Electroantennogram Responses to Female Sex Pheromones in Five Genera of Lymantriidae (Lepidoptera)

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Sex pheromones of European species of *Lymantria*, *Euproctis*, *Stilpnotia*, *Orgyia*, and *Dasychira* (Lymantriidae) have been cross-checked by recording male electroantennogram (EAG) responses to excised female pheromone glands. Within the same genus, there was invariably full reciprocity of the gland effects. Between different genera, however, in all species combinations investigated the males strongly preferred their own species. From this pattern it is concluded that the major pheromone constituents are different for the five genera. In accordance with these results, several species of *Lymantria* are either known or supposed to produce the same sexual attractant, *cis*-7,8-epoxy-2-methyloctadecane (disparlure), whereas for one species of *Orgyia* the sex pheromone was recently identified (Smith *et al.*, Science **188**, 63 [1975]) as *cis*-6-heneicosen-11-one. None of the additional lymantriid pheromones have yet been chemically defined. In EAG screening tests, some species of this family were specifically responsive to hydrocarbons related to *cis*-7,2-methyloctadecene, the olefinic precursor of disparlure.

The potential of disparlure (*cis*-7,8-epoxy-2-methyloctadecane) [1], the sex attractant produced by the female gypsy moth (*Lymantria dispar* L.) [1], as a tool for manipulating populations of this pest species has been increasingly investigated [2–5]. The same compound has recently [6] been found in sex pheromone glands of the female nun moth (*Lymantria monacha* L.), thus corroborating earlier reports of cross-attraction between these two species [7, 8], and of the strong attractancy of synthetic disparlure also for *L. monacha* males [9–13]. Males of other *Lymantria* species including the Indian gypsy moth (*L. obfuscata* WALK.) and two Japanese forms (*L. fumida* BUTL., *L. dispar* ssp. *japonica* MOTSCH.) are also strongly responsive to this compound [14, 15]. A ketone, *cis*-6-heneicosen-11-one, was recently [15a] identified as the sex pheromone of the female Douglas fir tussock moth (*Orgyia pseudotsugata* McDUNN.). With regard to the remaining Lymantriidae genera (comprising a total of approx. 3000 species), there is so far no report on the chemical nature of their sex pheromones, or the effects of synthetic compounds on male behaviour.

Once a few pheromone structures have been chemically identified within a lepidopterous family, the pheromones of additional species of this taxon may be approached by screening synthetic derivatives, altered with regard to characters likely to have changed during evolution. In certain other moth families, distinct small changes such as, *e.g.*, the alteration of chain length, the shift of a double bond, or the conversion to the opposite geometric isomer, have been evidenced [16–18] to be such evolutionary steps in the structure of the pheromone molecule. For the Lymantriidae, the two compounds listed above are the starting point for this kind of investigation.

In studies of this type, the interspecific effects of the naturally-produced pheromones of the species have to be considered in comparison to the effects of synthetic compounds. Some results of electroantennogram (EAG) cross-checking experiments are presented here which have relevance to recent reports [6, 19] on the occurence and isomeric nature of disparlure.

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Methods

As in similar studies in other moth families [20–22], abdominal tips of unmated females were cut off and placed in individual glass cartridges (5 cm long, i.d. 5.5 mm). For stimulation, the cartridges were positioned on the outlet of a compressed air system so that a defined air current (1.0 m/sec) could be directed for 1.0 sec over the pheromone gland towards the middle of the male antennal preparation. Series of such female gland preparations were made for each test species and replaced as needed. Between the experiments, the gland preparations were kept at $-18\,^{\circ}C$, thus maintaining their effectiveness up to several years.

The methods of male preparation, stimulation, and electrophysiological recording are essentially the same as described earlier [20]. All statements are based on the maximum electroantennogram (EAG) amplitude elicited during the time of 0.1 sec of stimulation.

Results and Discussion

Since the female gland preparations could be maintained over a period of time (see above), the experimental program is restricted mainly by the number of living males of different species available on the same day. Under constant conditions of stimulation, the same test program (a series of gland preparations of each species) was offered to male antennal preparations of each species. For a given combination of species, the degree of interspecific effectiveness of their pheromones is then indicated by the relative EAG amplitudes.

A typical example is shown in Fig. 1. As can be noted, the intraspecific responses can vary strongly depending on the responsiveness of the antennal preparation used, and on the pheromone content of the gland preparations. When evaluating the degree of interspecific reciprocity, it is therefore necessary to refer to those preparations which gave the relatively highest intraspecific responses (*e. g.*, of the two males and six females of *L. monacha* listed, the male 2, and the females 13, 21, and 23).

Of the six species in Fig. 1, the two *Lymantria* species show strong reciprocal responses, as do the two *Euproctis* species. Further experiments substantiate that in these species combinations there is full reciprocity, *i. e.*, the species differences were not distinguishable from individual differences found within the same species. *Stilpnotia salicis* and *Orgya antiqua*, on the other hand, clearly prefer their own species.

When these comparisons were extended to further species of the family (not shown in Fig. 1), some exhibited full reciprocity with one of the six il-

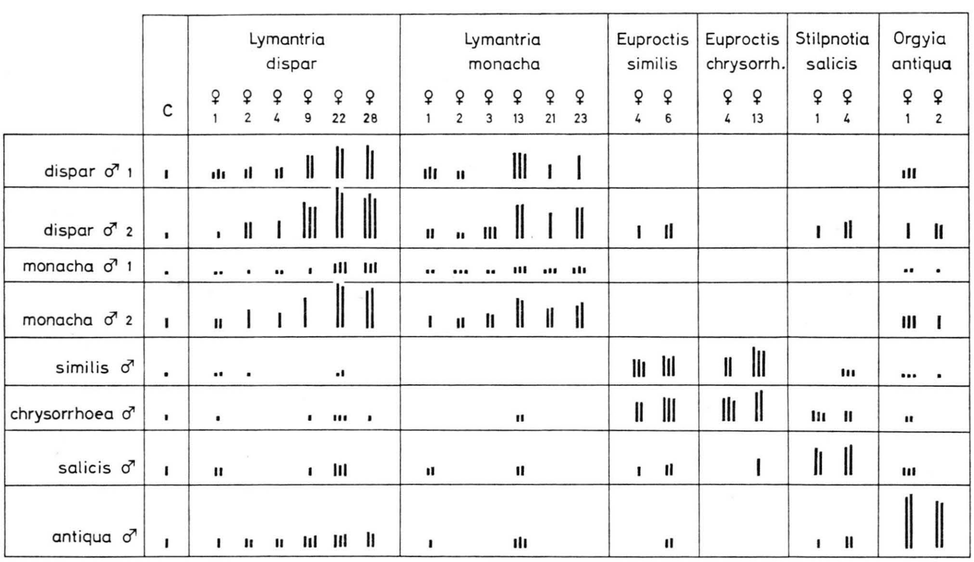

Fig. 1. Intra- and interspecific electroantennogram (EAG) responses to female sex pheromones for six species of Lymantriidae (Lepidoptera). On the same day (July 15, 1965), the individual pheromone gland preparations listed (♀, indicated by number) were presented to the eight antennal preparations (♂). Each bar represents the maximum amplitude for a single EAG response. Absence of a bar indicates that this individual combination was not tested. C is the control response to pure air current without pheromone. Up to three recordings are listed for a given ♂ – ♀ combination to illustrate that intraindividual variation does not exceed 15% of the total EAG amplitude. Within the same species combination, however, there can be extreme interindividual variation due to differences in pheromone content (♀) and in responsiveness (♂). Because of this variation, any species comparisons have to be evaluated separately for each individual ♂ and ♀. These evaluations are then based on relating the two interspecific responses (♂A♀B, ♂B♀A) to the two intraspecific ones (♂A♀A, ♂B♀B). As can be seen, the two *Lymantria* species show almost full reciprocity in the gland effects, as do the two *Euproctis* species. In all the other species combinations listed, the interspecific responses are much smaller than the intraspecific responses.

lustrated species (*e. g.*, *Orgyia gonostigma* F. or *O. ericae* GERM. with *O. antiqua*). Other lymantriids showed only weak affinity with these species, comprising a 'reaction group' of their own, such as the *Dasychira* species *pudibunda* L., *fascelina* L., *abietis* SCHIFF., and *selenitica* ESP. The overall pattern emerging from these experiments in the Lymantriidae corresponds to those which have been determined for other Lepidoptera families [20–22]; *i. e.*, monophyletic groups of species (often represented by one genus) comprise a 'reaction group'.

The results leave no doubt that the pheromone constituents responsible for the EAG effect are different for each of the five genera mentioned here (*Lymantria, Euproctis, Dasychira, Stilpnotia, Orgyia*). However, the observed pattern would be consistent with the assumption that species of the same genus produce the same major pheromone constituent, as has been verified [1, 6] for the two *Lymantria* species. Of various synthetic analogues tested in EAG and single cell measurements [23], dispar-disparlure was by far the most effective structure for both *L. dispar* and *L. monacha* [23]; this strongly suggests that the full EAG reciprocity of the gland preparations of the two species (Fig. 1) is indeed due to disparlure (and not to any other volatile substance).

On the other hand, EAG recordings do not necessarily reflect the effects of all the different pheromone constituents produced by a female moth, and of all the olfactory receptor types of the male antenna. Also for the two *Lymantria* species, the results leave open the possibility of species specific chemical signals in addition to the common disparlure message. Indeed, although in field experiments the two species show strong cross attractancy, in competitive tests there is a certain preference for the same species [7, 8]; the nature of these species differences is unknown (see also [6]).

Between different lymantriid genera, the gland preparations showed only weak, if any, effects (Fig. 1). It is not possible to say on the basis of these measurements whether these partial interspecific EAG effects (♀A on ♂B) are due to

a. a small amount of pheromone B (in addition to pheromone A) present in female A;
b. the presence of receptors specialized for A (in addition to those for B) on the antenna of male B; or
c. the effects of substance A on receptors B.

Thus, the weak EAG responses of, *e. g.*, *Stilpnotia salicis* or *Orgyia antiqua* to some *Lymantria* gland preparations (Fig. 1) could be responses to disparlure, or to other compounds liberated by the stimulus sources. Possibility (c.) is supported by the observations that those gland preparations which gave the highest intraspecific response are also preferred interspecifically (see Fig. 1), and by the recent findings that synthetic disparlure elicits weak EAG responses in male *S. salicis* and *O. antiqua* comparable to the *Lymantria* gland effects.

Due to its chiral centers at C_7 and C_8, disparlure is optically active [24]. Both enantiomers have recently [19] been synthesized in approx. 96% purity and tested on male gypsy moths (*L. dispar*) behaviourally and in EAG measurements [19]. Based on amounts necessary to elicit equivalent responses, in both tests the (+) isomer was at least 100 times more effective than the (−) isomer. Such tests have not yet been conducted in the male nun moth (*L. monacha*). The isomer proportions produced by *L. dispar* and *L. monacha* are not known. If in both species the females preferentially produce only one isomer (as has been shown for optically active pheromones in other insects [25]), it must be the (+) form; this follows from the large difference in effectiveness of the enantiomers (see above) considered with the full EAG reciprocity of the gland preparations (Fig. 1). The results, however, do not exclude other (less likely) possibilities; *e. g.*, that both species produce an approx. 1:1 racemic mixture and the *monacha* males respond more strongly to the (−) portion.

Could (−) disparlure be the pheromone in any of the additional lymantriids investigated? The synthetic disparlure formulations used in the present study were a 1:1 racemate. Their failure to elicit higher EAG responses clearly excludes the above possibility for the other genera investigated so far. *Trans*-disparlure and several *cis*-epoxyde analogues could similarly be excluded. However, some $C_{17}-C_{21}$ ketones and hydrocarbons were found to elicit remarkably high EAG responses in certain Lymantriidae genera, such as *Stilpnotia* or *Euproctis*. These compounds include 2-methylheptadecane (see below), and *cis*-7,2-methyloctadecene, the olefinic precursor [26–28] in the biosynthesis of disparlure.

In this context the close taxonomic relationship between the Lymantriidae and the Arctiidae (tiger moths) has to be considered. So far, the only arctiid pheromone which has been chemically identified is

2-methylheptadecane[29], isolated from females of New World species of *Holomelina* and *Pyrrharctia*[29]. Additional pheromones in this family are indicated by the results of earlier EAG cross-checking of species from 16 arctiid genera[22]. When these EAG comparisons were extended to species combinations between these two families, there was no case of full EAG reciprocity; however, the partial interspecific effects were higher for several lymantriid-arctiid combinations than for the majority of the lymantriid-lymantriid combinations. This corresponds well with the preference, by the respective lymantriid species, for hydrocarbons over disparlure and its analogues (see above).

Interspecific EAG responses between members of taxonomically-related Lepidoptera families have been demonstrated earlier. Cross checking experiments within 104 species of Saturniidae (emperor moths) and 25 species of Sphingidae (hawk moths) have resulted in respectively 19 and 7 groups of fully reciprocally-responding species[20–22]. Each of these 'reaction groups' was considered monophyletic on the basis of morphological characters. Whereas there were almost no EAG responses betwen the phylogenetically most ancient and most advanced species of the same family[20], EAG responses were high between the most ancient Saturniidae and Sphingidae species[20–22]. The same pattern has now been found for the Arctiidae and Lymantriidae. In both cases it supports the idea of a common origin for the two families.

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