Journal of Chemical Ecology, Vol. 21, No. 5, 1995

DIFFERENCES IN SEX PHEROMONE COMMUNICATION SYSTEMS OF CLOSELY RELATED SPECIES: *Spodoptera latifascia* (WALKER) AND *S. descoinsi* LALANNE-CASSOU & SILVAIN (LEPIDOPTERA: NOCTUIDAE)

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(Received October 27, 1994; accepted January 31, 1995)

Abstract—*S. latifascia* and *S. descoinsi* are closely related species that occur sympatrically over limited areas in French Guiana. We examined allopatric populations, *S. latifascia* originating from Barbados and *S. descoinsi* from French Guiana. Studies on nocturnal activity cycles showed temporal partitioning of female calling behavior, male sexual activity, and mating behavior. *S. descoinsi* were sexually active in the first half of the scotophase whereas *S. latifascia* were sexually active in the second half. Seven compounds (*Z*9-14:Ac, *Z*9,*E*12-14:Ac, Z11-16:Ac, *E*9,*E*12-14:Ac, *Z*9-14:Ald, *Z*9,*E*11-14:Ac and Z11-14:Ac) were identified in females of both *S. latifascia* and *S. descoinsi* extracts. *Z*9-14:Ac was a main pheromone component for the two species. The major difference between the pheromones of *S. latifascia* and *S. descoinsi* was the proportion of *Z*9,*E*12-14:Ac in the extracts: 7% for *S. latifascia* and 42% for *S. descoinsi*. The proportion of *Z*9,*E*12-14:Ac relative to the sum of *Z*9-14:Ac and *Z*9,*E*12-14:Ac in individual gland extracts was 4 ± 1% (mean ± standard deviation) for *S. latifascia* and 44.8 ± 6% for *S. descoinsi*. Electrophysiological studies showed no major differences between species in the morphology and physiology of the pheromone receptors of males. Receptors were identified for *Z*9-14:Ac and *Z*9,*E*12-14:Ac, but no receptor was found for the other compounds. In the wind tunnel, synthetic blends with *Z*9-14:Ac and *Z*9,*E*12-14:Ac gave the same behavioral responses as conspecific female extracts for the males of the two species. Some cross-attraction was observed with synthetic blends and female extracts. Nethertheless, previous field trapping experiments in French Guiana

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0098-0331/95/0500-0641$07.50/0

were species-specific and suggested differences in the attractivity of males. In the laboratory, *S. latifascia* and *S. descoinsi* could hybridize in both reciprocal crosses. Female *S. descoinsi* × male *S. latifascia* mating rate was significantly lower than for the reciprocal cross, and 26.7% of female *S. descoinsi* could not separate from male *S. latifascia* after mating. These copulatory problems may involve genital incompatibilities between males and females. Several barriers against interbreeding between *S. latifascia* and *S. descoinsi* seem to combine including differences in nocturnal activity cycles, pheromone differences, and genital barriers. The study of sympatric populations will be necessary to define the role of sex pheromones in the reproductive isolation of *S. latifascia* and *S. descoinsi*.

Key Words—Lepidoptera, Noctuidae, *Spodoptera latifascia*, *Spodoptera descoinsi*, pheromones, (*Z*)-9-tetradecenyl acetate, (*Z,E*)-9,12-tetradecadienyl acetate, sexual activity rhythms, cross-attraction, electrophysiology, reproductive isolation.

INTRODUCTION

When different species occur sympatrically, precopulatory (ecological or ethological) and/or postcopulatory barriers (mechanical isolation, gametic mortality or incompatibility, inviability or sterility of hybrid zygotes) are necessary to keep them reproductively isolated (Mayr, 1942; Dobzhansky, 1970). In moths, differences in pheromone communication systems between species may be an important barrier against interspecific matings. The specificity of the pheromone blend emitted by females is the most common factor limiting reproductive interactions (Roelofs and Cardé, 1974). In some cases, this specificity is not sufficient to prevent hybridization, and other mechanisms are required to ensure complete reproductive isolation. Hence, in order to understand the role of pheromones in reproductive isolation, it is critical to study their specificity.

Spodoptera latifascia (Walker) is a noctuid moth of the *Amphipyrinae* subfamily. It occurs from Brazil to the United States where it can cause damage to vegetable crops (King and Saunders, 1984; Godfrey, 1987). Field-trapping experiments with sexual attractants in French Guiana enabled us to discover a new population of *Spodoptera* morphologically close to *S. latifascia* but attracted by a different pheromone blend (Lalanne-Cassou et al., 1994). While *S. latifascia* males were mainly caught by binary mixtures of (*Z*)-9-tetradecenyl acetate (*Z*9–14 : Ac) with 4–24% of (*Z,E*)-9,12-tetradecadienyl acetate (*Z*9,*E*12–14 : Ac), the new population of males was attracted by mixtures containing 32–48% of *Z*9,*E*12–14 : Ac. Furthermore, males and females of this particular population could be distinguished from *S. latifascia* by small differences in wing and body coloration and by the morphology of the genitalia. The stability of these phenotypical differences, the sympatry of the two moth populations in limited areas, and the differences in sexual attractants led us to consider this population, which

seems to be endemic to French Guiana, as a new species. It was described and named *S. descoinsi* (Lalanne-Cassou et al., 1994).

The present work deals with a comparison of pheromone communication systems between allopatric populations of *S. latifascia* and *S. descoinsi*. Female calling rhythms and pheromone composition were determined for the two species. Male olfactory and behavioral responses to pheromone were studied by electroantennography and single sensillum recordings and by wind tunnel experiments, respectively. The potential role of these characters in the reproductive isolation of *S. latifascia* (SL) and *S. descoinsi* (SD) is discussed.

METHODS AND MATERIALS

Rearing of Insects. A SL culture was established from larvae collected in the field in Barbados in 1990 and a SD culture from adults caught by light traps in French Guiana in 1992. Larvae were fed on a semiartificial diet supplemented with cabbage and corn powders. They were maintained at 23°C and 70% relative humidity under a 12 : 12 hr light–dark regime. At pupation, males and females were separated in distinct rooms.

Female Calling Behavior and Mating Rhythms. At emergence, females were placed individually in 250-cm^3 plastic boxes for observation of calling behavior or placed with a male in 690-cm^3 plastic boxes for observation of mating periods. Preliminary studies showed no calling behavior during the photophase. Observations were then performed during the scotophase every 0.5 hr with a red lamp at a light intensity of 1 lux on 1- to 8-day-old females for SD and on 1- to 6-day-old females for SL. The day of emergence was designated day 1. Mating rates were estimated on groups of 15 pairs per box by dissecting females after death and searching for spermatophores transferred by males during copulation. Mating rhythms were observed during the scotophase every 0.5 hr on pairs formed with individuals of the same age. The origin of time measures for calling and mating was fixed at lights off.

Chemical Analysis. Pheromone glands of calling females were dissected during the scotophase between 3 and 7 hr after lights off for SD and between 6 and 10.5 hr after lights off for SL. Glands were individually soaked in 15 μl of hexane during 40 sec to study interindividual variation or extracted in batches of 15–30 in 250 μl of hexane during 2 hr to determine the average composition of pheromone in the populations and to detect minor compounds. Global extracts were analyzed by gas chromatography–mass spectrometry (GC-MS) in the electronic impact ionization mode on a Nermag R10-10C quadrupole interfaced with a Girdel 32 gas chromatograph. A WCOT capillary column (25 m × 0.32 mm ID Chrompack) with a polar phase FFAP-CB or a CPSil 88-CB column (60 m × 0.32 mm ID, Chrompack) was temperature programmed from 140 to 240°C

at 5°C/min. Compounds in the extracts were identified by comparison with retention times and mass spectra of synthetic standards analyzed under the same conditions. Individual gland extracts were subjected to GC analysis with a Girdel 300 or a Carlo Erba Fractovap 2900 chromatograph with flame ionization detectors and splitless and Ross injectors, respectively, for the Girdel 300 and the Carlo Erba. Helium was used as carrier gas at approximately 27 cm/sec linear velocity. A capillary column WCOT (25 m × 0.32 mm ID Chrompack) with a polar phase FFAP-CB was used. The temperature program for the Girdel 300 was 120 to 240°C at 3°C/min, with injector and detector temperatures at 240°C. The program for the Carlo Erba Fractovap 2900 was 55 to 140°C at 35°C/min, 140 to 210°C at 5°C/min with injector temperature at 225°C and detector temperature at 250°C. In each individual gland extract, the peaks for *Z*9,*E*12-14:Ac and *Z*9-14:Ac were identified by comparison with the retention times of synthetic standards analyzed under the same conditions.

Electrophysiology. Electrophysiological activity of SL and SD male antenna was measured by electroantennographic (EAG) and single sensillum recording (SSR) techniques as previously described by Lucas and Renou (1989). All experiments were carried out on whole insect preparations. Briefly, for EAGs, the apex of the antenna was excised and inserted into the recording electrode. The reference electrode was implanted in the neck of the insect. Both electrodes were connected to a Neurolog NL102 preamplifier through chloridized silver wires. The EAG responses were filtered (DC, 300 Hz) and amplified (×1000). In order to eliminate the differences in absolute sensitivity between individuals, corrected EAG data were calculated. For each individual, the response to a given compound was divided by the mean of the responses to all compounds tested (Renou et al., 1988). The responses of 10 males were measured for each species. Means of the corrected data and their confidence intervals were calculated. For SSRs, the tip recording technique of Kaissling and Thorson (1980) was used. Glass electrodes were filled with sensillar saline for the recording electrode and with hemolymph saline for the reference electrode. The reference electrode covered the tip of the antenna, the last segments of which had been cut. The tips of several sensilla were cut off using sharpened forceps, and the recording electrode was slipped over the cut end of one hair. The SSR responses were filtered (DC, 5000 Hz) and amplified (×1000). The recordings were stored on a PC-AT-compatible microcomputer via a DASH 16 (Metrabyte) analog-to-digital conversion board. Acquisition and analysis of the recordings were performed by specific programs developed in our laboratory and written in Asyst language (Macmillan Software Company).

The olfactory stimulations were delivered from Pasteur pipets containing a piece of filter paper on which 500 ng of diluted test compound had been deposited. The antenna was continuously flushed with humidified air (1.0 liter/min) and stimulations were achieved by blowing a puff of air (1 sec, 0.5 liter/min)

through the Pasteur pipet. Nineteen stimulus compounds were tested in a random order.

Male Sexual Activity and Behavioral Response to Pheromone. Male sexual activity was first tested with a simple bioassay. Four males, one to three days after the emergence, were kept together in boxes and maintained separated from females to prevent any contact with pheromone. A piece of filter paper impregnated with conspecific female gland extract at a dose of 0.5 female equivalent (FE) was introduced for 5 min into a box containing four males. A group of four males was tested every hour during the scotophase, and each group was tested only once. When sexually excited, the males behaved in a characteristic way by rapidly beating their wings (wing fanning), displaying genitalia, and displaying a pair of hairpencils situated on eversible epithelial pouches on the genital valvae. Wing fanning was chosen as a behavioral indicator of male sexual activity.

Male attraction to pheromone blends was studied in a 200 × 90 × 70-cm Perspex wind tunnel. The air speed was approximately 0.2 m/sec and the temperature 23°C. Red fluorescent tubes were placed above the tunnel to provide illumination of 0.3 lux. Tests were performed on 2- to 4-day-old males, between 3½ and 6 hr after the onset of the scotophase for SD, and between 7½ and 10 hr after the onset of the scotophase for SL. Males were placed on a wired cage at 1.5 m from the source made of a piece of filter paper impregnated with natural or synthetic pheromone. A dead male was fixed as a decoy on the source of pheromone. We chose seven phases of the behavioral response to describe male attraction to pheromone blend: (1) walks with or without wing fanning (W); (2) taking flight (Tf), males flew away from the wired cage; (3) orientation (O), males flew up the odor plume; (4) touching the source in flight without landing (T); (5) landing at the source (L); (6) precopulatory behavior (Pb), hairpencil or genital displays; (7) copulatory attempts *sensu stricto* (Ca).

Previous field-trapping experiments in French Guiana (Lalanne-Cassou et al., 1994), suggested to us to test two binary mixtures of *Z*9–14 : Ac and *Z*9,*E*12–14 : Ac at different doses: (1) SL 50 and SL 500: the ratio of *Z*9,*E*12–14 : Ac to *Z*9–14 : Ac was 5 : 95 at 50 and 500 ng, respectively, of *Z*9–14 : Ac; and (2) SD 50 and SD 500: the ratio of *Z*9,*E*12–14 : Ac to *Z*9–14 : Ac was 50 : 50 at 50 and 500 ng, respectively, of *Z*9–14 : Ac. Furthermore, female SD extracts were tested at a dose of 0.5 FE and female SL extracts at doses of 0.15, 0.5, and 1.5 FE. Each male was tested only once.

The percentage of male response was calculated for each of the seven behavioral steps as the number of males exhibiting a given behavior divided by the number of tested males. Differences in the behavioral responses to the extracts and the synthetic blends were analyzed by using χ^2 tests for each behavioral step (2 × 4 for SD and 2 × 7 for SL) with the threshold of significance set at 0.05. When heterogeneity was detected, all treatments were compared using 2

× 2 contingency tables. To minimize the risk of error arising from the increase of degrees of freedom, the threshold of significance was set to 0.01.

Chemicals. Compounds used as standards in the chemical analysis of the pheromone, as stimulus compounds in electrophysiological studies, or in wind tunnel experiments were all synthesized in the laboratory according to the procedures described by Mori (1981), Ramiandrasoa and Tellier (1990), and Renou et al. (1979, 1981). Their chemical purity was at least 98% as determined by gas chromatographic analysis.

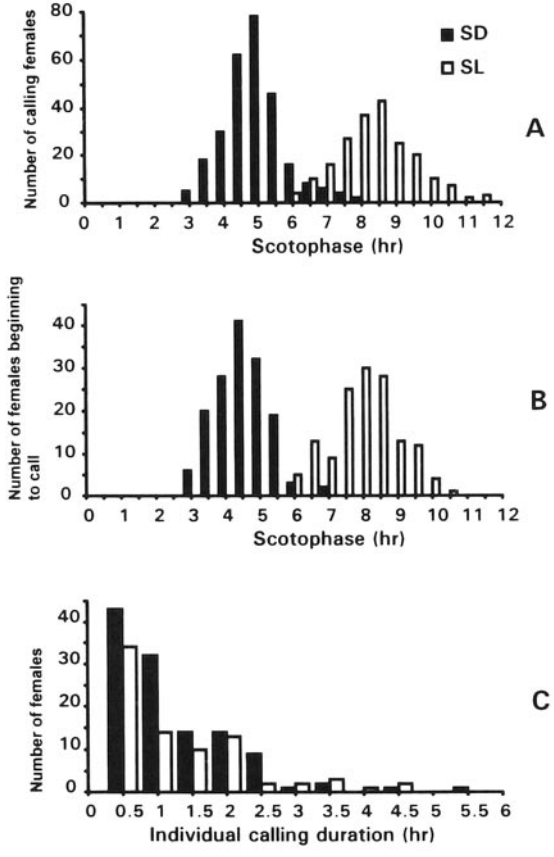

FIG. 1. Nocturnal calling periods of female SD and SL (A), beginning of calling behavior (B), and individual calling duration (C).

RESULTS

Female Calling Behavior. Calling behavior studies indicated marked temporal differences between species (Mann-Whitney test, $P < 0.001$) despite a slight overlap (Figure 1A). For SD, the maximum calling activity occurred in the first half of the scotophase, 5 hr after onset. Calling began at 4.5 $\pm$ 0.8 hr (mean $\pm$ SD) with a variation from 3 to 7 hr (Figure 1B). In contrast, for SL, the peak calling activity occurred in the second half of the scotophase (8.5 hr) (Figure 1A). Calling began at 8.2 $\pm$ 1 hr with a variation from 6 to 10.5 hr (Figure 1B). The beginning of calling was significantly different between SL and SD (Mann-Whitney test, $P < 0.001$). Individual calling durations (Figure 1C), 1.2 $\pm$ 0.9 hr and 1.3 $\pm$ 0.9 hr, respectively, for SD and SL, were not significantly different between species (Mann-Whitney test) at the 5% level of significance.

Mating Behavior. SL and SD matings occurred at significantly different times of the night (Mann-Whitney test, $P < 0.001$) despite an overlap of 2.5 hr (Figure 2A). Copulation durations (Figure 2B), 1.1 $\pm$ 0.7 hr and 1 $\pm$ 0.5 hr, respectively, for SL and SD, were not significantly different between species

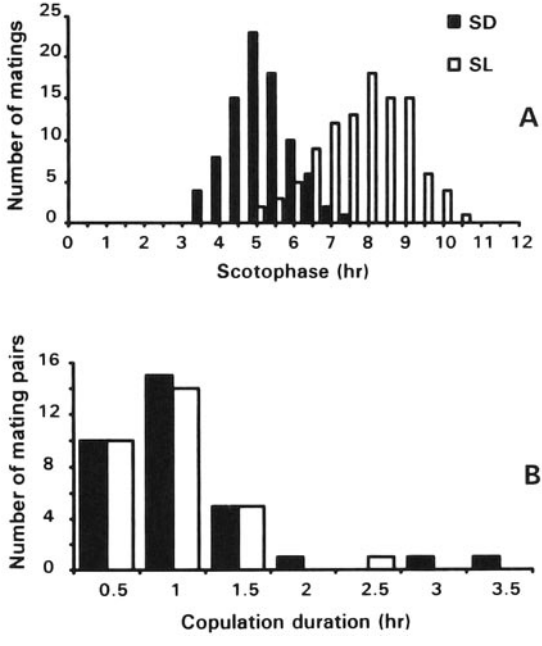

FIG. 2. Mating periods in SD and SL (A) and mating duration (B)

TABLE 1. MATING EFFICIENCY IN SL AND SD CROSSES[a]

Cross ♀ × ♂	Pairs (*N*)	Fertile matings (%)	Stuck pairs (%)
SD × SL	60	81.7b	0
SD × SD	60	83.3b	0
SL × SD	60	65.0b	0
SD × SL	60	10.0a	26.7

[a]Numbers followed by the same letter are not significantly different by χ^2 test at the 5% level of significance. Stuck pairs: male and female could not separate after copulation.

(Mann-Whitney test, $P > 0.05$). Fertile interspecific matings (giving offspring) could be obtained in both reciprocal crosses between SL and SD (Table 1). The SL × SD mating rate (65%) was not significantly different from those of the parental crosses (81.7% and 83.3% for SL × SL and SD × SD, respectively), (χ^2 test, $P > 0.05$). However, SD × SL mated less effectively than the reciprocal (SL × SD) and the parental crosses. Only 10% of pairs gave offspring and 26.7% of females could not separate from males after copulation.

Pheromone Composition. Analysis of an extract of 23 female SD glands showed the presence of six acetates and one aldehyde (Table 2). *Z*9–14:Ac [mass spectrum, *m/z* 41, 43 (100%), 53, 55, 61, 67, 81, 82, 96, 109, 194 (M-$CH_3COOH)^+$] and *Z*9,*E*12–14:Ac [mass spectrum, *m/z* 41, 43 (100%), 55, 61, 67, 68, 79, 81, 93, 95, 107, 121, 135, 149, 192 (M-$CH_3COOH)^+$] were found to be the main pheromone compounds in the extract with 41% of *Z*9–14:Ac and 42% of *Z*9,*E*12–14:Ac. Minor compounds could be identified as *Z*11–

TABLE 2. PERCENTAGES OF COMPOUNDS IN GLAND EXTRACTS FROM FEMALE SD AND SL

Compounds	SD (%) (23 females)	SL (%) (14 females)
*Z*9–14:Ald	2	8
*Z*9–14:Ac	41	75
*Z*11–14:Ac	<1	<1
*Z*9,*E*12–14:Ac	42	7
*E*9,*E*12–14:Ac	3	1
*Z*9,*E*11–14:Ac	1	1
*Z*11–16:Ac	11	8

16:Ac (11%) [mass spectrum, *m/z* 41, 43, 55 (100%), 61, 67, 81, 82, 96, 110, 123, 137, 151, 222 (M-CH_3COOH)$^+$], *E*9,*E*12-14:Ac (3%) [mass spectrum, *m/z* 41, 43 (100%), 55, 61, 67, 68, 79, 81, 93, 95, 107, 121, 135, 149, 192 (M-CH_3COOH)$^+$], *Z*9-14:Ald (2%) [mass spectrum, *m/z* 41, 43, 55 (100%), 67, 69, 81, 95, 98, 107, 121, 192 (M-CH_3COOH)$^+$], *Z*9,*E*11-14:Ac (1%) [mass spectrum, *m/z* 41, 43, 55, 61, 67 (100%), 79, 82, 93, 95, 107, 121, 135, 149, 192 (M-CH_3COOH)$^+$] and *Z*11-14:Ac (<1%) [mass spectrum, *m/z* 41, 43 (100%), 55, 61, 68, 82, 96, 110, 127, 194 (M-CH_3COOH)$^+$]. The retention times of these compounds were identical to those of synthetic references both with the WCOT column (*Z*9-14:Ac 4.3 min, *Z*9,*E*12-14:Ac 5 min, *Z*11-16:Ac 6.6 min, *E*9,*E*12-14:Ac 5.3 min, *Z*9-14:Ald 3 min, *Z*9,*E*11-14:Ac 5.9 min, and *Z*11-14:Ac 4.5 min) and with the more polar CPSil 88-CB column (*Z*9-14:Ac 11 min, *Z*9,*E*12-14:Ac 12.3 min, *Z*11-16:Ac 13.8 min, *E*9,*E*12-14:Ac 12.8 min, *Z*9-14:Ald 9.8 min, *Z*9,*E*11-14:Ac 13.7 min, and *Z*11-14:Ac 11.2 min). These same pheromone compounds were identified in the female SL extract (14 glands). *Z*9-14:Ac was the major pheromone compound (75%). *Z*11-16:Ac (8%), *Z*9-14:Ald (8%), *Z*9,*E*12-14:Ac (7%), *E*9,*E*12-14:Ac (1%), *Z*9,*E*11-14:Ac (1%), and *Z*11-14:Ac (<1%) were found as minor compounds. The pheromone compositions of SL and SD differed by the proportion of *Z*9-14:Ald in the extracts (2% for SD and 8% for SL), but the main difference was the proportion of *Z*9,*E*12-14:Ac (42% for SD and 7% for SL).

To confirm the differences found between the global extracts of female SL and SD, we analyzed individual gland extracts. The proportions and quantities of *Z*9-14:Ald were low for the two species. Therefore, we chose to calculate the percentage of *Z*9,*E*12-14:Ac relative to the sum of *Z*9-14:Ac and *Z*9,*E*12-14:Ac {[*Z*9,*E*12-14:Ac/(*Z*9-14:Ac + *Z*9,*E*12-14:Ac)] × 100}. This percentage averaged 4 ± 1% (mean ± SD) and ranged from 2 to 7.4% for SL, while it averaged 44.8 ± 6% and ranged from 29.1 to 59.2% for SD (Figure 3). Individual variation was high for the two species with coefficients of variation [CV = (SD/mean) × 100] reaching 26% for SL and 13.3% for SD. Despite this intraspecific variation, SL and SD distributions were significantly different (Mann-Whitney test, $P < 0.01$) and did not overlap. Percentages were stable among generations, allowing data pooling. *Z*9-14:Ac amounts averaged 61.1 ± 53 ng for SL and 56.5 ± 49.5 ng for SD. They were highly variable in the individual gland extracts (CV = 86.9% for SL and 87.6% for SD), but not significantly different between species (Mann-Whitney test, $P > 0.05$) (Figure 4).

Electrophysiology. *Z*9,*E*12-14:Ac was the most active compound for both SL and SD males (corrected EAG responses SD: 2.0; SL: 2.5) (Figure 5). *Z*9-14:Ac showed significant lower levels of responsiveness for the two species (one-way ANOVA, Newman-Keuls test, $P < 0.05$), whereas this compound

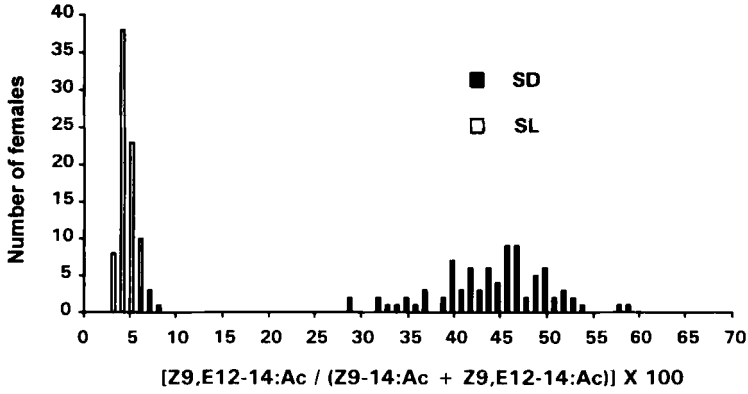

FIG. 3. Percentages of *Z*9,*E*12–14 : Ac relative to the sum of *Z*9–14 : Ac and *Z*9,*E*12–14 : Ac measured by gas chromatography in 84 individual gland extracts of female SD and 83 individual gland extracts of female SL.

was in equivalent proportion to *Z*9,*E*12–14 : Ac in the pheromone of female SD and was the major compound in the pheromone of female SL. *Z*9–12 : Ac also gave strong responses, although it is absent from the pheromone of the two species. Other compounds, except *Z*9,*E*12–14 : OH for male SD, had no significant EAG activity when compared to the control. Among them Z11–16 : Ac, present for 11% in the pheromone of female SD and for 8% in the pheromone

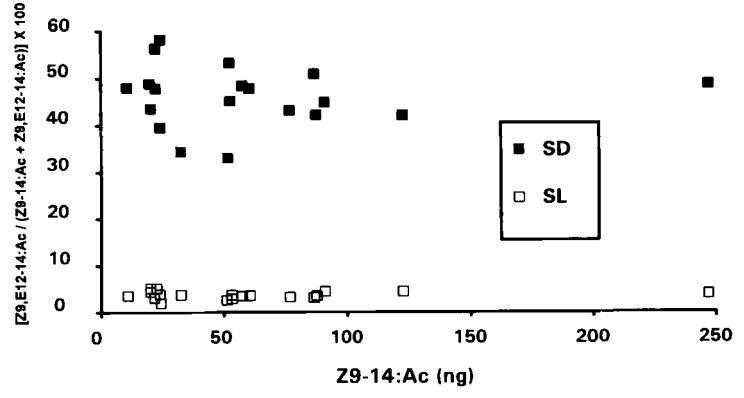

FIG. 4. Relation between the percentage of *Z*9,*E*12–14 : Ac relative to the sum of *Z*9–14 : Ac and *Z*9,*E*12–14 : Ac, and the quantity of *Z*9–14 : Ac from 23 individual female SD extracts and 19 individual female SL extracts.

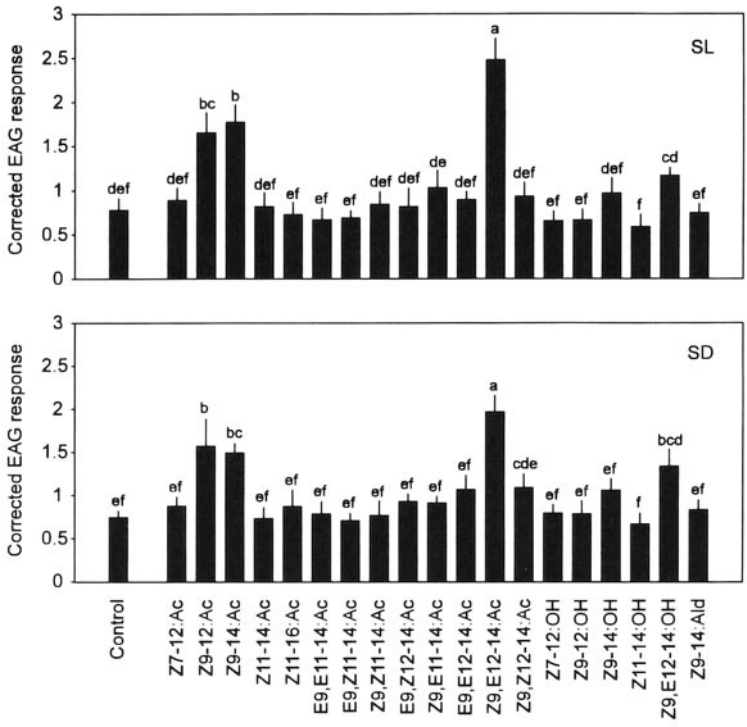

FIG. 5. EAG recordings of male SL and SD with a series of 19 pheromone compounds or analogs and pure air as control. Each compound was tested at 500 ng. Mean of the responses of 10 males. Error bars represent 95% confidence intervals. Bars associated with the same letter are not significantly different at the 5% level of significance (one-way ANOVA, Newman-Keuls test).

of female SL, showed no significant responses compared to the control (corrected EAG responses SD = 0.9; SL = 0.7).

Antennae are filiform in SD and SL. Observation of their morphology under light microscopy demonstrated the presence of at least two classes of sensilla trichodea on the unscaled ventral side of the antenna. These hairs were separated according to their length and location. Each flagellar segment exhibited the same sensillar organization. The longer hairs were located on the lateral parts of the ventral sensory area. They are referred to as the lateral hairs (LHs). Shorter hairs were located medioventrally on the flagellar segments and are referred to as medial hairs (MHs).

The activity of 30 LHs in SL and 43 in SD was recorded (Figure 6). In each LH, the firing activity clearly consisted of two amplitude classes of action potentials, indicating the presence of at least two neurons. The cell firing high-amplitude action potentials, up to 2.8 mV, is referred to as cell A. Action potentials emitted by the other cell, referred to as the B cell, were much smaller (50% of the amplitude of the A cell action potentials). In SD, the amplitude of B-cell spikes was smaller than in SL and was often hardly discriminated from the noise. In both SD and SL, A cells responded specifically to Z9,*E*12–14 : Ac. B cells were less specific since they had two key-compounds, Z9–14 : OH and Z9,*E*12–14 : OH. Moreover in SL, B cells also slightly responded to a third alcohol, Z9–12 : OH, and to a minor pheromone compound, Z9–14 : Ald.

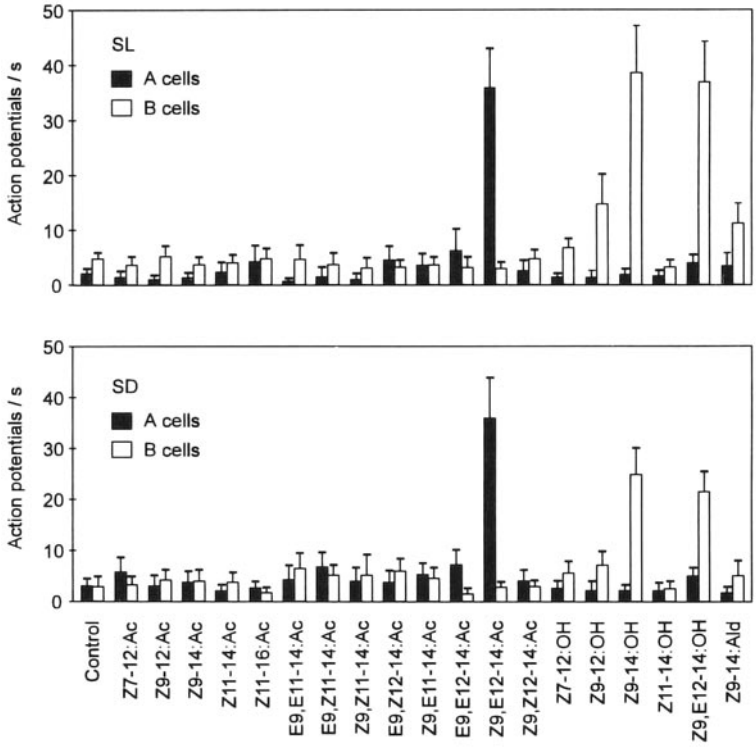

FIG. 6. Response profiles obtained by single sensillum recordings of A and B cells in long lateral hairs of SL (N = 30 hairs) and SD (N = 43 hairs) to 19 pheromone compounds or analogs. Each compound was tested at 500 ng, on 11–30 different hairs for SL and on 11–40 hairs for SD. Vertical bars represent 95% confidence intervals.

MHs appeared to be in smaller number and to be slightly shorter in SD than in SL. Electrical contacts were very unstable in SD. For these reasons, the activity of only three MHs was recorded in SD and Z9–14 : Ald, Z11–16 : Ac, and *E*9,*E*12–14 : Ac were not tested on these hairs because these compounds were not available at the time of the experiments. Analysis of the activity of MHs in both SL and SD always revealed the presence of only one class of action potential amplitudes, with a mean amplitude of 1.4 mV (Figure 7). Equal responses were recorded to the two pheromone compounds Z9,*E*12–14 : Ac and Z9–14 : Ac. None of the other compounds increased the firing activity.

No other sensillar type was discovered. In particular, unlike *S. littoralis*

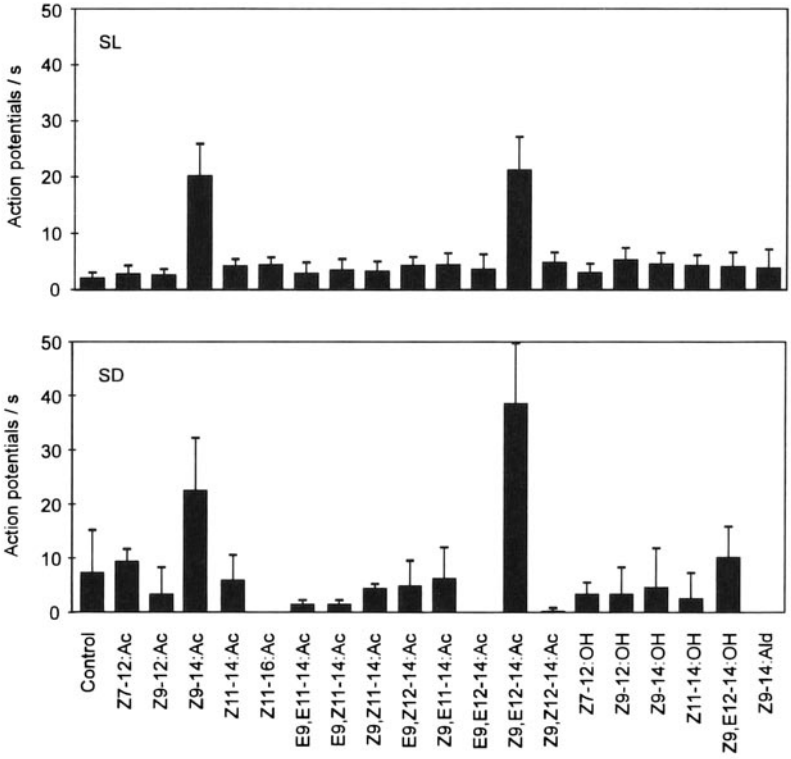

FIG. 7. Response profiles obtained by single sensillum recordings of short medial hairs of SL (N = 23 hairs) and SD (N = 3 hairs). Nineteen pheromone compounds or analogs were tested for SL and 16 compounds for SD. Each compound was tested at 500 ng, on 10–22 hairs for SL and on 2–3 hairs for SD. Z9–14 : Ald, Z11–16 : Ac and *E*9,*E*12–14 : Ac were not tested in SD. Vertical bars represent 95% confidence intervals.

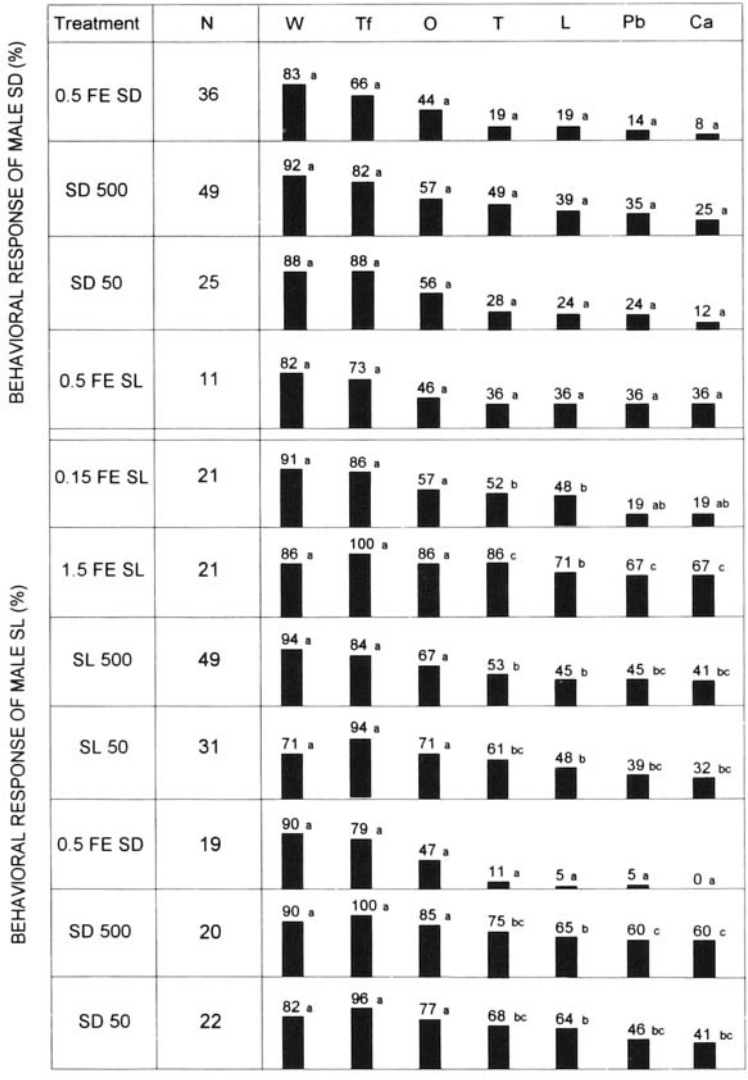

FIG. 8. Behavioral response of male SD and SL to female extracts and synthetic blends in the wind tunnel. For each species, the percentages in the same column followed by the same letter are not significantly different (χ^2 2 × 4 test, $P > 0.05$; χ^2 2 × 2 test, $P > 0.01$ for SD and χ^2 2 × 7 test, $P > 0.05$; χ^2 2 × 2 test, $P > 0.01$ for SL).

(Ljungberg et al., 1993), the most lateral LHs had the same response pattern as other LHs.

Male Sexual Activity and Behavioral Response to Pheromone. Male SL and SD showed distinct peaks of "wing-fanning" response to the pheromone of their conspecific females. The peak occurred at 4–5 hr in the scotophase for SD and at 9 hr for SL. Periods of male sexual activity were significantly different between species (Mann-Whitney test, $P < 0.001$) although they widely overlapped. SL and SD were simultaneously sexually active from 4 to 9 hr. The levels of responsiveness were high for the two species ranging from 20 to 89% for SD and from 34 to 88% for SL. However, wind-tunnel experiments showed low frequencies of behavioral response of male SD (Figure 8). Especially with the 0.5 FE SD treatment, only 8% achieved the complete behavioral sequence until "copulatory attempts." Blends with synthetic components SD 500 and SD 50 were as effective in eliciting behavior as the female extract. No significant difference was observed between these blends. SD males were also attracted by the female SL extract (0.5 FE SL) and completed the behavioral sequence until "copulatory attempts." In that case, frequencies of behavioral categories were not significantly different from all other treatments. Wind-tunnel experiments on male SL revealed that the female extract 1.5 FE SL was more effective than the 0.15 FE SL extract in the last steps of the behavioral sequence ("touching," "precopulatory behavior," and "copulatory attempts"). Blends with synthetic compounds SL 500 and SL 50 showed no significant difference and induced similar levels of behavioral response as female extracts (except SL 500, which gave less "touching" than 1.5 FE SL extract). SL males also showed behavioral response to heterospecific extracts and blends. No significant difference was observed between SD 500 and SD 50 that gave similar levels of behavioral response as conspecific blends and extracts. However, the female extract 0.5 FE SD elicited lower "touching," "precopulatory behavior," and "copulatory attempts" than the other treatments.

DISCUSSION

Female calling behavior studies in SL and SD showed temporal partitioning of nocturnal calling activities. Female SD called in the first half of the scotophase, whereas female SL called in the second half. Mating activities showed the same pattern. Peaks of male sexual activity were distinct between species and concomitant with their respective female ones, but sexual activity periods were longer than in the population of females, showing more substantial overlaps between SL and SD. Some SD males were found to be responsive at the time of the peak calling activity of female SL. Some SL males showed sexual activity at the peak calling activity of female SD. These results suggest that differences

mainly observed in the nocturnal activities of female SL and SD are not sufficient to allow a complete premating isolation between species. A similar case of temporal partitioning of mating activities was observed in *S. frugiperda* between the corn and rice strains. While corn strain females mate in the first two thirds of the scotophase, rice strain females mate in the last one third. This difference in mating rhythms seems to be a strong barrier against interbreeding between the two strains (Pashley et al., 1992).

The same compounds were identified in the female SL and SD extracts. The main difference in the pheromone composition between species was the proportion of *Z*9,*E*12–14:Ac in the extracts: 7% for SL and 42% for SD. Teixeira et al. (1989) identified *Z*9–14:Ac and *Z*9,*E*12–14:Ac as pheromone compounds of female SL. The analysis of the proportion of *Z*9,*E*12–14:Ac relative to *Z*9–14:Ac from individual gland extracts showed that in spite of intraspecific variation, the two species were completely discriminated. While the proportions ranged from 2 to 7.4% for SL, they ranged from 29.1 to 59.1% for SD. Quantities of *Z*9–14:Ac were highly variable between individuals of a given species but not significantly different between species. Differences in pheromone composition between closely related species that depend more on relative proportions of compounds than on different chemical structures have frequently been observed in moths. In the *Yponomeuta* complex, *Y. evonymellus* and *Y. cagnagellus* share the same pheromone compounds, and pheromones mainly differ by the amount of *E*11–14:Ac relative to the *Z* isomer (*Z*11–14:Ac) (Löfstedt and Van Der Pers, 1985). Similarly, in the sibling taxa *Diachrysia chrysitis* and *D. tutti*, pheromone compositions differ by the proportions of *Z*5–10:Ac and *Z*7–10:Ac almost in opposite ratios (Löfstedt et al., 1994).

Electrophysiological studies showed no major differences in the morphology and physiology of the pheromone receptors on the antennae of male SL and SD. Two size classes of sensilla trichodea were identified for both species. While longer hairs were located on the lateral parts of the ventral side (LHs), shorter hairs were distributed medioventrally (MHs). This sensillar organization, found in other noctuid moths such as *Agrotis exclamationis* (Hansson et al., 1986), *Mamestra suasa* (Lucas and Renou, 1991), or *M. brassicae* (Renou and Lucas, 1994), was correlated with sensitivity to different compounds. Receptors to *Z*9–14:Ac and *Z*9,*E*12–14:Ac were identified for SL and SD. *Z*9,*E*12–14:Ac induced the strongest EAG response even for SL, where it is a minor pheromone compound. For the two species, receptors to *Z*9,*E*12–14:Ac were found in the two sensillar types, while receptors to *Z*9–14:Ac were only identified on MHs. No receptor was found for the minor compounds of the pheromone except in SL for *Z*9–14:Ald slightly detected by B cells on LHs. Although not produced by female SL or SD, *Z*9–12:OH, *Z*9–14:OH, and *Z*9,*E*12–14:OH activated olfactory neurons of B cells on LHs. The role of these compounds in the behavior

of males is unknown. Z9–12 : Ac gave strong EAG responses for SL and SD, although it did not increase the firing activity of any of the two sensillar types.

The flight-tunnel bioassay showed that for the two species, binary mixtures with Z9–14 : Ac and Z9,E12–14 : Ac were as effective in eliciting reproductive behavior as female extracts. Hence, these chemicals were critical to the expression of male behavior. Some mutual cross-attraction was observed between SL and SD with synthetic compounds and female extracts. Thus, male behavioral response was initiated whatever the proportion of Z9,E12–14 : Ac relative to Z9–14 : Ac. However, for male SL, the female SD extract elicited significantly fewer behavioral responses than any other treatment, particularly than SD 50 and SD 500. In the SD extract, another (other) component(s), seems to be responsible for these low responses. Further tests are required to confirm this hypothesis. The high levels of mutual cross-attraction with synthetic blends were not in accordance with previous field-trapping experiments. Traps with binary mixtures of Z9–14 : Ac and Z9,E12–14 : Ac, in different proportions and in which positions were rerandomized every night, were tested on pasturelands surrounded by the forest in French Guiana (Matoury) during January 1982 and July–August 1983 (Lalanne-Cassou et al., 1994). These traps caught specifically male SL or SD. Field experiment results suggest differences in male attractivity between species. The flight-tunnel bioassay did not confirm these differences that may occur in the timing of behavioral sequences or in the way of approaching or landing on the trap. Similar cases of cross-attraction in the wind tunnel were found for several species of *Orgyia* (Grant, 1977), for the *Euxoa declarata* group (Byers et al., 1981), and for several members of the stored-product complex of phycitine moths (Phelan and Baker, 1986). The above authors supposed that these interspecific attractions were favored by the similarity of the pheromones used by the closely related species. Grant et al. (1975) observed interspecific courtship between the Indian meal moth, *Plodia interpunctella*, and the almond moth, *Cadra cautella*, but several isolating mechanisms before and during courtship prevented cross-mating. In the small ermine moths (*Yponomeuta*), wind-tunnel experiments also showed a high level of cross-attraction, particularly between *Y. evonymellus* and *Y. vigintipunctatus*, two species with very similar pheromones (Hendrikse, 1986). However, field-trapping experiments with synthetic attractants were species-specific in the natural habitat of the respective species (Löfstedt et al., 1991). The case of *Y. evonymellus* and *Y. vigintipunctatus* is, however, different from that of SL and SD because these species are reproductively isolated by their asynchronous occurrence and differences in habitats (Löfstedt and Van Der Pers, 1985).

In the laboratory, if confined in boxes, SL and SD hybridized and produced fertile offspring in both reciprocal crosses. However, mating frequency was significantly lower in the cross SD × SL than in the reciprocal one. Several

pairs SD × SL could not separate after copulation. Differences in the morphology of genital structures between SL and SD (Lalanne-Cassou et al., 1994) suggest that failure to separate could be linked to genital incompatibilities between males and females. However, further studies are required to understand the importance of morphological features in male–female mating success.

No individual with intermediary morphology between French Guiana populations of SL and SD has been found in the field so far. During the two trapping campaigns in French Guiana in 1982 and 1983, 476 SL and 254 SD of 730 males caught by sexual attractants could be identified in regard to the morphology of the genitalia (Lalanne-Cassou et al., 1994). On the several dozens of individuals caught by light traps, no hybrid form between SL and SD has been found either (Lalanne-Cassou, personal communication).

In the laboratory, with SL individuals originating from Barbados, we found a combination of several mechanisms involved in the reproductive isolation of the species: (1) differences in nocturnal activity cycles may be a partial premating barrier; (2) pheromone specificity has not been proved by flight-tunnel experiments, but it seems to be an effective isolating factor in the field (Lalanne-Cassou et al., 1994); (3) genital differences might constitute a mechanical barrier against interbreeding; and (4) other isolating factors such as differences in host plants might be involved.

Reproductive isolation is generally thought to evolve as an incidental by-product of divergence between allopatric populations. If, upon secondary contact, unfit hybrids are produced, increased isolation will occur. Butlin (1985, 1987) called this process "reinforcement." He distinguished it from the "reproductive character displacement" process of divergence in the mate recognition systems of species already reproductively isolated. In the case of SL and SD, no hybrid morph has been found in French Guiana so far, suggesting that reinforcement is less likely to occur. However, a detailed study of French Guiana sympatric populations, including both enzymatic and pheromonal approaches, is necessary to determine the potential for gene flow between populations and to draw conclusions on the role of sex pheromones in the reproductive isolation. The comparison between allopatric and sympatric populations will be critical to understanding the evolutionary history of the species.

Acknowledgments—We wish to thank Marguerite Leverve for technical assistance. We are grateful to Dr. Christer Löfstedt for reviewing the paper. We also thank Drs. Françoise Lemeunier, Frédéric Marion-Poll, Michel Renou, Didier Rochat, and Pierre Zagatti for comments and advice for the statistical analysis of the data.

REFERENCES

BUTLIN, R.K. 1985. Speciation by reinforcement, pp. 84–113, *in* J. Gosálvez, C. López-Fernández, and C. García de la Vega (eds.). Orthoptera, Vol. 1. Fundacion Ramon Areces, Madrid.

BUTLIN, R.K. 1987. Speciation by reinforcement. *Trends Ecol. Evol.* 2:8–13.

BYERS, J.R., UNDERHILL, E.W., STECK, W.F., CHISHOLM, M.D., and TEAL, P.E.A. 1981. Biosystematics of the genus *Euxoa* (Lepidoptera: Noctuidae) XV. Sex pheromone cross attractancy among the three related species of the *declarata* group. *Can. Entomol.* 113:235–243.

DOBZHANSKY, T. 1970. Genetics of the Evolutionary Process. Columbia University Press, New York.

GODFREY, G.L. 1987. Noctuidae, pp. 549–578, *in* S.W. Stehr (ed.). Immature Insects. Kendall/Hunt. Dubuque, Iowa.

GRANT, G.G. 1977. Interspecific pheromone responses of tussock moths and some isolating mechanisms of eastern species. *Environ. Entomol.* 6:739–742.

GRANT, G.G., SMITHWICK, E.B., and BRADY, U.E. 1975. Courtship behavior of phycitid moths. II. Behavioral and pheromonal isolation of *Plodia interpunctella* and *Cadra cautella* in the laboratory. *Can. J. Zool.* 53:827–832.

HANSSON, B.S., LÖFSTEDT, C., LÖFQVIST, J., and HALLBERG, S. 1986. Spatial arrangement of different types of pheromone-sensitive sensilla in a male moth. *Naturwissenschaften* 73:269–270.

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

KAISSLING, K.E., and THORSON, J. 1980. Insect olfactory sensilla: Structural, chemical and electrical aspects of the functional organization, pp. 261–282, *in* D.B. Satelle, L. Hall, and J.G. Hildebrand (eds.). Receptors for Neurotransmitters, Hormones and Pheromones in Insects. Elsevier, Amsterdam.

KING, A.B.S., and SAUNDERS, J.L. 1984. The Invertebrate Pests of Annual Food Crops in Central America. Overseas Development Administration, London.

LALANNE-CASSOU, B., SILVAIN, J.F., MONTI, L., and MALOSSE, C. 1994. Description d'une nouvelle espèce de *Spodoptera* de Guyane française: *S. descoinsi* (Lepidoptera: Noctuidae: Amphipyrinae), découverte grâce à des attractifs sexuels. *Am. Soc. Entomol. Fr.* 30:25–32.

LJUNGBERG, H., ANDERSON, P., and HANSSON, B.S. 1993. Physiology and morphology of pheromone-specific sensilla on the antennae of male and female *Spodoptera littoralis* (Lepidoptera: Noctuidae). *J. Insect Physiol.* 39:253–260.

LÖFSTEDT, C., and 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., HERREBOUT, W.M., and 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.

LÖFSTEDT, C., HANSSON, B.S., TÖTH, M., SZOCS, G., BUDA, V., BENGTSSON, M., RYRHOLM, N., SVENSSON, M., and PRIESNER, E. 1994. Pheromone differences between sibling taxa *Diachrysia chrysitis* (Linnaeus, 1758) and *D. tutti* (Kostrowicki, 1961) (Lepidoptera: Noctuidae). *J. Chem. Ecol.* 20:91–109.

LUCAS, P., and RENOU, M. 1989. Responses to pheromone compounds in *Mamestra suasa* (Lepidoptera: Noctuidae) olfactory neurones. *J. Insect Physiol.* 35:837–845.

LUCAS, P., and RENOU, M. 1991. Mise en évidence d'un neurone récepteur au (*Z*)-hexadéc-11-én-1-al, composé minoritaire de la phéromone, chez *Mamestra suasa* (Lepidoptera: Noctuidae). *C.R. Acad. Sci. Paris* 312:71–76.

MAYR, E. 1942. Systematics and the Origin of Species. Columbia University Press, New York.

MORI, K. 1981. The synthesis of insect pheromones, pp. 1–184, *in* J. Apsimon (ed.). The total synthesis of natural products, Vol. 4. Wiley-Interscience, New York.

PASHLEY, D.P., HAMMOND, A.M., and HARDY, T.N. 1992. Reproductive isolating mechanisms in fall armyworm host strains (Lepidoptera: Noctuidae). *Ann. Entomol. Soc. Am.* 85:400–405.

PHELAN, P.L., and BAKER, T.C. 1986. Cross-attraction of five species of stored-product Phycitinae (Lepidoptera: Pyralidae) in a wind tunnel. *Environ. Entomol.* 15:369–372.

RAMIANDRASOA, F., and TELLIER, F. 1990. Stereoselective synthesis of a potential sex pheromone of *Stenoma cecropia* (Lepidoptera). *Synth. Commun.* 20:333–344.

RENOU, M., and LUCAS, P. 1994. Sex pheromone reception in *Mamestra brassicae* L. (Lepidoptera): Responses of olfactory receptor neurones to minor components of the pheromone blend. *J. Insect Physiol.* 40:75–85.

RENOU, M., DESCOINS, C., PRIESNER, E., GALLOIS, M., and LETTERE, M. 1979. Le tétradécène-7 *Z* al-1, constituant principal de la sécrétion phéromonale de la Teigne de l'Olivier: *Prays oleae* Bern (Lepidoptère: Hyponomeutidae). *C.R. Acad. Sci. Paris* 288:1559–1562.

RENOU, M., DESCOINS, C., PRIESNER, E., GALLOIS, M., and LETTERE, M. 1981. Etude de la phéromone sexuelle de la Teigne du Poireau *Acrolepiopsis assectalla. Entomol. Exp. Appl.* 29:198–208.

RENOU, M., LALANNE-CASSOU, B., DORÉ, J.C., and MILAT, M.L. 1988. Electroantennographic analysis of sex pheromone specificity in neotropical Catocalinae (Lepidoptera: Noctuidae): A multivariate approach. *J. Insect Physiol.* 34:481–488.

ROELOFS, W.L., and CARDÉ, R.T. 1974. Sex pheromone in the reproductive isolation of Lepidopterous species, pp. 96–114, *in* M.C. Birch (ed.). Pheromones. North Holland Publishing, Amsterdam.

TEIXEIRA, A.L., DE OLIVEIRA, J.S., VILELA, E.F., DE LIMA, E.R., and REIS, H.O. 1989. Sex pheromone components of *Spodoptera latifascia* Walker (Lepidoptera: Noctuidae). *Rev. Ceres* 36:373–381.