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

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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,
Sex pheromones in Yponomeuta

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 synchronous, 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. plumbeus 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. rorellus, Y. mistrutus, Y. padellus, 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

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 speculation and the evolution of reproductive isolation in the ermine moths.

<table>
<thead>
<tr>
<th>Yponomeuta species</th>
<th>Host-plant</th>
<th>Plant family</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Y. evonymellus</em> (L.)</td>
<td>Prunus padus L.</td>
<td>Rosaceae</td>
</tr>
<tr>
<td><em>Y. cagnagellus</em> (Hübner)</td>
<td>Euonymus europaeus L.</td>
<td>Celastraceae</td>
</tr>
<tr>
<td><em>Y. mahalebellus</em> Guenée</td>
<td>Prunus mahaleb L.</td>
<td>Rosaceae</td>
</tr>
<tr>
<td><em>Y. malinellus</em> Zeller</td>
<td>Malus sp.</td>
<td>Rosaceae</td>
</tr>
<tr>
<td><em>Y. padellus</em> (L.)</td>
<td>Crataegus sp.</td>
<td>Rosaceae</td>
</tr>
<tr>
<td><em>Y. rorellus</em> (Hübner)</td>
<td>Prunus spinosa L.</td>
<td>Rosaceae</td>
</tr>
<tr>
<td><em>Y. irrorellus</em> (Hübner)</td>
<td>Prunus domestica L.</td>
<td>Rosaceae</td>
</tr>
<tr>
<td><em>Y. plumbeus</em> (Denis &amp; Schiffermüller)</td>
<td>Sorbus aucuparia L.</td>
<td>Rosaceae</td>
</tr>
<tr>
<td><em>Y. vigintipunctatus</em> (Retzius)</td>
<td>Sedum telephium L.</td>
<td>Crassulaceae</td>
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</tbody>
</table>

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 reproductive isolation within a group of moths.

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

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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*, *Y. evonymellus*, *Y. cagnagellus*, *Y. plumbellus* and *Y. irrorellus* (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 attraction 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 palaena* (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.).

*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
Sex pheromones in Yponomeuta

Amount (µg)

compound

Subtracted

erally, acetylation of alcohols in moth pheromone glands is (corresponding alcohols) are present in large amounts. Gen-

been found in this species although the potential precursors (corresponding alcohols) are present in large amounts. Gen-

tractive lure than virgin females in field trapping experiments. 

and a mixture of Z11-14:OH and Z9-12:OAc was a more at-

orth America some years ago (Herrebout & Menken 1990),

E11-14:OH, and Z11-14:OH attracts some males in the field

unpubl.). Analysing 
technical mixture is 100 by definition in each series. Bars accompanied

of 14:OAc, 14:OH, 16:OAc, and 16:OH, but 14:OAc on its

produce three 16-carbon acetates and 14:OAc, but no clear evi-

one receptor cell responded to El1-14:OAc and Z11-14:OAc in 8 out of the 9 species

ments of nine 

species (modified from Van Der Pers 1982)

The role of El1-14: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 El1-14:OAc in 8 out of the 9 species studied. In Y. malinellus one receptor cell responded to El1-14: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 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).

Yponomeuta evonymellus
Tortrix viridana
Archips xylosteana

Yponomeuta vigintipunctatus
Aphelia paleana
Dichrorampha petiverella

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=20). Average trap catch of Y. evonymellus with the reference bait was 80 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 pher-

moone bouquet. 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 

E11-14:OAc, Z11-14:OAc, and 

E11-14:OH, and Z11-14:OH, 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).

Y. irrorellus
Y. evonymellus
Y. padellus
Y. vigintipunctatus
Y. rorellus
Y. mahalebellus
Y. cagnagellus

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)
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’ (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/T denotes increased/decreased amount of E isomer, Δ/11 (14) denotes delta 11 desaturation of 14 carbon acyl moiety, Ac denotes acetylation, β-oxid stands for β-oxidation (chain-shortening), etc.

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/T denotes increased/decreased amount of E isomer, Δ/11 (14) denotes delta 11 desaturation of 14 carbon acyl moiety, Ac denotes acetylation, β-oxid stands for β-oxidation (chain-shortening), etc.

The 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-
decenoate (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 A9-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 A11-desaturation of stearic acid followed by chain shortening, and not from the large pool of Z9-16:acyl produced by A9-desaturation of palmitic acid (Bjostad & Roelofs 1983).

Whereas A11-desaturation of 16:acyl only produces Z11-16:acyl, A11-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 A11-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

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 A11-desaturation and chain-shortening (within the frame). The fatty acids are selectively reduced and acetylated to give alcohols and acetates, active as pheromone components.
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 (Dijkstra 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 sympathy, 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. irorellus, 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. irorellus 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 A11-desaturation), and Y. rorellus has no unsaturated pheromone components at all (A11-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
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-population 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; Lofstedt 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-Hebroek 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). Instead, Feder et al. (1990) and references therein have convinced 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; Hendriks & 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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