PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Moth Pheromone Genetics and Evolution

Christer Lofstedt

Phil. Trans. R. Soc. Lond. B 1993 **340**, 167-177
doi: 10.1098/rstb.1993.0055

Moth pheromone genetics and evolution

CHRISTER LÖFSTEDT

Department of Ecology, Lund University, S-223 62 Lund, Sweden

SUMMARY

Sex pheromone communication in moths is a well investigated case of mate-finding by chemical signals, but the evolutionary causes of the great complexity and diversity of these signals are still not generally agreed on. In the present paper, I argue that there is no reason to dismiss species recognition as a possible cause of evolutionary change in moth sex pheromones. Admittedly, selection for species recognition cannot explain all of the diversity in sex pheromones and the data supporting this contention are weak, but the alternative causes suggested, invoking mate choice between conspecifics as the mechanism of sexual selection, has so far no empirical support. Finding and analysing genes responsible for mate choice is important to corroborate any theory of sexual selection and speciation. In this respect genetic dissection of moth pheromone communication has provided important progress. Mendelian genes controlling differences in mate choice and in the production of mate recognition signals have been found. Polymorphic pheromone systems give the population biologists unique possibilities to study mate choice and selection at the genotype level in nature.

1. SPECIFICITY OF MOTH SEX PHEROMONES

Mate-finding is in many insects mediated by pheromones. Sex pheromones in Lepidoptera is probably the best investigated case, but even so the evolutionary causes of the great complexity and diversity of these signals are not generally agreed on. During the 1960s and 70s the interpretation of the species-specific moth sex pheromones in terms of reproductive isolation was largely non-controversial. According to the ruling paradigm at that time (reviewed by Cardé & Baker (1984)), interspecific competition plays a major role in the partitioning of pheromone communication channels in moths (see, for example, Comeau (1971); Minks *et al.* (1973); Sanders (1971); Roelofs & Cardé (1974); Cardé *et al.* (1977); Greenfield & Karandinos (1979); Löfstedt & Van Der Pers (1985)). If the competing species are closely related, pheromone differences may also provide premating reproductive isolation mechanisms that prevent hybridization (Roelofs & Cardé 1974; Cardé *et al.* 1977). This paradigm with its emphasis on the adaptive nature of the species specificity of sex pheromones has, however, been challenged in several recent papers.

As advocates of the so called recognition concept of species, Paterson and colleagues (Paterson 1978, 1985; Lambert *et al.* 1987) question the existence of any such reproductive isolation mechanisms. They point out that to qualify as actual mechanisms of reproductive isolation, pheromone differences must evolve to prevent hybridization between sympatric taxa which are not already reproductively isolated. Instead, according to Paterson, pheromones act to facilitate matings between individuals with a common mate recognition system, not to prevent hybridization with other species.

West-Eberhard (1984), on the other hand, did not explicitly disapprove the existence of reproductive isolation mechanisms, but she still argued that selection for 'species recognition is a much less important factor in the evolution of insect communication than formerly believed. . . . The use of competitive signals as species recognition cues, when it occurs, may often be a secondary function of characters diverged under sexual selection prior to contact'.

Thus, neither of the two critical voices regard selection for species recognition an important factor driving the evolution of mate recognition systems, but for very different reasons. The proponents of the recognition concept of species, maintain that specific mate-recognition systems are under strong stabilizing selection and as a consequence they show very little variation. According to this paradigm their complexity and diversity can primarily be viewed as adaptations to the physical environment. West-Eberhard, on the other hand, contends that intraspecific social competition constitutes a major, directional, selection pressure causing changes in these systems. Although I have found both views helpful advancing my thinking about pheromone communication, I am not willing to accept any of them in their orthodox forms. Instead, in the present paper I will argue that selection for species recognition is still worthwhile considering as an explanation for the pattern of variation in moth sex pheromone communication systems. Selection for species recognition comprises both reinforcement and reproductive character displacement but a clear distinction should be made between the two processes (Butlin 1987*a*,*b*). I will also show that that in spite of

Phil. Trans. R. Soc. Lond. B (1993) **340,** 167–177
Printed in Great Britain

strong stabilizing selection in the local population there may still be considerable geographic variation in mate recognition systems. Secondly, I will discuss the genetics of a change of the sender and the receiver in the evolution of communication systems. This problem is important regardless of which set of selective forces one favours as the mechanism behind a process of divergence. Finding and analysing genes responsible for mating preference is considered one of the biggest challenges facing experimental evolutionary genetics, and despite the richness of theories concerning sexual selection and speciation not a single gene has yet been found that qualifies as a 'female choice gene' (see Ritchie 1992). I hope, however, to show that research on pheromone communication offers some relevant progress in this respect.

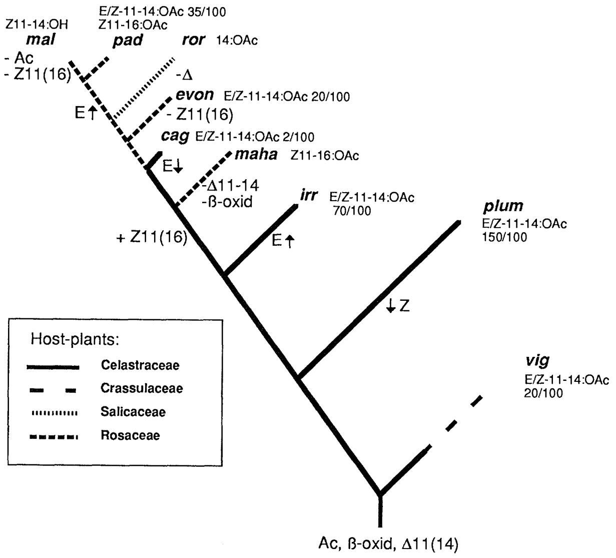


Figure 1. Phylogenetic tree of European small ermine moths *Yponomeuta* sp. based on allozyme variation at 51 loci. The figure also incorporates information on host-plants and pheromone composition of the respective species. Proposed shifts in pheromone composition and host plants superimposed on a phylogenetic 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. (Reprinted from Löfstedt *et al.* (1991), with the permission of Springer-Verlag.)

2. SELECTION FOR SPECIES RECOGNITION

When assigning adaptive explanations to the species specificity of communication systems it is important to consider both phylogenetic aspects and the geographical conditions of the divergence of the systems. Paterson (1985) pointed out that the evolution of differences between species with the primary function of reproductive isolation requires that the diverging populations initially should be able to form fertile (but inferior) hybrids and that this requires sympatry (or parapatry) during the selective process. (For a balanced and stimulating application of Paterson's recognition concept to moth sex pheromone communication, see Linn & Roelofs (1992)). True reproductive isolation mechanisms can thus only develop between closely related and coexisting populations, as a result of selection against gene flow. Butlin (1987*a*,*b*) made the important distinction between this process, called reinforcement, and the divergence of the mating systems of populations which are already reproductively isolated. The latter process of divergence he called reproductive character displacement and both processes qualify as proper subsets of the general term 'selection for species recognition'. Thus, in my opinion, paucity of examples confirming reinforcement does not automatically demonstrate that selection for species recognition is unimportant, as long as the possibility of selection against wastage of reproductive effort in the absence of gene flow remains untested.

Few cases of reproductive character displacement and none of reinforcement have been unequivocally demonstrated in the case of moth pheromone communication. This may be because these processes are extremely rare or because too few studies were explicitly designed to demonstrate any of them. The evolution of the European small ermine moths of the genus *Yponomeuta* (Lepidoptera: Yponomeutidae) has been studied for more than 15 years. Nine species of small ermine moths occur in the western Palearctic region and traditionally some of them presented great difficulties to the taxonomist, as they are difficult to distinguish by morphological characters. This well-studied group of moths provides a rare opportunity to investigate the significance of sex pheromones in speciation and interspecific interactions (for a full reference, see Löfstedt *et al.* (1991)).

All nine species have been studied by allozyme analysis at more than 50 larval and adult loci. From the absence of heterozygotes at diagnostic loci in samples taken from sympatry and the species-specific allozyme patterns, Menken (1980, 1989) concluded that all nine are genuine biological species. This conclusion is also corroborated by recent work on morphological characters (Povel 1984, 1986). A phylogenetic tree was constructed based on the allozyme variation, using 'Jelly', a modified version of Rogers (1984)algorithm. Host plants and pheromones of the nine species may be superimposed on the phylogenetic tree to form a basis for a discussion of pheromone divergence in relation to speciation and species recognition (figure 1).

Intra- and interspecific crossattraction of male small ermine moths in response to calling females was observed in a flight tunnel by Hendrikse (1986). She found that between 50 and 90% of the males were able to locate their conspecific females. However, significant interspecific crossattraction was also observed. The highest level of mutual crossattraction was observed between *Y. evonymellus* (L.) and *Y. vigintipunctatus* (Retzius) (50–60%). These two species

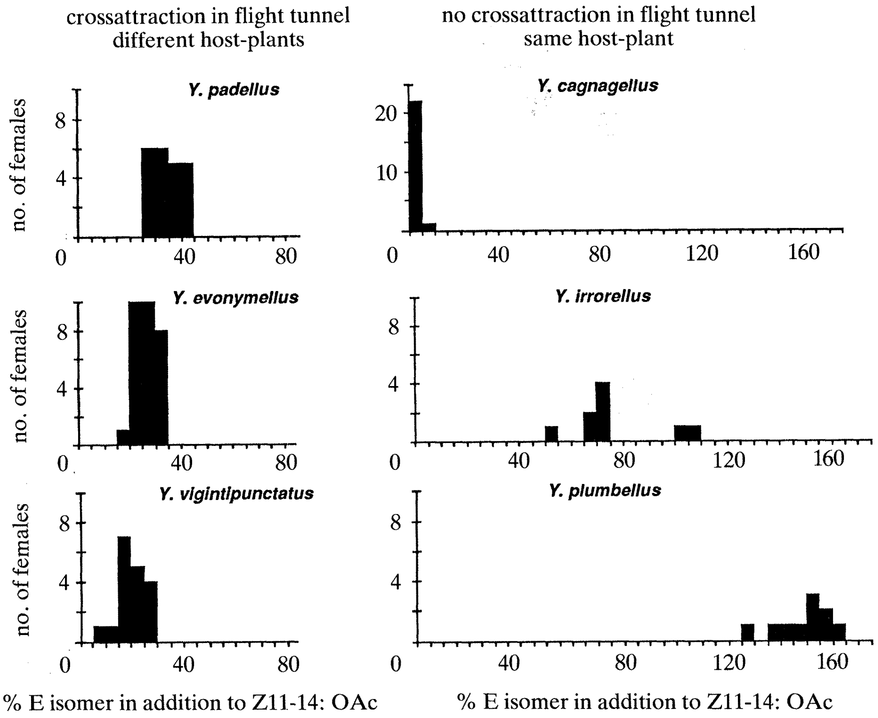


Figure 2. Frequency diagrams of (*E*)- to (*Z*)-11-tetradecenyl acetate ratios produced by individual females of six species of small ermine moths (*Yponomeuta*). Three of the species share the same hostplant and show no crossattraction in a flight tunnel, whereas three of them inhabit different habitats and show significant crossattraction in the flight tunnel. (Reprinted from Löfstedt *et al.* (1991) with the permission of Springer-Verlag.)

seem to use almost identical pheromones. The synthetic pheromones, however, in spite of their similarity, were still species specific when tested in the natural habitat of the respective species, i.e. the *Y. vigintipunctatus* pheromone trapped no *Y. evonymellus* and vice versa. No (or very limited) crossattraction was observed between species which normally share the same habitat and corresponding differences in the pheromone composition of these species was demonstrated (figure 2).

In the laboratory members of the so-called '*padellus*-complex' (namely *Y. cagnagellus* (Hübner), *mahalebellus* Guenée, *malinellus* Zeller, *padellus* (L.), and *rorellus (Hübner)*) may hybridize and produce offspring, the fertility and fecundity of which differs by combination (Menken 1980; Van Drongelen & Van Loon 1980; Hendrikse 1988). Under natural conditions pheromone differences would prevent hybridization despite the genetic compatibility of the taxa, due to lack of crossattraction.

Males of some *Yponomeuta* species possess specialized receptors on their antennae for compounds which are not pheromone components of the own species, but which function as primary pheromone components in most of their close relatives. Interestingly, field experiments provide clear evidence for the action of such compounds as behavioural antagonists, i.e. when added to an active pheromone blend they cause a dramatic reduction in trap catch. For instance males of *Y. rorellus*, in which females seem to have lost the possibility to produce unsaturated pheromone components, have receptors for the unsaturated tetradecenyl acetates. Addition of as little as 1% of Z11–14:OAc to the pheromone of female *Y. rorellus* reduces the attraction of conspecific males to a small percentage of what was obtained with the reference bait (Löfstedt *et al.* 1990). It is hard to envisage the evolution of this apparently specialized detection of and response to non-pheromonal compounds without the involvement of interspecific interaction as a major selective force.

Thus, in my opinion, the European small ermine moths provide the best possible evidence for the adaptive significance of differences in pheromone composition between closely related species, without the examination of geographical variation in pheromones of potentially interfering species and without studies of selection in currently polymorphic populations.

3. VARIATION IN A COMMUNICATION SYSTEM UNDER STABILIZING SELECTION

Regardless of which type of selection one advocates as a major evolutionary force the mechanism for change in a communication system constitutes a problem. First of all, a change in a signal requires coordinated changes in the sender and the receiver. Genetic, neurobiological and biochemical constraints may prevent such coordinated changes. Pheromone components used within a species, a genus or even a family of moths are remarkably similar in chemical structure, which demonstrates the conservative design of the

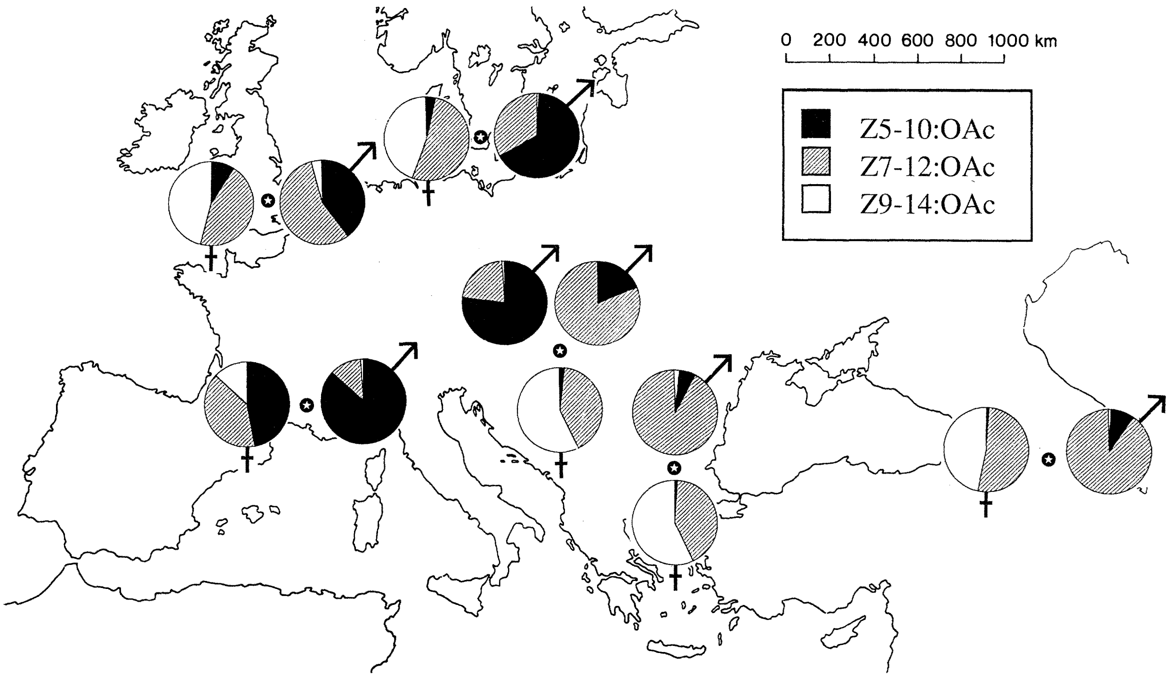


Figure 3. Geographical variation in the relative composition of the female produced sex pheromone of the turnip moth *Agrotis segetum* and in the relative abundance of different types of pheromone sensitive sensilla on the male antenna.

communication systems. Secondly, many communication systems may be under strong stabilizing selection and any deviation from the population norm is strongly selected against. This may prevent the spread of any mutant that is not associated with a strong selective advantage.

Geographical variation in the sex pheromone of the turnip moth *Agrotis segetum* Schiff. (Lepidoptera: Noctuidae) demonstrates stabilizing selection within the local populations, but significant variation in the communication system occurring between populations. The sex pheromone of the turnip moth *Agrotis segetum* has been reported as a mixture of three homologous acetates (*Z*)-5-decenyl acetate (Z5-10:OAc), (*Z*)-7-dodecenyl acetate (Z7-12:OAc) and (*Z*)-9-tetradecenyl acetate (Z9-14:OAc) (Bestmann *et al.* 1978; Arn *et al.* 1980, 1983; Tóth *et al.* 1980; Löfstedt *et al.* 1982). The composition of the pheromone with respect to proportions of these three compounds varies widely within and between populations, but all three compounds are necessary for maximal trap catches in all European populations tested (Arn *et al.* 1983; Löfstedt *et al.* 1986; Hansson *et al.* 1990; Tóth *et al.* 1992). Interestingly, there is covariation between the frequency of receptors for Z7-12:OAc and Z5-10:OAc and the abundance of the same components in the pheromone glands of females in the populations (figure 3). The proportion of receptors tuned to Z7-12:OAc is higher in the eastern populations, where Z7-12:OAc constitutes a major part of the female pheromone gland secretion, than in the population from France, where Z5-10:OAc appears to be a more important pheromone component. This may be taken as evidence for stabilizing selection in the local population, despite the large differences observed between populations. The exact meaning of this, however, is not clear, as the actual behavioural response and not the frequency of receptors is the accurate measure of male response types. Receptors for the major pheromone component are generally the most abundant on the antennae of males, but at present we do not know the mechanism that correlates receptor frequency with blend discrimination. A first step towards the construction of a wire-diagram of pheromone receptors in *A. segetum* and their projection into glomeruli in the antennal lobe has just been taken (Hansson *et al.* 1992).

In an extensive field-trapping program using different combinations of the three pheromone components, attraction of male *A. segetum* was studied on eleven sites in Europe, west Asia and Africa (Tóth *et al.* 1992). The largest difference in attraction of males appeared between the two populations monitored south of the Saharan desert and the rest of the populations. This study confirms the occurrence of behaviourally significant geographic variation in a sex pheromone communication system, but it does not explain why the variation occurs. Field-trapping of *A. segetum* in Europe with pure Z5-10:OAc caused attraction of non-specific male moths, whereas the pure Z5-10:OAc appeared specific in the populations in southern Africa, which points towards reproductive character displacement. However, this interpretation remains an hypothesis which requires further testing.

Recent studies in our laboratory revealed that *A. segetum* from Zimbabwe use Z7-12:OAc in addition to Z5-10:OAc in its pheromone, albeit in much lower relative amounts than the European populations.

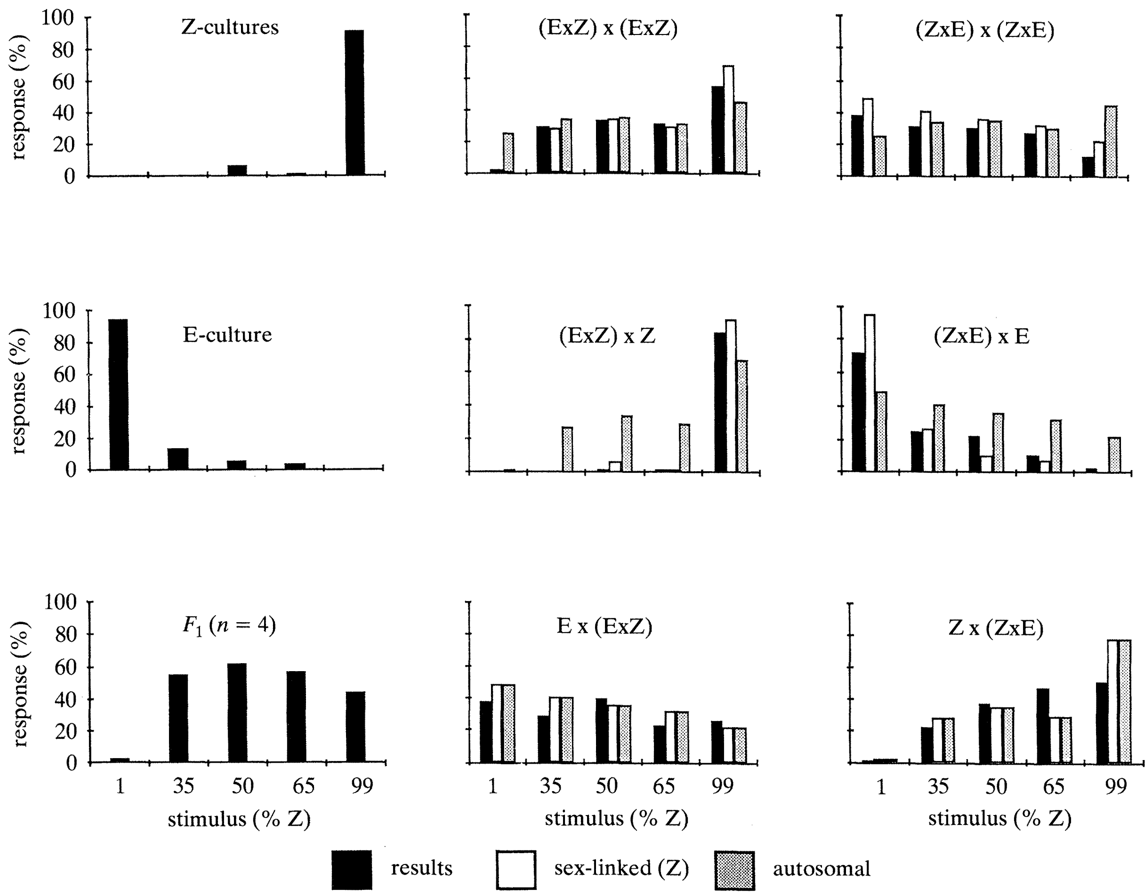


Figure 4. Proportion of male *Ostrinia nubilalis* from E and Z strains, reciprocal F_1 crosses, F_2 and backcrosses responding to different blends of (E)- and (Z)-11-tetradecenyl acetate (data originally from Roelofs *et al.* (1987)). Data on Z cultures is the average of the responses of a bivoltine and a univoltine strain, data on F_1 males is the average responses of the four possible (reciprocal) crosses between the two Z strains and the one E strain. $(E \times Z) \times (E \times Z)$ denotes the F_2 males obtained from originally mating E-strain females with Z-strain males, $(E \times Z) \times Z$ denotes males obtained from backcrossing F_1 females with Z-strain males. The rest of the crosses are assigned accordingly. The predicted response frequencies, if differences in response between parental strains and F_1 hybrids were determined by two alleles at a Z-linked or an autosomal locus, are displayed for comparison. This shows that difference in behavioural responses between males of the two strains are best explained as controlled by two alleles at a Z-linked locus. (Reprinted from Löfstedt (1990) with the permission of Kluwer Academic Publishers.)

Furthermore, extended behavioural studies revealed that the attraction of *A. segetum* to the three-component synthetic pheromone can be increased some 50% by addition of a small amount of (Z)-5-dodecenyl acetate, another monounsaturated acetate produced by female turnip moths (W. Wu & C. Löfstedt, unpublished data). Studies of specificity, using live females and optimized synthetic blends, will now be carried out both in the laboratory and in the field. Differences in pheromones between populations can potentially be demonstrated using live females, but full chemical characterization and quantitative determination of variation is necessary for a complete understanding of how and why the differences may have evolved. At the present state, the data on geographical variation in the pheromone of *A. segetum* anyhow demonstrates that stabilizing selection at the local level does not hinder populations isolated by distance to diverge with respect to important elements of their mate-recognition system. This is not compatible with the concepts of Paterson and his colleagues, at least not in their orthodox form.

4. PHEROMONE GENETICS

A comprehensive model of pheromone evolution must incorporate an understanding of how pheromone communication systems are genetically controlled. The communication systems of the small ermine moths were not tractable to genetic dissection because of the problems involved with rearing the moths. Studies of the pheromone genetics of *A. segetum* have only just begun in my research group at Lund University. However, a model of the genetic architecture of pheromone communication systems has emerged from studies of other moths, especially the European corn borer *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae). Although only a few species have been studied so far, it is interesting to note that several other studies suggest that this model may hold for other moths as well.

Pheromone polymorphism in *O. nubilalis* is controlled by at least three major loci; the female production locus, the male behavioural response locus and a third locus that controls the organization of the male pheromone sensitive sensilla. The autosomally inherited production locus, originally reported by Klun & Maini (1979), seems to control the specificity of the reductase involved in a late step in the pheromone biosynthesis (Roelofs *et al.* 1987). The reductase is responsible for the selective conversion of E11- and Z11-14:acyl precursors to pheromone components. Both E- and Z-strain females as well as hybrids have these precursors in approximately the same ratios, but *in vivo* experiments have confirmed that Z-strain females reduce the Z11-14:Acyl and E-strain females the E11-14:Acyl to the corresponding alcohols, which are subsequently acetylated to form the acetate pheromone components. Hybrid females are heterozygous at the production locus and possess both the E and the Z form of the reductase, and as a consequence they produce a mixture of E11- and Z11-14:OAc. An autosomal locus with two alleles has been found to control pheromone polymorphism also in the cabbage looper *Trichoplusia ni* (Haynes & Hunt 1990) and in two sibling tortricid species of the genus *Ctenopseustis* (S. P. Foster, personal communication).

The male behavioural response in *O. nubilalis* is controlled by a sex-linked locus (inherited on the Z chromosome) with two alleles (figure 4). In Lepidoptera males are homogametic (ZZ) and females are heterogametic (ZW). In two species of the tortricid genus *Ctenopseustis* a Z-linked gene controlling male electrophysiological response to pheromone was reported by Hansson *et al.* (1989) and flight tunnel experiments seem to support a Z-linked locus determining male behavioural response as well (S. P. Foster, S. J. Muggleston, B. O. Bengtsson, C. Löfstedt, unpublished data). A Z-linked response locus could also account for the pattern of pheromone response reported for hybrids between the two pheromone morphs of *Zeiraphera diniana* (Priesner & Baltensweiler 1987; Löfstedt 1990). In *O. nubilalis* we found that an autosomal locus with two alleles controls the organization of the olfactory sensilla on the antennae of the males, but male cornborers seem to respond behaviourally to different pheromone blends as determined by the alleles present at their Z-linked behavioural response locus, regardless of their receptor outfit (Roelofs *et al.* 1987).

In *Drosophila* loci controlling important elements of olfaction are found on the X-chromosome (Siddiqi 1987) and in the butterfly *Colias* loci controlling the visual signals used by males in mating were also found on the Z chromosome (Grula & Taylor 1980). Different explanations for the prevalence of sex-linked 'communication-genes' have been suggested; for instance, sex-linkage may facilitate the evolution of new pheromone strains (Grula & Taylor 1980; Cardé & Baker 1984), but none of the suggested mechanisms help to explain the occurrence of Z-linked pheromone response genes in moths. Sex-linked genes may be more easily demonstrated in breeding experiments and in *Drosophila* the X chromosome may organize as much as 25% of the genome, but in the Lepidoptera the Z chromosome probably carries less than 5% of the genetic material (the modal chromosome number (n) in Lepidoptera is 31 (Robinson 1971)). It has also been suggested that sex-linked loci may evolve faster than autosomal ones because they experience more intense selection in their hemizygous state in the heterogametic sex (see, for example, Ewing (1969)), but pheromone response is expressed in the homogametic male sex only. Under these conditions there should be no difference between a Z-linked and an autosomal locus with respect to intensity of selection (Charlesworth *et al.* 1987). It is generally believed that the number of cell divisions differs between sperms and eggs. This has bearings on the rate of molecular evolution at different loci. Errors in DNA replication and repair are probably the major source of mutations. Miyata *et al.* (1987) demonstrated that different numbers of germ-cell divisions between males and females result in different mutation frequencies between autosomes and sex chromosomes. Mutation rates at Z-linked loci may be four-thirds times that of an autosomal locus. Thus, if mutation rate was the limiting factor in evolution, Z-linked loci would be more likely to change than autosomal ones. However, the higher mutation rate at Z-linked loci has no greater influence on the rate of evolution than an increase of population size by a factor of four-thirds and does not explain the frequent cases of Z-linked response genes. Thus, in the case of moth pheromone communication, we are left with a number of intriguing examples of sex-linked inheritance with no adaptive explanation for its prevalence.

Close linkage between genes affecting female pheromone production and male response would help in building up linkage disequilibrium between alleles specifying new types at these loci, and would thereby have a strong effect on the evolutionary process that leads a population to substitute one pheromone blend for another. In *O. nubilalis* as well as in *Ctenopseustis* sp., signal production seems to be autosomally controlled, whereas behavioural response to the pheromone is determined by a sex-linked locus. To investigate whether the autosomal loci affecting pheromone production in females and the organization of antennal pheromone sensitive sensilla in *O. nubilalis* are closely linked to each other, a recombination experiment was performed. This showed unequivocally that the genes determining the variation in pheromone production and organization of male olfactory sensilla are not closely linked and probably inherited on different chromosomes (Löfstedt *et al.* 1989).

Hybridization experiments with the two pheromone strains of *O. nubilalis* demonstrated that canalization may hide considerable genetic variation at loci influencing pheromone biosynthesis (J. Zhu, C. Löfstedt & B. O. Bengtsson, unpublished data; Löfstedt 1991). Despite the narrowly defined pheromone component ratios produced by females of the parental E and Z strains, pheromone component ratios produced by females heterozygous at the major production locus showed a bimodal distribution. A mutation in the reductase system of the cornborer may thus expose significant variation in the production of ratios to selection, as was observed in heterozygote females in our experiments. The variation in heterozygotes was partly interpreted as the segregation of two different Z-reductase alleles, one resulting in high %E and another in low %E females. The precise ratio of isomers produced by hybrid females may be very important for the survival of a mutant in an originally monomorphic population. The hybridization experiments also demonstrated other effects that could not be attributed to the two reductase alleles just mentioned, but should be the effects of modifier genes. The concept of canalization was originally introduced by Waddington (1940; referenced by Scharloo (1991)) in the context of developmental biology. With the use of the term canalization he emphasized that the endproducts of development in an organism (i.e. the tissues) are of distinct types with no intergradation. Deviations from the preferred developmental paths are corrected for by regulatory processes. In the case of *O. nubilalis* the existence of two reductases with clear geometric specificity may in the homozygotes prevent the expression of existing variation in the earlier and later steps of the pheromone biosynthesis.

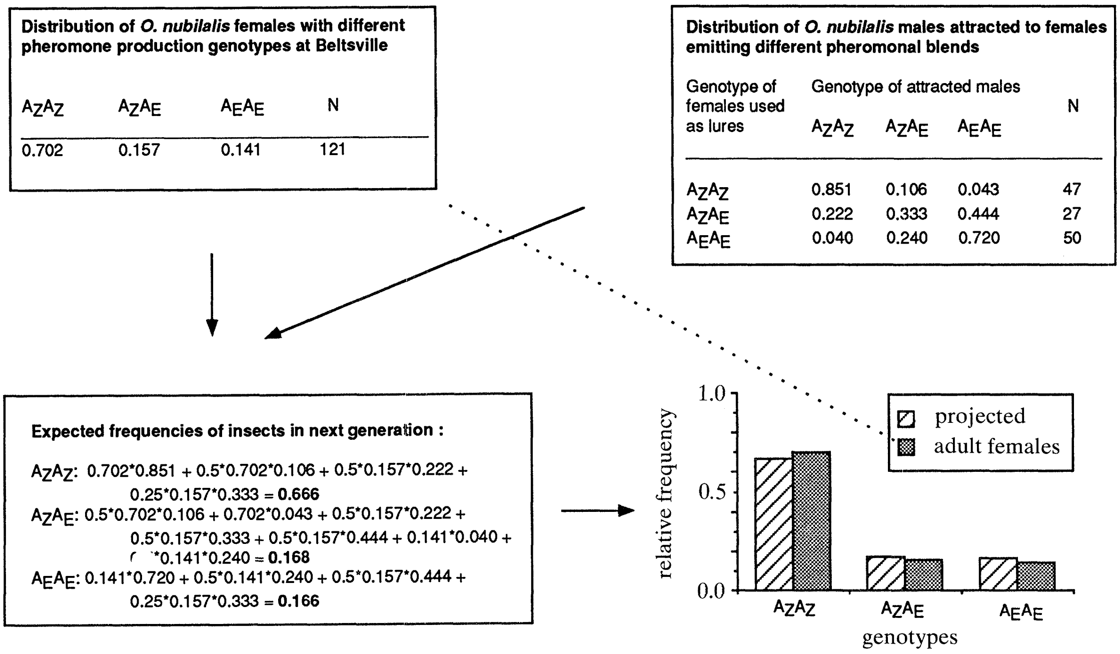
Although the evolutionary change of a communication system requires a more-or-less synchronized change of the sender and the receiver, the striking diversity of moth pheromones confirms that divergence is still possible. In the light of what we know about pheromone genetics today and in considering the asymmetry of sexual selection, the most likely scenario for a change starts with a mutation that results in the production of a new female signal. The survival of female mutants in the population, awaiting matching mutations of male preference, is facilitated by the wide response windows of males. The biological environment, i.e. coexisting species with interfering pheromones (rather than the physical environment focused by the Paterson school), should be the major external selective pressure having an influence on such a polymorphic pheromone communication system.

5. SELECTION IN A POLYMORPHIC POPULATION

Natural selection can affect the composition of the pheromone (as expressed in female production and male response window) in three ways.

1. Directional selection may shift the composition of the signal by favouring individuals at one end of the distribution. In this way the mean will change and the variance may decrease.
2. Stabilizing selection will favour the population norm and decrease the variance.
3. Disruptive (or diversifying) selection may favour extreme individuals relative to intermediate ones, because of their better performance. This could be the result of density or frequency dependent selection or of the presence of two favourable communication niches.

This categorization (according to Simpson (1944) and Mather (1953), cited by Endler (1986)) applies to both quantitative (continuously varying) and polymorphic traits. Quantitative as well as polymorphic variation has been documented in pheromone communication systems (for a review, see Löfstedt (1990)).



Distribution of *O. nubilalis* females with different pheromone production genotypes at Beltsville

A_ZA_Z	A_ZA_E	A_EA_E	N
0.702	0.157	0.141	121

Distribution of *O. nubilalis* males attracted to females emitting different pheromonal blends

Genotype of females used as lures	Genotype of attracted males			N
	A_ZA_Z	A_ZA_E	A_EA_E	
A_ZA_Z	0.851	0.106	0.043	47
A_ZA_E	0.222	0.333	0.444	27
A_EA_E	0.040	0.240	0.720	50

Expected frequencies of insects in next generation :

A_ZA_Z: 0.702*0.851 + 0.5*0.702*0.106 + 0.5*0.157*0.222 +
0.25*0.157*0.333 = **0.666**
A_ZA_E: 0.5*0.702*0.106 + 0.702*0.043 + 0.5*0.157*0.222 +
0.5*0.157*0.333 + 0.5*0.157*0.444 + 0.141*0.040 +
[illegible]*0.141*0.240 = **0.168**
A_EA_E: 0.141*0.720 + 0.5*0.141*0.240 + 0.5*0.157*0.444 +
0.25*0.157*0.333 = **0.166**

Figure 5. Testing the constancy of genotype proportions in a polymorphic population of the European Cornborer *Ostrinia nubilalis* at Beltesville, Maryland, U.S.A. Using the data of Klun & Huettel (1988) on crossattraction between the different pheromone morphs, Bengtsson & Löfstedt (1990) calculated the genotype frequencies in the projected generation and found these to be remarkably close to the frequencies found for the three genotypes among adult females. Thus, the experimental data demonstrates assortative mating without selective differences between the genotypes.

Stabilizing selection should always be in operation to match the senders and receivers. Interference with pheromone communication in other species may exert directional or occasionally disruptive selection. Different forms of selection will operate on the communication systems simultaneously, but only directional selection will necessarily change the mean of the character. Thus it is far from self-evident how natural selection will effect pheromone communication systems, but pheromonally variable populations of moths may give the biologists opportunities to study selection at the genotype level in nature.

Klun & Huettel (1988) analysed crossattraction in a mixed Z- and E-strain *O. nubilalis* population in Maryland, U.S.A. They determined the genotype of attracted males at the pheromone production locus by pheromone analysis of the female offspring produced by matings with females of known genotype, and showed that there is strong assortative mating in the population with respect to this locus. Bengtsson & Löfstedt (1990) extended the analysis of their data and found no indication of any male sexual selection in the population with respect to the production locus. With the observed frequency of matings between different genotypes, the same genotype distribution was generated in the next generation (figure 5), which implies that the mixed population is genetically stable. However, this stability may be highly relative. It is perfectly possible that some or all pheromone-polymorphic populations are evolutionarily unstable in the sense that they would become monomorphic if they were left to evolve unaffected by migration from surrounding populations.

Unfortunately the data set of Klun & Huettel did not contain any information on the male response locus. Male behavioural response type can not be determined directly, as is possible for female pheromone production. Glover *et al.* (1990) have demonstrated a polymorphic allozyme locus (triose phosphate isomerase) which is linked to the behavioural response locus on the Z chromosome. The E strain investigated by Glover and coworkers was fixed for a fast moving TPI allele, but Z-strain insects were polymorphic with both the fast and a slower moving form. Thus this approach can not be used to unequivocally assign pheromone response type to individual males. The development of DNA probes for the actual response locus would allow the extension of selection studies to include also the male character.

6. CONCLUDING REMARKS

(*a*) *Evolution of pheromones is mediated by assymetric sexual selection*

There are several reported cases of narrowly defined pheromone communication channels, manifested in low within- and between population variation in pheromone composition. Female moths within a species normally produce a well-defined pheromone signal, whereas males respond to a much larger range of signals. Sexual selection theory helps us to identify the nature of this variation. As sexual selection acts more strongly on males than females, it is likely that the narrow variation in female pheromone production reflects small but real individual variation. On the contrary, the wider response window of males is probably a characteristic which is shared by all males in the population and maximal sensitivity is for all males tuned to the average female produced signal (Löfstedt 1990). Owing to the asymmetry of selection on characters related to mating success, male response to sex pheromones will track changes in the the female produced signal. This is in contrast to many other systems, in which the male-produced signal will track female response (choice) (Phelan 1992).

Whereas sexual selection theory is useful explaining how sex pheromones in moths may have diverged and diversified, the common distinction between sexual selection and natural selection does not contribute to an understanding of why certain designs evolve and not others. I find this dichotomy dating back to Darwin (1859), rather confusing and misleading. In my opinion sexual selection is best treated as a subset of natural selection (in a general sense), not only for semantic reasons. The common currency of all types of natural selection is differential reproduction. As long as other individuals reproduce, selection on survival (i.e. natural selection in a strict sense) is significant only if it leads to differential reproduction. The same holds for sexual selection, whether the mechanism is intra- or intersexual, whether it is a choice of a conspecific mate or a choice between conspecifics.

Two reasons are frequently given for treating sexual selection separately from mortality selection (a term which I prefer to natural selection *sensu stricto*)†: (i) sexual selection may lead to the evolution of exaggerated secondary male characters which intuitively should be selected against by increased mortality; and (ii) sexual selection (of the Fisherian runaway type) has a potential for generating very rapid changes of character states. In moth sex pheromone communication the specific signal is a female character and thus the first argument does not apply. Of course the Fisherian run-away model may still provide a mechanism for a change, but it does not provide an evolutionary cause and other forms of sexual selection may generate rapid change as well.

(*b*) *Selection for species recognition has empirical support*

West-Eberhard (1984) recognized four possible causes of evolutionary change in female calling signals: (i) species recognition; (ii) protective advantage of facilitating sex recognition in species with hyperaggressive males; (iii) female advantage of attracting more than one male; and (iv) mutual sexual selection due to a significant male parental investment. Although I agree with West-Eberhard that selection for species recognition does not explain all of the

---

† Endler (1986) divides natural selection into sexual and non-sexual selection, the latter category is subdivided into mortality selection and fecundity selection. I find this terminology clearer and more productive than the dichotomy between sexual and natural selection.

diversity and complexity of mate recognition signals, I think that 'the case is not ready for the jury'.

As far as we know, crossattraction of moths is a rare event in nature, but this does not immediately prove anything. Absence of cross-attraction may indicate that interspecific interference is an unimportant selection pressure or that significant selection in the past has effectively eliminated crossattraction ('the ghost of competition past', see Connel (1980)). Selection studies in currently polymorphic communication systems as well as investigations of geographic variation in mate-recognition systems are needed to thoroughly test the hypotheses of selection for species recognition, resulting in reproductive character displacement as well as reinforcment, as an evolutionary cause of the species-specific composition of moth pheromones. I hope, however, that I have been able to show at least comparative evidence in favour of selection for species recognition in moth sex pheromone communication, whereas there is no evidence in support of any of the other three hypothetical causes. The second hypothesis involving 'hyperaggressive males' does not to my knowledge apply to Lepidoptera. With respect to the testing of hypotheses three and four it would be valuable to have data on the following.

1. How many female moths (individuals and species) are simultaneously calling in a given habitat?
2. How many males (individuals and species) are simultaneously looking for a mate?
3. What is the variance in male and female mating success, respectively?
4. What proportion of males or females is mated during their lifetime?
5. How long time does it take before a male or female gets mated?
6. Do female moths actively choose among the males attracted?
7. What is the incidence or significance of multiple matings?
8. What is the premium in terms of reproductive success for a female who attracts a male sooner than another female does?

Such data should give an idea of the strength of competition for mates by means of sex pheromones. At present I see no reason to dismiss selection for species recognition as a possible cause of moth sex pheromone specificity because of weak empirical support when the alternative hypotheses have no support at all.

(*c*) *Major genes control mate recognition signals and mate choice in moths*

Understanding the genetic control of mate choice is an important step to corroborate any theory concerning processes of sexual selection and speciation. Studies with the two-spot ladybird *Adalia bipunctata* have indicated that complex behaviours like alternative choices of mating partner could have a relatively simple genetic control (Majerus & O'Donald 1986), but recent experiments could not reproduce the original results (see Ritchie (1992) and references therein). Thus, at present the most promising studies in search for genes controlling mate choice may be those on moth pheromone communication. In hindsight the control of pheromone production differences by allelic differences at a major locus is not surprising, as two forms of an enzyme, producing distinct biosynthetic end-products, can be controlled by different alleles at one locus. The mechanism for the control of pheromone response by a major Z-linked locus in *O. nubilalis* is not known, but behavioural response differences between sibling species of the tortricid genus *Ctenopseustis* may be controlled in a similar way as mentioned above.

The small number of genes controlling major differences in moth mate-recognition systems should not represent a constraint on the evolution of these systems, but rather the opposite. A mutation at one of the major loci may reveal additional (quantitative) variation due to modifier genes, as was shown in *O. nubilalis*. This variation may then be subject to gradual selection and facilitate the spread of a mutant at the major locus.

(*d*) *Moth pheromone communication systems provide unique opportunities for the study of natural selection at the genotype level in nature!*

Considerable selective differences may have gone unnoticed in the analysis of sexual selection in the mixed population of *O. nubilalis* because of limitations of the data set and the limited power of the tests used (Bengtsson & Löfstedt 1990). Nevertheless, our study indicated how selection on communication systems can be investigated at the genotype level using a combination of empirical experiments and a selection-component analysis. To further measure the strength of sexual selection in pheromonally variable populations would be an important contribution to a better understanding of how communication systems evolve.

It is advantageous to address general questions about the evolution of communication systems using moth pheromones as models as there is a wealth of information available on their structure and function (for an excellent review see Baker (1989)). The pheromone signal composition can be analysed and manipulated under experimental conditions. But despite the hundreds of moth pheromones investigated since the early 1960s, the chemical and biological characterization of pheromones is far from trivial. A number of cases have demonstrated the importance of working with fully identified pheromones, when testing behavioural, ecological and evolutionary hypotheses about pheromone communication. With this caveat, genetic dissection of pheromone communication in moths and studies of selection in variable pheromone systems in nature may provide a better understanding of not only the evolution of species specific sex pheromones, but theories on mate choice in general.

This work was supported by grants from the Swedish Research Councils NFR and SJFR and the Bank of Sweden Tercentenary Foundation. I am thankful to Dr R. Butlin, Dr C. Linn and Dr J. Endler for helpful reviews and critiscism, and to Siana Laforest for criticism and linguistic revision of an earlier draft of this manuscript.

REFERENCES

Arn, H., Städler, E., Rauscher, S. *et al.* 1980 Multicomponent sex pheromone in *Agrotis segetum*: Preliminary analysis and field evaluation. *Z. Naturforsch.* **35C**, 986–989.

Arn, H., Esbjerg, P., Bues, R. *et al.* 1983 Field attraction of *Agrotis segetum* males in four European countries to mixtures containing three homologous acetates. *J. chem. Ecol.* **9**, 267–276.

Baker, T.C. 1989 Sex pheromone communication in the Lepidoptera: New research progress. *Experientia* **45**, 248–262.

Baker, T.C. & Cardé, R.T. 1979 Courtship behavior of the oriental fruit moth (*Grapholitha molesta*): Experimental analysis and consideration of the role of sexual selection in the evolution of courtship pheromones in the Lepidoptera. *Ann. ent. Soc. Am.* **72**, 173–188.

Bengtsson, B.O. & Löfstedt, C. 1990 No evidence for selection in a pheromonally polymorphic moth population. *Am. Nat.* **136**, 722–726.

Bestmann, H.J., Vostrowsky, O., Koschatzky, K.-H. *et al.* 1978 (*Z*)-5-Decenylacetat, ein Sexuallockstoff für Männchen der Saateule *Agrotis segetum* (Lepidoptera). *Angew. Chem.* **10**, 815–816.

Butlin, R. 1987*a* Speciation by reinforcement. *Trends Ecol. Evol.* **2**, 8–13.

Butlin, R. 1987*b* Species, speciation, and reinforcement. *Am. Nat.* **130**, 461–464.

Cardé, R.T. & Baker, T.C. 1984 Sexual communication with pheromones. In *Chemical ecology of insects* (ed. W. J. Bell & R. T. Cardé), pp. 355–383. London: Chapman and Hall.

Cardé, R.T., Cardé, A.M., Hill, A.S. & Roelofs, W.L. 1977 Sex pheromone specificity as a reproductive isolating mechanism among the sibling species *Archips argyrospilus* and *A. mortuanus* and other sympatric tortricine moths (Lepidoptera: Tortricidae). *J. chem. Ecol.* **3**, 71–84.

Charlesworth, B., Coyne, J.A. & Barton, N.H. 1987 The relative rates of evolution of sex chromosomes and autosomes. *Am. Nat.* **130**, 113–146.

Comeau, A. 1971 *Physiology of sex pheromone attraction in Tortricidae and other Lepidoptera (Heterocera)*. Ph.D. thesis, Cornell University.

Connel, J.H. 1980 Diversity and the coevolution of competitors, or the ghost of competition past. *Oikos* **35**, 131–138.

Darwin, C. 1859 *On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life.* London. John Murray.

Endler, J.A. 1986 *Natural selection in the wild.* Princeton University Press.

Ewing, A.W. 1969 The genetic basis of sound production in *Drosophila pseudoobscura* and *D. persimilis*. *Anim. Behav.* **17**, 555–560.

Glover, T., Campbell, M., Robbins, P. & Roelofs, W. 1990 Sex-linked control of sex pheromone behavioral responses in European corn-borer moths (*Ostrinia nubilalis*) confirmed with TPI marker gene. *Arch. Insect Biochem. Physiol.* **15**, 67–77.

Greenfield, M.D. & Karandinos, M.G. 1979 Resource partitioning of the sex communication channel in clearwing moths (Lepidoptera: Sesiidae) of Wisconsin. *Ecol. Monogr.* **49**, 403–426.

Grula, J.W. & Taylor, O.R. Jr 1980 The effect of X-chromosome inheritance on mate-selection behavior in the sulphur butterflies, *Colias eurytheme* and *C. philodice*. *Evolution* **34**, 688–695.

Hansson, B.S., Löfstedt, C. & Foster, S. 1989 Z-linked inheritance of male olfactory response to sex pheromone components in two species of tortricid moths, *Ctenopseustis obliquana* and *Ctenopseustis. sp. Ent. exp. appl.* **53**, 137–145.

Hansson, B.S., Tóth, M., Löfstedt, C., Szöcs, G., Subchev, M. & Löfqvist, J. 1990 Pheromone variation among eastern European and a western Asian population of the turnip moth *Agrotis segetum*. *J. chem. Ecol.* **16**, 1611–1622.

Hansson, B.S., Ljungberg, H., Hallberg, E. & Löfstedt, C. 1992 Functional specialization of olfactory glomeruli in a moth. *Science, Wash.* **256**, 1313–1315.

Haynes, K.F. & Hunt, R.E. 1990 Interpopulational variation in emitted pheromone blend of the cabbage looper moth, *Trichoplusia ni*. *J. chem. Ecol.* **16**, 509–519.

Hendrikse, A. 1986 Intra- and interspecific sex pheromone communication in the genus *Yponomeuta*. *Physiol. Ent.* **11**, 159–169.

Hendrikse, A. 1988 Hybridization and sex-pheromone responses among members of the *Yponomeuta padellus*-complex. *Ent. exp. appl.* **48**, 213–223.

Klun, J.A. & Maini, S. 1979 Genetic basis of an insect chemical communication system: The European corn borer. *Environ. Ent.* **8**, 423–426.

Lambert, D.M., Michaux, B. & White, C.S. 1987 Are species self-defining? *Syst. Zool.* **36**, 196–205.

Linn, C.E. & Roelofs, W.L. 1992 Pheromone communication in the moths and its role in the speciation process. In *Speciation and the recognition concept: theory and application* (ed. D. H. Lambert & H. Spencer). Baltimore and London: Johns Hopkins University Press.

Löfstedt, C. 1990 Population variation and genetic control of pheromone communication systems in moths. *Ent. exp. appl.* **54**, 199–218.

Löfstedt, C. & Van Der Pers, J.N.C. 1985 Sex pheromones and reproductive isolation in four European small ermine moths. *J. chem. Ecol.* **11**, 649–666.

Löfstedt, C., Van Der Pers, J.N.C., Löfqvist, J. *et al.* 1982 Sex pheromone components of the turnip moth, *Agrotis segetum*: chemical identification, electrophysiological evaluation and behavioural activity. *J. chem. Ecol.* **8**, 1305–1321.

Löfstedt, C., Löfqvist, J., Lanne, B.S., Van Der Pers, J.N.C. & Hansson, B.S. 1986 Pheromone dialects in European turnip moths *Agrotis segetum*. *Oikos* **46**, 250–257.

Löfstedt, C., Hansson, B.S., Roelofs, W.L. & Bengtsson, B.O. 1989 No linkage between genes controlling female pheromone production and male pheromone response in the European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Pyralidae). *Genetics* **123**, 553–556.

Löfstedt, C., Hansson, B.S., Dijkerman, H.J. & Herrebout, W.M. 1990 Behavioral and electrophysiological activity of unsaturated analogues of the pheromone tetradecyl acetate in the small ermine moth *Yponomeuta rorellus*. *Physiol. Ent.* **15**, 47–54.

Löfstedt, C., Herrebout, W.M. & Menken, S.B.J. 1991 Sex pheromones and their potential role in the evolution of reproductive isolation in small ermine moths (Yponomeutidae). *Chemoecology* **2**, 20–28.

Majerus, M.E.N., O'Donald, P., Kearns, P.W.E. & Ireland, H. 1986 Genetics and evolution of female choice. *Nature, Lond.* **321**, 164–167

Mather, K. 1953 The genetical structure of populations. *Evol. Symp. Soc. exp. Biol.* **7**, 66–95.

Menken, S.B.J. 1980 Inheritance of allozymes in *Yponomeuta* II. Interspecific crosses within the padellus complex and reproductive isolation. *Proc. Kon. Ned. Acad. Wet. (C)* **83**, 425–431.

Menken, S.B.J. 1989 Electrophoretic studies on populations, host races, and sibling species in insect pest species. In *Electrophoretic studies in insect pests* (ed. H. Loxdale & D. Hollander), pp. 181–201. Oxford: Clarendon Press.

Minks, A.K., Roelofs, W.L., Ritter, F.J. & Persoons, C.J. 1971 Reproductive isolation of two tortricid moth species by different ratios of a two-component sex attractant. *Science, Wash.* **180**, 1073–1074.

Miyata, T., Hayashida, H., Kuma, K., Mitsuyasu, K. & Yasunaga, T. 1987 Male-driven molecular evolution: a model and nucleotide sequence analysis. *Cold Spring Harb. Symp. quant. Biol.* **LII**, 863–867

Paterson, H.E.H. 1978 More evidence against speciation by reinforcement. *S. Afr. J. Sci.* **74**, 369–371.

Paterson, H.E.H. 1985 The recognition concept of species. In *Species and speciation* (ed. E. S. Vrba) pp. 21–29. Pretoria, South Africa: Transvaal Museum Monograph No. 4.

Phelan, P.L. 1992 Evolution of sex pheromones and the role of asymmetric tracking. In *Evolutionary perspectives on the chemical ecology of insects* (ed. B. D. Roitberg & M. B. Isman), pp. 265–314. New York: Chapman and Hall.

Phelan, P.L. & Baker, T.C. 1987 Evolution of male pheromones in moths: reproductive isolation through sexual selection. *Science, Wash.* **235**, 205–307.

Povel, G.D.E. 1984 The identification of the European small ermine moths, with special reference to the *Yponomeuta padellus*-complex (Lepidoptera: Yponomeutidae). *Proc. Kon. Ned. Acad. Wet. (C)* **87**,149–180.

Povel, G.D.E. 1986 Pattern detection within the *Yponomeuta padellus*-complex of the European small ermine moths (Lepidoptera: Yponomeutidae). I. Biometric description and recognition of groups by numerical taxonomy. *Proc. Kon. Ned. Acad. Wet. (C)* **89**, 425–41.

Priesner, E. & Baltensweiler, W. 1987 Studien zum Pheromon-Polymorphismus von *Zeiraphera diniana* Gn. (Lep., Tortricidae). *J. appl. Ent.* **104**, 433–448.

Ritchie, M.G. 1992 Setbacks in the search for mate-preference genes. *Trends Ecol. Evol.* **7**, 328–329.

Robinson, R. 1971 Lepidoptera genetics. Oxford: Pergamon Press.

Roelofs, W.L. & Cardé, R.T. 1974 Sex pheromones in the reproductive isolation of lepidopterous species. In *Pheromones* (ed. M. C. Birch), pp. 96–114. Amsterdam: North-Holland Publishing Company.

Roelofs, W.L., Glover, T., Tang, X.-H., Sreng, I., Robbins, P., Eckenrode, C. Löfstedt, C., Hansson, B. & Bengtsson, B. O. 1987 Sex pheromone production and perception in European corn borer moths is determined by both autosomal and sex-linked genes. *Proc. natn. Acad. Sci. U.S.A.* **84**, 7585–7589.

Rogers, J.S. 1984 Deriving phylogenetic trees from allele frequencies. *Syst. Zool.* **33**, 52–63.

Sanders, C.J. 1971 Daily activity patterns and sex pheromone specificity as sexual isolating mechanisms in two species of *Choristoneura* (Lepidoptera: Tortricidae). *Can. Ent.* **103**, 498–502.

Siddiqi, O. 1987 Neurogenetics of olfaction in *Drosophila melanogaster*. *Trends Genet.* **3**, 137–142.

Scharloo, W. 1991 Canalization – genetic and developmental aspects. *A. Rev. Ecol. Syst.* **22**, 65–93.

Simpson, G.G. 1954 *Tempo and mode in evolution*. New York: Columbia University Press.

Tóth, M., Jakab, J. & Novàk, L. 1980 Identification of two components from the sex pheromone system of the white-line dart moth, *Scotia segetum* (Schiff.) (Lep., Noctuidae). *Z. angew. Ent.* **90**, 505–510.

Tóth, M., Löfstedt, C., Blair, B.W. *et al.* 1992 Attraction of male turnip moths *Agrotis segetum* (Lepidoptera: Noctuidae) to sex pheromone components and their mixtures at 11 sites in Europe, Asia and Africa. *J. chem. Ecol.* **18**, 1337–1347.

Waddington, C.H. 1940 *Organisers and genes*. Cambridge University Press.

Van Drongelen, W. & van Loon, J.J.A. 1980 Inheritance of gustatory sensitivity in F1 progeny of crosses between *Yponomeuta cagnagellus* and *Yponomeuta malinellus*. *Ent. exp. appl.* **24**, 199–203.

West-Eberhard, M.J. 1984 Sexual selection, competitive communication and species-specific signals in insects. In *Insect communication* (ed. T. Lewis), pp 281–324. London: Academic Press.