Reproductive Isolation of Two Tortricid Moth Species by Different Ratios of a Two-Component Sex Attractant

Abstract. *Two tortricid moth species,* Adoxophyes orana *and* Clepsis spectrana, *utilize the same two isomers,* cis-*9- and* cis-*11-tetradecenyl acetate, as their sex attractant. Communication between the sexes of each species is separated effectively by a different blending of both compounds by the females and a different response to these blends by the males.*

Reproductive isolation of moth species with the same sex pheromone can be based on small differences in the quantities eliciting optimum attraction. Thus, largest catches of the European corn borer and oblique-banded leafroller moths are obtained by different doses of the *cis*-11 isomer of tetradecenyl acetate (TDA) (*1*). On the other hand, the addition of other compounds to the main attractant can increase the attractiveness for one moth species and inhibit the attraction of males of another (*2*).

We now report on two tortricid moth species, the summer fruit tortricid, *Adoxophyes orana,* and the leafroller, *Clepsis spectrana.* They coexist in nature; their flight period as well as their period of activity during the 24-hour cycle overlap almost completely. They probably use a mixture of the same two chemicals as their sex attractant and apparently maintain reproductive isolation because of a different sensitivity of the males to different chemical ratios.

The sex pheromones for *A. orana* have been reported to be *cis*-9-TDA and *cis*-11-TDA (*3*), the females producing a mixture of these compounds in a molar ratio of about 9:1. Male antennal responses (*4*) to a series of C_{12}, C_{14}, and C_{16} acetates and alcohols showed that *cis*-9-TDA elicited the strongest response with *A. orana,* whereas *cis*-11-TDA elicited the strongest response with *C. spectrana* (Fig. 1). Secondary activity was indicated for *cis*-11-TDA with *A. orana,* and for *cis*-9-TDA with *C. spectrana.* These compounds elicited relatively good antennal responses; recovery rates to base-line response were slow compared with those for the other standards. These results indicate possible biological significance for these two compounds.

Evidence for the presence of *cis*-9- and *cis*-11-TDA in *C. spectrana* females was obtained by a combination of gas chromatography and electroantennography (EAG) (*5*). Crude extract from 40 abdominal tips from female *C. spectrana* was injected onto three different gas chromatography columns [5 percent DC-200 silicone (Dow Corning), 5 percent diethylene glycol succinate, and 6 percent cyclohexanedimethanol succinate], and the effluents were collected each minute in capillary tubes. Electroantennographic assay of these collection tubes showed that antennal activity was only present in samples collected at 9 to 10 minutes, at 12 to 14 minutes, and at 14 to 16 minutes from the respective columns. These intervals correspond to the retention times on these columns for the standards *cis*-9-TDA (9.2, 12.0, and 14.3 minutes, respectively) and *cis*-11-TDA (9.4, 12.8, and 15.0 minutes, respectively). Gas chromatographic analysis (5 percent diethylene glycol succinate) of the combined active fractions showed a large peak at the retention time of *cis*-11-TDA (16.2 minutes) and a shoulder at the retention time for *cis*-9-TDA (15.4 minutes).

Field studies in which various ratios of the two chemicals were used gave results in full agreement with the above-described data for the two species. Total catches collected from three different apple orchards are shown in Fig. 2. Males of *A. orana* were attracted to mixtures containing predominantly *cis*-9-TDA, whereas males of *C. spectrana* were attracted to mixtures containing mainly *cis*-11-TDA. A difference in the male response is noteworthy; *cis*-9-TDA alone (*6*) did not attract males of either species, whereas *cis*-11-TDA alone did attract *C. spectrana* males in small but significant numbers. The males of these two species are quite sensitive to different ratios of the two chemicals, and probably would not be lured to females of the wrong species.

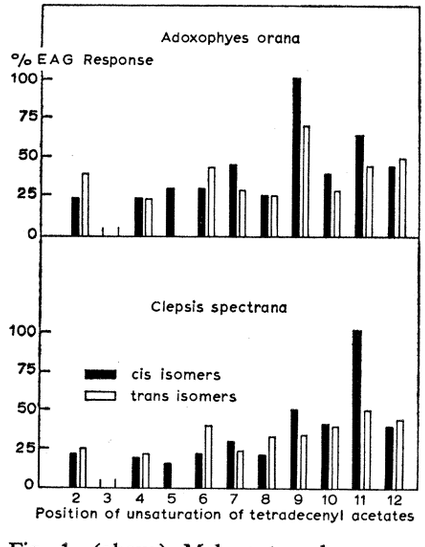

Fig. 1. (above) Male antennal response to monounsaturated tetradecenyl acetate standards; EAG, electroantennogram. Average control response is 0 to 1 percent.

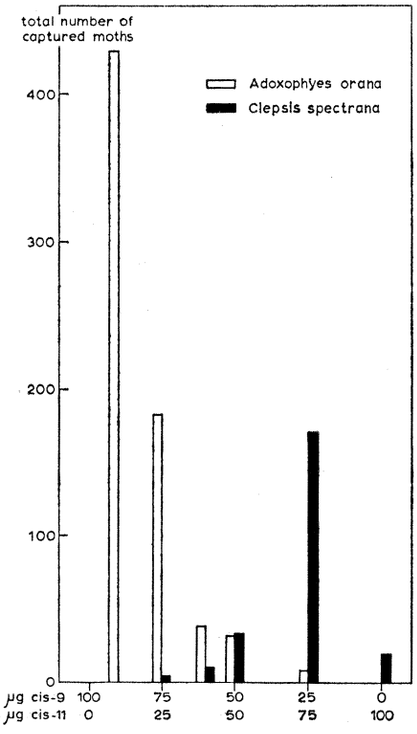

Fig. 2. (right) Total number of *A. orana* and *C. spectrana* moths caught in three different orchards from 5 August to 15 September 1972 (two traps with each chemical ratio in each orchard).

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4. W. L. Roelofs and A. Comeau, *J. Insect. Physiol.* **17**, 1969 (1971). The EAG setup at the Central Laboratory, TNO, is similar to that described by Roelofs and Comeau, except that a custom-made preamplifier (10^{12} ohms) and amplifier (100-fold) fed signals to a Philips PM 3200 oscilloscope and a Honeywell 906T Visicorder. The test chemicals were at least 95 percent pure.
5. W. L. Roelofs and A. Comeau, in *International IUPAC Congress of Pesticide Chemistry, Second, 1971*, A. S. Tahori, Ed., vol. 3: *Chemical Releasers in Insects* (Gordon and Breach, New York, 1971), pp. 91-114.
6. *Cis*-9- and *cis*-11-tetradecenyl acetate were synthesized by the method of D. R. Howton and R. A. Stein [*J. Lipid Res.* **10**, 631 (1969)]. Both compounds were purified by column chromatography to get geometrically pure material [R. L. Anderson and E. J. Hollenbach, *ibid.* **6**, 577 (1965)]. The purity was checked by gas-liquid chromatography and infrared spectrometry and was more than 98 percent.
7. We thank S. Voerman (Laboratory for Research on Insecticides, Wageningen, Netherlands) for providing test chemicals and D. J. de Jong (Experimental Station for Fruit Growing, Wilhelminadorp, Netherlands) for providing the *C. spectrana* moths.
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Estrogen Formation by the Isolated Perfused Rhesus Monkey Brain

Abstract. *Perfusion of two isolated brains from immature male rhesus monkeys with [^{3}H]androstenedione resulted in the identification of free and conjugated [^{3}H]estrone and free [^{3}H]estradiol from the perfusates. In the dissected cerebral tissues, estrogens were recovered only from the hypothalamus and limbic system. The production of estrogens from androstenedione during the 40-minute perfusions in these two experiments totaled 1.58 and 2.83 nanograms.*

The in vitro conversion of androstenedione to estrone and of testosterone to estradiol has been demonstrated with the use of homogenates of hypothalamic and limbic tissues from immature and adult rhesus monkey brains. In addition, biotransformation of androstenedione to estrone, and in some cases to estradiol, has been observed with the same tissues from human fetuses and from adult rats and rabbits (*1*). The crucial actions that estrogens exert centrally, such as control of gonadotropins, sexual behavior, and perhaps sexual differentiation of the hypothalamus (*2*), make on-site hormone synthesis particularly intriguing. Estrogens formed in the brain at their site of action would not be subject to systemic dilution and metabolic degradation before exerting a local effect. Confirmation in vivo of estrogen formation in the brain was sought to determine the applicability of the above in vitro observations to a physiological system.

The substrate, 7α-[^{3}H]androstenedione (specific activity, 7.7 c/mmole; Amersham/Searle) was purified by paper chromatography (in a ligroin, 96 percent methanol system). The techniques of perfusion and monitoring were as described by White *et al.* (*3*). The experimental subjects were two prepubertal male monkeys weighing 2.5 kg each. In order to minimize the amount of extraneous tissues perfused, the dissection included removal of the lower jaw, tongue, skin, salivary glands, orbital tissues, and muscles of the head. Thus, the experimental preparations consisted only of the isolated cranial vault, brain, and pituitary. After cannulation of the carotid arteries and separation of the head and spinal cord at the foramen magnum, perfusion was established at approximately 20 ml/min with intra-arterial pressures of 85/50 mm-Hg and normal electroencephalogram (EEG) patterns. In the first experiment, 350 μc of [^{3}H]androstenedione in 2 ml of absolute ethanol were slowly injected into the 250-ml perfusion volume. The injection was soon followed by a flat EEG and a rising arterial pressure over the 40-minute perfusion period. The brain, on sectioning, showed edema without hemorrhage. In order to minimize the effects of ethanol in the second study, the [^{3}H]androstenedione was slowly injected in 0.5 ml of 50 percent ethanol. The perfusion pressure and flow remained similar to that in the period before injection, and no edema developed. The EEG lost fast wave components which were regained by the end of the 40-minute perfusion period. On sectioning, no edema or hemorrhage were present. Immediately after perfusion, the hypothalamus, limbic system, the pituitary, and the cortex tissues were dissected and homogenized (*1*), except that, in the case of the second monkey, the pons was also studied.

The perfusate from each monkey was extracted with 20 volumes of a mixture of ethanol and ether (3 : 1). For tissues, the extraction was carried out with three 50-ml portions of the above-mentioned solvents. After evaporation, lipid precipitation was performed by resuspending the residue in 50 ml of 70 percent methanol and storing the samples overnight at −20°C. They were then centrifuged for 15 minutes at 8000*g*; the supernatant was decanted and evaporated to dryness; the residue was reconstituted in 50 ml of water, which was then extracted with three 50-ml portions of ether. The aqueous or conjugated fractions were hydrolyzed with 0.15 ml of Glusulase (glucuronidase, 26,925 units; sulfatase, 7,050 units; Endo Laboratories). The hydrolyzates were adjusted to *p*H 5 by the addition of 5 ml of acetate buffer and stored at 37°C overnight. They were extracted then with three 50-ml portions of ether.

Table 1. Aromatization of perfused [^{3}H]androstenedione (350 μc; specific activity, 7.7 c/mmole) by rhesus monkey brain. Estrogen was identified in the perfusate (250 ml). For crystallization, 10 mg of nonlabeled estrogen was used. Abbreviations: (c), conjugated fraction; dpm, disintegrations per minute; N, final crystallization; N-1, next to final crystallization; ML, mother liquor.

Product	Monkey No.	Total estrogen characterized			Specific activities (dpm/mg)			
					N−1		N	
		(pmole)	(pg)	(dpm)	Crystal	ML	Crystal	ML
[3-methyl-^{3}H]-estrone	1	4.98	1340	84090	8629	8434	8409	8109
	2	4.08	1096	67670	7175	6840	6767	6475
[3-methyl-^{3}H]-estrone (c)	1	0.06	15	950	96	96	95	93
	2	1.21	325	2045	2088	2032	2045	2004
[3-methyl-^{3}H]-estradiol	1	0.08	23	1430	142	146	143	141
	2	0.13	25	2210	223	217	221	233