *Y. rorellus*. The likely biosynthetic routes to the ermine moth pheromones involving the fatty acid intermediates mentioned are shown in Figure 8.

There is a general correspondence between the presence of unusual fatty acyl groups in the pheromone glands of all ermine moths (Fig. 7) and the production of pheromone components (Fig. 1). However, the presence of a certain fatty acyl precursor does not necessarily mean that the corresponding alcohol or acetate is produced. Thus we have not been able to demonstrate Z11-16:OAc in *Y. cagnagellus* although there seems to be a large pool of Z11-16:acyl available. Z9-14:acyl has been found in all of the species, but we have only been able to demonstrate Z9-14:OAc in *Y. padellus*. In both cases selectivity of the reductase responsible for the production of an alcohol/acetate from the acyl moiety might explain these findings. In the case of Z9-14:OAc there might also be different pools of Z9-14:acyl, one produced by Δ9-desaturation of 14:acyl and the other produced by chain-shortening of Z11-16:acyl. The second pool might be the only one available for pheromone biosynthesis, as has been found in the cabbage looper moth *Trichoplusia ni,* where Z9-16:-, Z7-14:-, and Z5-12:acyl moieties involved in pheromone biosynthesis come from Δ11-desaturation of stearic acid followed by chain shortening, and not from the large pool of Z9-16:acyl produced by Δ9-desaturation of palmitic acid (Bjostad & Roelofs 1983).

Whereas Δ11-desaturation of 16:acyl only produces Z11-16:acyl, Δ11-desaturation of 14:acyl produces both the E- and Z-isomers. The reason for this is not known. One explanation could be that there is a specific E11-desaturase. Another explanation could be that the desaturase interacting with 16:acyl produces only the Z11 product, whereas another enzyme interacting with the 14:acyl produces a mixture of E and Z isomers. The occurrence of two or more specific Δ11-desaturases has been postulated by Wolf & Roelofs (1987) in some other Lepidoptera. Regulation of the E/Z-ratio is a critical step in the biosynthesis of the species-specific sex pheromones (Bjostad & Roelofs 1986; Roelofs & Wolf 1988). We found no precise correspondence between the isomer ratios among the acetates and their acid precursors. Thus at least some of the differences could be due to species-specific reduction of the acids to alcohols, as the ratios between pheromone gland alcohols usually corresponds well to the ratios between the acetates.

The results of the precursor analysis underline the biochemical similarity of the small ermine moth pheromones. The limited number of differences in distinct biochemical reaction steps unfortunately provides low resolution when pheromone precursors are used for phylogenetic analyses. It appears that the phylogenetic tree constructed on allozyme data may not be the most parsimonious with respect to either pheromone changes or host-plant shifts. For instance, it seems as if Z11 desaturation of hexadecanoic acid may have been lost several times in our tree and that shifts from the Celastraceae to the Rosaceae as host-plants have taken place more than once. However, this may very well have been the case. Certain biosynthetic steps can probably be turned on and off by mutations in regulatory genes, without involving the actual

loss or acquisition of the structural genes involved, and there is evidence that a shift in host from (ancestral) *Euonymus* to *Prunus* is not so difficult (Peterson *et al.* 1990). Furthermore there is a remarkable parallelism in the pattern of parasitoid host suitability and the phylogenetic tree based on allozyme variation. All species that are unsuitable as hosts for *Diadegma armillata* (Gravenhorst) (*i.e. Y. cagnagellus, Y. mahalebellus* and *Y. plumbellus*), have diverged relatively early in the evolution of the genus according to the tree. The more recently evolved *Yponomeuta* show a reduced or lost ability to encapsulate eggs of *D. armillata*. Apparently, after divergence of *Y. cagnagellus* a change in the common ancestor of *Y. evonymellus, Y. padellus, Y. malinellus* and *Y. rorellus* has made them suitable as hosts for the parasitoid (Dijkerman 1990). Thus, our further argument is based on the calculated phylogenetic tree, which we believe is the best available for *Yponomeuta*.

Pheromones, reproductive isolation and the process of speciation

Pheromone changes can be involved in the speciation process in different ways. They may be (one of) the key events giving rise to separation of populations in sympatry, or they may bring about complete isolation between populations, which have already diverged in allopatry. Under disruptive selection for the manner in which a population exploits its resources, a stable polymorphism might be built up with initially high levels of gene exchange between the diverging groups. If there is ecological specialisation, reinforcement due to increased assortative mating might occur, eventually leading to speciation (see Tauber & Tauber 1989 for an overview). Alternatively, pheromone differences may arise as by-products of selection for specific mate recognition systems (SMRS). When two moth populations meet after a period of divergence in allopatry they may still have sufficiently similar sex pheromones to allow cross-attraction and interpopulational matings. If the populations already have diverged to the point where inviable or sterile offspring are being formed, character displacement may reduce the wastage of reproductive effort (Butlin 1987).

With the phylogenetic tree as a background, we can outline a scenario of pheromone changes during the evolution of European small ermine moths (Fig. 6): The similarity of the pheromones in *Y. irrorellus, Y. plumbellus*, and *Y. vigintipunctatus* suggests that a mixture of 14:OAc, Z11-14:OAc, E11-14:OAc, and their corresponding alcohols is a primitive ermine moth pheromone. Reproductive character displacement, resulting in diverging E/Z-ratios, probably took place when the ancestral species of *Y. irrorellus* and *Y. plumbellus* diverged. The very low amount of E-isomer in the *Y. cagnagellus* pheromone evolved in the same way. The pheromone of *Y. evonymellus* is almost identical to that of *Y. vigintipunctatus*. The fact that these species occurred in different habitats, and have only partly overlapping generations and periods of sexual activity (Hendrikse 1978, 1979; Herrebout *et al.* 1976) explains the absence of disruptive selection on the pheromone composition.

The pheromone differences within the so-called "*padellus*-complex" probably contributed to the speciation process, as viable offspring can still be produced among these species. *Y. malinellus, Y. mahalebellus,* and *Y. rorellus* all seem to have lost pheromone components in comparison with their close relatives. *Y. malinellus* does not produce 14-carbon acetates (no acetylation), *Y. mahalebellus* has no unsaturated 14-carbon compounds (no 14-carbon chain Δ11-desaturation), and *Y. rorellus* has no unsaturated pheromone components at all (Δ11-desaturation lost completely). Our analyses of biosynthetic precursors confirm the loss or suppression of critical biosynthetic routes in these species. The occurrence of receptors of E11- and Z11-14:OAc on the male antennae of all European small ermine moths (Van Der Pers 1982), including *Y. rorellus*, supports the interpretation that the *Y. rorellus* pheromone evolved from a more complicated pattern by loss of unsaturated pheromone components. It is interesting to note the antagonistic effect of Z11-14:OAc on the attraction of *Y. rorellus*. In contrast, some other tetradecenyl analogues (including E6-14:OAc, E7-14:OAc, E12-14:OAc and Z12-14:OAc, which have not been reported as pheromone components of any sympatric species) were as attractive to *Y. rorellus* males as the natural pheromone (Löfstedt *et al.* 1990). This indicates that the specific antagonistic effect of Z11-14:OAc on attraction of *Y. rorellus* may have evolved due to selection against hybridisation.

However, one should be careful in assigning adaptive antagonistic effects to pheromone components. If an *Yponomeuta* species and a tortricid moth do not have different pheromones this may lead to significant cross attraction but hardly to mating and certainly not to fertilisation. Thus, pheromone differences between such species can not have evolved by reinforcement. If the species interfere with each others pheromone communication this interference may still constitute a significantly strong selection pressure to cause divergence in the communication systems. However, whether the antagonistic effects of the minor pheromone components of *Y. evonymellus* and *Y. vigintipunctatus* on several tortricids (Fig. 3) are adaptive or incidental, remains a matter of speculation at the present stage of our knowledge.

The loss of pheromone components in the derived species mentioned above agrees with what Kaneshiro (1980) found in this study of sex communication in Hawaiian *Drosophila*. He argued that in general, derived species should have simpler species-recognition mechanisms than ancestral ones. If females of the derived ermine moth population did not produce essential components of the ancestral pheromone, they did not attract ancestral males effectively. On the other hand, derived males could still be attracted to ancestral females. If hybrids were less fit, then the asymmetric premating reproductive isolation evolved into complete reproductive isolation as was suggested earlier for *Y. rorellus* (Löfstedt *et al.* 1986a).

Mayr (1963) suggested that population bottlenecks associated with a genetic revolution might be important in speciation. *Y. rorellus* is almost monomorphic at some 75 enzyme loci (Menken 1987). The mean proportion of heterozygous loci per individual is 0.003 in *Y. rorellus*, compared with 0.061–0.136 in other ermine moths (Menken 1982, 1987). Indeed, such a low level of heterozygosity is expected for a species which has (repeatedly) been through a bottleneck. In this context we also found it interesting that Thorpe (1929) and Gershenson (1967) reported the aberrant haploid n = 29 number of chromosomes for *Y. rorellus*, whereas the rest of the examined European ermine moths were reported to have

n = 31 (which is the modal chromosome number for Lepidoptera). Changes in the karyotype affect recombination and may give sterile heterozygotes. However, we could not confirm this suggested karyotype change among *Yponomeuta* species. Instead we found an unusual haploid chromosome number, n = 29A + AA^wZ in females and n = 30A + ZZ in males, in *all* of the investigated species (AA^wZ is s sex chromosome trivalent, containing a w-chromosome translocated to an autosome to form an A^w-chromosome, Nilsson *et al.* 1988). The hypothesis regarding a "genetic revolution" at a population bottleneck in the early history of *Y. rorellus* can of course still be true, but is not supported by visible evidence of a karyotype change.

The idea of speciation by a genetic revolution and passage through a bottleneck has been criticised on both theoretical and empirical grounds. Barton & Charlesworth (1984) found neither empirical nor theoretical support for rapid evolutionary divergence in extremely small populations. The probability that a founder population will undergo a stochastic transition, to form a new selective equilibrium that is reproductively isolated from its ancestral population, is low. According to Barton & Charlesworth (1984) a similar level of isolation is more easily achieved by crossing a series of small selective barriers than a single large one.

Divergence in pheromone systems can proceed by parallel selection on female and male characteristics (see for instance de Jong 1988, and references therein), provided there is enough heritable variation for these characters within the population. Our analyses of the pheromone produced by individual female ermine moths confirm that there is significant within-populational variation in the E/Z-isomer ratios and the high level of repeatability for this character in *Y. padellus* indicates that the variation may be under genetic control (Du *et al.* 1987).

A dramatic "instantaneous" change of a pheromone and resulting *quantum speciation* (Grant 1971) has been suggested as a speciation mechanism in sibling species of moths using opposite geometric isomers of pheromone components (Roelofs & Comeau 1969). In the genetically best investigated moth so far, the European corn borer *Ostrinia nubilalis,* it was found that the factors controlling differences in pheromone production and response between the so-called E- and Z-strains, are inherited independently on different chromosomes (Roelofs *et al.* 1987; Löfstedt *et al.* 1989). This also has important bearings for our understanding of the potential role of pheromones in speciation in *Yponomeuta.* The probability that two critical mutations, changing female production and male response respectively, will occur and match is very low, but not zero. First, a mutation can be maintained for a long time in a population even if it is selected against; second, gene flow can greatly increase effective population size and thus the probability that both types of mutations arise and are brought together if they arise in different populations. The future survival and propagation of these mutants is of course much dependent on the mating success of mutant individuals and the exact mode of inheritance (Lanier & Burkholder 1974; Roelofs *et al.* 1987). If host races of *Y. padellus* really exist and are incipient species these very situations are much in need of research along the lines formulated above.

Conclusions

Plants of the family Celastraceae are believed to be the ancestral hosts of small ermine moths, but six of the European *Yponomeuta* have shifted host-plants at least once (Gerrits-Heybroek *et al.* 1978). Over the past 10 years or so modelling has made clear that host race formation is possible under conditions that are far less stringent than was previously thought (Tauber & Tauber 1989). Indeed, Feder *et al.* (1990 and references therein) found convincing empirical evidence for the existence of host races in *Rhagoletis pomonella.*

In ermine moths female calling behaviour is stimulated by the presence of host-plants (Herrebout & Van De Water 1982; Hendrikse & Vos-Bünnemeyer 1987). However, under experimental conditions absence of host-plants will not inhibit calling, but only retard it. If in the field host-plant choice (for oviposition) normally precedes calling activity, the influence of the host-plant on reproductive isolation might be much stronger than suggested by the experiments (Menken *et al.* 1991). At the moment crucial data for this hypothesis are still lacking, therefore an important role can be assigned to the specific sex pheromones in this respect.

The cross-attraction between allochronic species in the laboratory, and the formation of hybrids in the laboratory between species that are reproductively isolated by pheromone differences, is evidence for the role of pheromones as reproductive isolation mechanisms among *Yponomeuta.* The occurrence of pheromone components with an antagonistic behavioural effect on other species, provides further support in favour of interspecific selection as an important evolutionary force in the evolution of species-specific pheromones among the small ermine moths.

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