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Chemical communication in heliothine moths

III. Flight behavior of male *Helicoverpa zea* and *Heliothis virescens* in response to varying ratios of intra- and interspecific sex pheromone components

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Summary. 1. *Helicoverpa zea* males flew upwind and successfully contacted the source when presented with 2-component blends consisting of their principal conspecific sex pheromone component, (Z)-11-hexadecenal, plus small amounts of (Z)-9-tetradecenal, a key secondary component in the *Heliothis virescens* blend which has heretofore been considered antagonistic to *H. zea* pheromone-mediated behavior. Neurophysiological studies of *H. zea* antennal receptor neurons and central interneurons had suggested that this unexpected antagonistic effect on behavior might occur.

2. When the amount of (Z)-9-tetradecenal in the blend reached 15% relative to the principal component its effect did become antagonistic with significantly more *H. zea* males remaining quiescent. Five-to-fifteen percent (Z)-9-tetradecenal is emitted by *H. virescens* in its pheromone blend, levels that evoked optimal upwind flight and source contact in *H. virescens* males.

3. As suggested by studies of *H. virescens* antennal receptor neurons, *H. virescens* males were unresponsive to the reciprocal inter-specific blend, comprised of (Z)-11-hexadecenal plus various percentages of (Z)-9-hexadecenal.

4. Receptors that allow such mutual replacement of compounds might permit significant shifts in pheromone systems; a single mutation that drastically alters the female sex pheromone blend could still be carried in a population due to the successful attraction of normal males by mutant females.

Key words: Sex pheromones – Antennal neurons – (Z)-11-hexadecenal – *Helicoverpa zea* – *Heliothis virescens*

Introduction

The sex pheromone blends of *Heliothis virescens* and *Helicoverpa zea* have been thoroughly examined chemically and behaviorally, beginning with Roelofs et al. (1974) and Tumlinson et al. (1975). The blend compositions have since been further refined (Klun et al. 1979, 1980a, b; Tumlinson et al. 1982; Pope et al. 1982, 1984). The behavioral studies required to verify the pheromonal activity of the compounds, e.g. to show that each of the emitted compounds contributes to evoking a response in conspecifics (as per the definition of the word 'pheromone' beginning with Karlson and Lüscher 1959) have included both wind tunnel and field trapping tests. The results have been somewhat variable, and some disagreement has arisen as to whether some of the secondary components can evoke subtle effects in combination with the rest of the blend of emitted compounds (Klun et al. 1980a, b; Vetter and Baker 1983, 1984; Ramaswamy et al. 1985; Teal et al. 1986). While some issues remain unresolved, workers generally agree on the following: 1) the principal component of the sex pheromone of both species is (Z)-11-hexadecenal (Z11-16:AL) (Roelofs et al. 1974; Tumlinson et al. 1975; Klun et al. 1979, 1980a, b; Vetter and Baker 1983, 1984; Ramaswamy et al. 1985; Teal et al. 1986); 2) the most behaviorally important secondary component of *H. virescens* is (Z)-9-tetradecenal (Z9-14:AL) (Roelofs et al. 1974; Tumlinson et al. 1975; Vetter and Baker 1983; Teal et al. 1986); and 3) the most important secondary component of *H. zea* is (Z)-9-hexadecenal (Z9-16:AL) (Klun et al. 1980b; Vetter and Baker 1984).

In *H. zea*, two other compounds found in the glands, (Z)-7-hexadecenal and hexadecanal (Klun et al. 1980b), have not been shown to evoke any additional behavioral response when combined with the two components of this species' pheromone blend, despite extensive testing. For *H. virescens*, other compounds identified (Klun et al. 1980a) from female glands that exert more subtle but significant effects when combined with the first two components are hexadecanal (Klun et al. 1980a; Vetter and Baker 1983; Ramaswamy et al. 1985; Teal et al. 1986), tetradecanal, (Z)-9-hexadecenal, and (Z)-7-hexadecenal (Klun et al. 1980a; Teal et al. 1986). Working in the field or laboratory, several groups could not confirm the behavioral activities of some or all of these compounds

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(Hartstack et al. 1980; Vetter and Baker 1983; Ramaswamy et al. 1985), but this is not surprising considering the relatively subtle nature of their effects.

Thus both species' pheromone blends consist primarily of a mixture of 2 essential components, the shared principal component Z11-16:AL, and a different secondary component, either Z9-14:AL (*H. virescens*) or Z9-16:AL (*H. zea*). Interestingly, despite the numerous tests that have been performed over the years, few have varied the blend ratios of these two most important components in each species' blend and recorded the responses from conspecific males (Roelofs et al. 1974, for *H. virescens*; Vetter and Baker 1984, for *H. zea*).

Inter-specific effects have also been examined. Shaver et al. (1982) demonstrated that adding trace amounts of Z9-14:AL to the two-component *H. zea* blend caused a severe reduction in trap catch of *H. zea* males. Other studies also indicated that placement of *H. virescens* females in traps with *H. zea* females significantly reduced the capture of *H. zea* males (Haile et al. 1973; Roach 1975; Carpenter et al. 1984; Lopez and Witz 1988). The antagonistic role of Z9-14:AL toward *H. zea* males, and hence its role in preventing interspecific mating mistakes, was confirmed again in pheromone disruption experiments. Area-wide emission of Z9-14:AL with its subsequent habituation effect on moths in the area resulted in *H. zea* males being attracted to, and coupling with, *H. virescens* females (Hendricks et al. 1982).

It was thus surprising when Almaas et al. (1991, preceding paper) found that *H. zea* males appear not to possess any antennal receptors that are specifically responsive to Z9-16:AL, but receptors tuned to only Z11-16:AL and Z9-14:AL, respectively. Likewise, in the antennal lobe, the projection interneurons were primarily activated by antennal stimulation with either Z11-16:AL or Z9-14:AL (Christensen et al. 1989; Christensen et al. 1991). However, both the receptor neurons and projection interneurons driven by Z9-14:AL, also responded to Z9-16:AL, but at higher concentrations. At first glance it seemed reasonable that *H. zea* males should possess the Z9-14:AL-sensitive receptor neurons and CNS pathways in order to detect and respond behaviorally in antagonistic fashion to this *H. virescens* component. However, the neuronal responses to Z9-16:AL at higher concentration led us to the idea that the receptor neurons tuned to Z9-14:AL might also mediate information from Z9-16:AL, promoting upwind flight, since Z9-14:AL is not emitted by *H. zea* females. This would mean that low activation of these receptor neurons (by Z9-16:AL) might mediate attraction, whereas high activation (by Z9-14:AL) could interrupt attraction. We decided to test this idea using wind tunnel experiments, and in addition we tested a complete series of blend ratios of each species' two most important components, since the information on blend ratio responses has been negligible.

Materials and methods

Moths. The *H. virescens* colony originated in T.C.B.'s laboratory in 1980 and the *H. zea* colony was obtained from Mycogen Corporation (San Diego, CA) in 1990. Both *H. virescens* and *H. zea* were reared on a modified pinto-bean diet (Shorey and Hale 1965) and separated, according to sex, as pupae. Male pupae were maintained in environmental chambers at 25 °C on a 14:10 L:D cycle. Moths were segregated according to age in separate cages and fed on an 8% sucrose solution. Moths between the ages of 4–8 days were used for experiments, and each experiment was conducted between the 5th and 8th h of scotophase (Vetter and Baker 1983, 1984).

Prior to scotophase on the day of flight, individual males were placed in cylindrical 6×6 cm diameter wire-screen cages. These cages were placed on plastic retaining trays (20 cages per tray) which were returned to the environmental chamber. At least 1 h prior to the flight period the trays containing the wire cages were removed from the environmental chamber and placed in the wind-tunnel in order to afford the moths a period of acclimation to the conditions therein.

Wind tunnel. The wind tunnel utilized in this series of experiments was based on a design modified after Miller and Roelofs (1978). Details of the tunnel construction can be found in Kuenen and Baker (1982). Conditions in the tunnel were maintained as follows: 24–26 °C, 60% R.H., 0.5 lux, with a wind-speed of 40 cm/s.

Pheromone test blends. Z11-16:AL, Z9-14:AL, and Z9-16:AL were obtained from highly concentrated stock solutions maintained in our laboratory (T.C.B.). Purity of the starting compounds was found to be greater than 99% by capillary gas chromatography (G.C.) on a Varian model 3740 G.C. using a 30 m DB-225 column. Mixtures were prepared with respect to the concentration of the principal component in the pheromone blend of each species, Z11-16:AL (100%). The percentage of the secondary component, either Z9-14:AL or Z9-16:AL, present in each mixture was confirmed by G.C. analysis of a sample of each test blend. Final concentrations of the test solutions were 0.1 µg/µl (referring to the amount of Z11-16:AL), the secondary component being present in its appropriate ratio.

A 10 µl micropipette was used to load the test solution onto a cotton, dental wick (Vetter and Baker 1984), which was then placed in the center of a 15×15 cm metal plate 15 cm above the tunnel floor. A clip was attached to the bottom of the wick to provide stability when approached by males. The test wick and stand were 34 cm from the upwind end of the tunnel. Treatments in each of the 5 experiments were tested using a randomized complete-block design. Each time that the order of treatments required that a higher secondary component concentration be used or that a change in the secondary component be made, the metal plate and stand were removed and rinsed with acetone before the next treatment commenced in order to avoid cross-contamination.

Behavioral observations. Moths were released singly 2.6 m downwind of the test-source by inverting their wire-screen cages and placing them in the center of a metal plate on an adjustable stand in the path of the plume from the test-source. The behavior of the moths in response to the test stimulus was recorded by an observer standing at the rear of the wind tunnel. Behaviors were broken down into 4 sequential categories: 1) takes flight (or remains in cage); 2) performs casting flight, keeping station near the take-off plate while attempting to 'lock-on' to the plume; 3) flies upwind in the plume; 4) locates source and lands. Moths were given 1 min to leave their wire cage. Those that did not were checked for their ability to fly. Any moth that appeared incapable of flight was not scored in these experiments. Those moths that were capable of flight but that did not respond to the test plume were scored as 'non-responders'. The numbers of moths performing each behavior were compared by using a χ^2 2×2 test of independence with Yates' correction. Statistical significance was determined at the $P<0.05$ level.

Results

H. virescens and *H. zea* males responded to a wide range of their two-component conspecific blends by flying up-

Table 1. Percentage success of *Heliothis virescens* males in performing 4 sequential behaviors in response to a series of synthetic binary pheromone mixtures containing the most important constituents of the conspecific female blend: 1 μg Z11-16:AL (100%) and varying amounts of Z9-14:AL relative to Z11-16:AL. Maximal 'upwind flight' and 'source contact' were attained between 5% and 50% Z9-14:AL. Percentages in columns having no letters in common are significantly different according to a χ^2 2×2 test of independence with Yates' correction ($P<0.05$)

Treatment	*N*	Take flight	Cast	Upwind flight	Contact source
+0.1% Z9-14:AL	72	86% b	13% c	0% c	0% c
+1.0% Z9-14:AL	75	97% a	29% b	7% bc	1% c
+5.0% Z9-14:AL	74	99% a	46% ab	20% a	16% ab
+15% Z9-14:AL	69	100% a	61% a	32% a	29% a
+50% Z9-14:AL	68	99% a	54% a	26% a	16% ab
+100% Z9-14:AL	76	93% ab	43% ab	18% ab	10% b

Table 2. Percentage success of *Helicoverpa zea* males in performing 4 sequential behaviors in response to a series of synthetic binary pheromone mixtures containing the most important constituents of the conspecific female blend: 1 μg Z11-16:AL (100%) and varying amounts of Z9-16:AL relative to Z11-16:AL. Maximal 'upwind flight' and 'source contact' were attained to blends containing between 1% and 5% Z9-16:AL. Blends containing 15% (and more) or 0.1% (and less) Z9-16:AL elicited significantly fewer source contacts. Percentages in columns having no letters in common are significantly different according to a χ^2 2×2 test of independence with Yates' correction ($P<0.05$)

Treatment	*N*	Take flight	Cast	Upwind flight	Contact source
100% Z11-16:AL	47	91% a	47% c	17% c	4% c
+0.1% Z9-16:AL	46	93% a	70% ab	26% bc	4% c
+1.0% Z9-16:AL	54	100% a	67% abc	43% ab	28% ab
+5% Z9-16:AL	43	100% a	79% a	56% a	44% a
+15% Z9-16:AL	45	100% a	76% ab	50% a	22% b
+50% Z9-16:AL	44	95% a	59% abc	30% bc	20% b
+100% Z9-16:AL	45	96% a	56% bc	24% bc	11% bc

Table 3. Percentage success of *Helicoverpa zea* males in performing 4 sequential behaviors in response to a series of synthetic binary pheromone mixtures. Interestingly a high level of upwind flight, not significantly different from the positive control (+1.0% Z9-16:AL), and a tendency toward higher source contact were observed in males responding to the +1.0% Z9-14:AL blend. This latter component is not released by conspecific females and at higher concentrations is antagonistic to *H. zea* males. Percentages in columns having no letters in common are significantly different according to a χ^2 2×2 test of independence with Yates' correction ($P<0.05$)

Treatment	*N*	Take flight	Cast	Upwind flight	Contact source
100% Z11-16:AL	68	88% bc	53% ab	22% bc	7% bc
+0.001% Z9-14:AL	71	94% abc	48% b	18% c	7% bc
+0.01% Z9-14:AL	72	86% cd	54% ab	19% c	1% c
+0.1% Z9-14:AL	64	94% abc	50% ab	19% c	2% c
+1.0% Z9-14:AL	68	97% ab	60% ab	38% ab	18% b
+15% Z9-14:AL	62	73% d	6% c	2% d	0% c
+1.0% Z9-16:AL	68	100% a	66% a	46% a	38% a

Table 4. Percentage success of *Helicoverpa zea* males in performing 4 sequential behaviors in response to a series of synthetic binary pheromone mixtures. A blend containing 0.9% Z9-14:AL confirmed a result from a previous experiment (Table 3) that the non-specific component, Z9-14:AL, was mutually replaceable with the usual secondary component, Z9-16:AL, to *H. zea* males. Percentages in columns having no letters in common are significantly different according to a χ^2 2×2 test of independence with Yates' correction ($P<0.05$)

Treatment	*N*	Take flight	Cast	Upwind flight	Contact source
100% Z11-16:AL	43	100% a	49% bc	14% c	0% d
+0.1% Z9-14:AL	42	98% a	43% c	26% bc	2% cd
+0.6% Z9-14:AL	41	98% a	54% abc	27% bc	12% bcd
+0.9% Z9-14:AL	39	97% a	69% abc	46% ab	23% ab
+1.0% Z9-14:AL	40	98% a	60% abc	33% bc	15% bc
+3.0% Z9-14:AL	36	94% a	44% bc	22% bc	11% bcd
+1.0% Z9-16:AL	42	100% a	76% a	62% a	40% a

wind and touching the source (Tables 1 and 2). However, despite the somewhat broad tuning of the response, males of both species did exhibit a clear optimum response that was centered close to the blend that is produced by their conspecific females (Klun et al. 1979, 1980a, b; Pope et al. 1982, 1984; Teal et al. 1986). *H. virescens* males exhibited maximum upwind flight and source contact in response to blends containing 5–15% Z9-14:AL with significantly fewer males touching sources containing 1% or less, or more than 50% Z9-14:AL in 1 μg Z11-16:AL (Table 1). *H. zea* males exhibited maximum upwind flight and source contact in response to blends containing 5% Z9-16:AL, with significantly fewer males touching sources containing 15% or more, or 0.1% or less, of Z9-16:AL (Table 2).

Surprisingly, in an initial test to see whether or not *H. zea* males would respond to blends in which the conspecific Z9-16:AL was replaced with Z9-14:AL, males did exhibit a tendency to fly upwind in greater numbers to a 1% Z9-14:AL blend than to the 1 μg Z11-16:AL one-component control, and they clearly exhibited reduced activity (significantly more males did not leave the cage) in response to blends containing 15% of this important component (the level at which it is found in calling *H. virescens* females) (Table 3). A second experiment using a finer-grained series of percentages, stepped down from 3%, confirmed the significant increase in upwind flight and source contact by *H. zea* males in response to blends containing Z9-14:AL, a compound their own females are not known to emit, compared to the 1 μg Z11-16:AL-only control (Table 4). These levels of source contact in response to blends containing the *H. virescens* component were not significantly different from those obtained with the *H. zea* conspecific blend with the appropriate conspecific component Z9-16:AL replacing Z9-14:AL at 1% in the blend (Table 4).

Interestingly, *H. virescens* males remained completely unresponsive to blends in which Z9-14:AL was replaced with the inter-specific *H. zea* component, Z9-16:AL (Table 5). This lack of response continued even when blends were severely overloaded with this inter-specific compound, containing up to equal amounts of Z9-16:AL

Table 5. Percentage success of *H. virescens* males in performing 4 sequential behaviors in response to a series of synthetic binary pheromone blends containing 100% Z11-16:AL (1 μg) and varying amounts of the *H. zea* secondary component, Z9-16:AL. For *H. virescens* males the conspecific secondary component, Z9-14:AL, was not mutually replaceable with Z9-16:AL at the levels tested in this experiment. Percentages in columns having no letters in common are significantly different according to a χ^2 2×2 test of independence with Yates' correction ($P<0.05$)

Treatment	*N*	Take flight	Cast	Upwind flight	Contact source
+15% Z9-14:AL	41	100% a	56% a	34% a	29% a
+1.0% Z9-16:AL	46	91% a	11% b	0% b	0% b
+5.0% Z9-16:AL	43	88% a	9% b	0% b	0% b
+15% Z9-16:AL	43	93% a	5% b	0% b	0% b
+50% Z9-16:AL	44	98% a	7% b	0% b	0% b
+100% Z9-16:AL	43	100% a	7% b	0% b	0% b

and Z11-16:AL (100% Z9-16:AL, Table 5). Thus the reaction of *H. virescens* males to blends in which the conspecific secondary component is replaced with an inter-specific one is unlike that of *H. zea* males. *H. virescens* males require Z9-14:AL to be present in the blend, and Z9-16:AL cannot substitute for this component even at the elevated proportions that we tested in this experiment.

Discussion

Studies of receptor neurons (Almaas et al. 1991) as well as antennal lobe projection neurons (Christensen et al. 1989; Christensen et al. 1991) in *H. zea* males have suggested that Z9-14:AL at low concentrations might be able to replace Z9-16:AL in the *H. zea* pheromone blend. Nevertheless, when flight tunnel behavioral experiments (Tables 2–4) confirmed that these compounds were indeed somewhat mutually replaceable in their ability to evoke upwind flight, the results were surprising, since all previous reports have supported the idea that Z9-14:AL exclusively acts antagonistically to *H. zea* pheromone-mediated behavior (Haile et al. 1973; Roach 1975; Shaver et al. 1982; Carpenter et al. 1984; Lopez and Witz 1988).

The ability to replace Z9-16:AL with Z9-14:AL in *H. zea* males, both at the behavioral and receptor cell level, has major evolutionary importance. We hypothesize that such low-specificity receptors can readily accept and respond to molecules formed as a result of a mutation affecting the emitted blend. In contrast to previous evolutionary models (cf. Roelofs and Comeau 1969; Lanier and Burkholder 1974), even a major mutation affecting the pheromone biosynthetic pathway need not be lost due to the lack of a simultaneous similar mutation in the receiver. Rather, receptor neurons would allow for adequate levels of mate-finding to continue among mutant senders and normal receivers, despite the altered blend. Given appropriate selection pressures, communication among mutant senders and normal receivers could even be favored, and we believe that this type of change may have shaped the current *H. zea* communication system.

The difference between pheromone blend components of *H. zea* and *H. virescens* is the lack of the two 14-carbon aldehydes in *H. zea* females that are produced by *H. virescens* (Klun et al. 1979, 1980a, b). Based on information about the biosynthetic pathways creating other lepidopterous sex pheromone blends, such as that of another noctuid, *Trichoplusia ni*, (Bjostad et al. 1987), it is reasonable to assume that a mutation (Haynes and Hunt 1990) reducing the activity of a chain-shortening enzyme (R. Jurenka and W. Roelofs, pers. comm.) might explain the loss of these shorter aldehydes. If it is accepted that *H. zea* females at one time had the capability to produce the shorter aldehydes and emit a blend similar to that of *H. virescens*, what needs to be explained is how mutant females lacking the shorter aldehydes could begin to be favored by selection at the expense of normal 14-carbon-aldehyde-producing females.

Without at all implying that a speciation event took place (reinforcement), but instead only a shift in the communication system related to reproductive character displacement (Butlin 1987; Löfstedt 1991), we hypothesize that at one time *H. zea* and *H. virescens* both used blends of Z11-16:AL and Z9-14:AL as their sex pheromone components. These two components were perhaps used at different ratios, with *H. zea* using far lower percentages of Z9-14:AL than *H. virescens*. Looking at blends containing only the principal component and Z9-14:AL, the responses of *H. zea* and *H. virescens* males shown in our data (Tables 1, 3, 4) take on the form of a classical partitioning of a range of blend ratios of the same components, as occurs in tortricid moths, for instance (Roelofs and Brown 1982). Perhaps in the past, after prolonged sympatry, mating mistakes due to the imperfect specificity imparted by these blend ratios would have favored *H. zea* females that produced less Z9-14:AL, attracting fewer *H. virescens* males. *H. zea* males would also have been favored that were hypersensitive to this compound in blends, resulting in arrestment of upwind flight before reaching the erroneous *H. virescens* female emitting it. Mutant *H. zea* females producing little or no chain-shortened 14-carbon aldehydes would also be favored, because mating mistakes with *H. virescens* males would be reduced to zero; male *H. virescens*' require Z9-14:AL to be present in the blend in order to fly upwind. Importantly, *H. zea* males would continue to be attracted to mutant *H. zea* females because their receptor neurons tuned to Z9-14:AL also accommodate the Z9-16:AL molecules that are still produced by mutant *H. zea* females, resulting in the proper ratio of neuronal firing of the two receptor types. Too much firing of the Z9-14:AL/Z9-16:AL receptor neuron, as would occur to the *H. virescens* blend containing 15% Z9-14:AL and emitted at over 3-times the blend rate of *H. zea* (Pope et al. 1984), would cause the ratio of firing ascending to the deutocerebrum to be too rich in the secondary component, and upwind flight would be arrested even before take-off (Table 3).

Our results are consonant with previous studies showing antagonism to *H. virescens* female-emitted Z9-14:AL

by *H. zea* males, if one understands that the combined amounts of Z9-14:AL and Z9-16:AL in the traps exceeded the optimum *H. zea* Z9-16:AL/Z11-16:AL ratio for male response (Vetter and Baker 1984; this study). They also are not at odds with results from atmospheric permeation experiments using Z9-14:AL (Hendricks et al. 1982). Habituation of the Z9-16:AL/Z9-14:AL sensory pathway would permit some attraction of *H. zea* males to Z11-16:AL alone (as does occur even in clean air; Vetter and Baker 1984; Carpenter et al. 1984; this study), and this would be all that would be detected by habituated *H. zea* males contacting an *H. virescens* plume.

On the other hand, our results do not at first glance agree with those of Shaver et al. (1982), who showed that even 0.1–1.0% of Z9-14:AL added to the synthetic *H. zea* two-component blend significantly reduced captures of *H. zea* males. Our study is slightly different in that we did not mix both Z9-16:AL and Z9-14:AL as they did, but rather created only binary mixtures. Nevertheless, if these 2 compounds share a common sensory pathway, such low amounts of Z9-14:AL, even when blended with the 3% of Z9-16:AL normally in lures for this species, should not have caused such severe antagonism. It is possible that males in the field differ in their relative sensitivity to these compounds compared to animals from laboratory cultures that have been buffered from selection pressures for many years. There is an indication in another noctuid species, *Agrotis segetum*, that the proportions of antennal neurons sensitive to secondary components can change rapidly following even only a few generations of laboratory rearing (Bill Hansson, pers. comm.), and so results from antennal neuron recordings and possibly from behavioral studies as well must be at this point taken with some caution as we learn about the malleability of sensory pathways.

This caveat notwithstanding, the positive behavioral responses to, and faithful reproduction of, information through the sensory pathways about a inter-specific pheromone component as if it were a conspecific one, is intriguing. A communication system that had heretofore been assumed to differ quite significantly from another sympatric species due to the strong antagonism caused by a single compound not used in its system now is revealed to contain the flexible neuronal hardware and the behavioral wherewithal for responding positively to this same component as if it were a conspecific one. The phenomenon of low-specificity receptors as promoters of major evolutionary shifts in chemical communication systems should be looked for in other species. Indeed the results of Löfstedt et al. (1990) on the *Yponomeuta rorellus* system might be re-evaluated in terms of this hypothesis. Males of this species possess antennal receptors that respond most strongly to interspecific unsaturated 14-carbon acetates that their conspecific females do not emit (Löfstedt et al. 1986). These receptors also display cross-reactivity but they are less sensitive to the unusual saturated 14-carbon acetate that *is* emitted by their females and alone comprises their pheromone system. A mutation eliminating the activity of the desaturase enzyme in females (Löfstedt et al. 1986) could have been carried in the population and finally favored under the pressure of mating mistakes with individuals from sympatric species, all facilitated by the existence of low-specificity receptors in *Y. rorellus* males that still proliferate.

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