Individual Variation in Sex Pheromone Component Ratios in Two Populations of the Redbanded Leafroller Moth, *Argyrotaenia velutinana*[1]

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ABSTRACT

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Gas chromatographic analyses of pheromone component ratios from 381 individual female tip extracts from field and laboratory populations of redbanded leafroller moths revealed that a narrow-variance signal (coefficient of variance = 9.7%) was used by both populations. Although all measured ratios of (*E*)-/(*Z*)-11-tetradecenyl acetates for both populations fell within 4-15% for the *E*-component, the untransformed means and standard deviations for the field and lab insects were 9.1±1.8 and 7.0±1.4%, respectively (difference significant at $P<0.01$). The field insects contained an average of 139±81 ng of pheromone/female compared to 107±58 ng/female for the laboratory insects. No significant relationships were found between insect body weights, quantity of pheromone, or component ratios.

The importance of precise blending of components comprising the sex pheromone of various moth species in a number of families is now well established. Differences of a few percent in component ratios can translate into widely differing behavioral responses by male moths. Such is the case for many of the tortricid moths that we have studied that employ (*E*)- and (*Z*)-11-tetradcenyl acetates (E11 and Z11-14:Ac's) as pheromone constitutents (Cardé et al. 1977). It appears that precise ratios of these acetates constitute at least one part of the specific mate recognition systems of closely related sympatric species (Roelofs 1979).

The proximate physiologic mechanisms governing blend production and perception by an insect species remain unknown. However, both processes appear to be under direct genetic control (Lanier and Burkholder 1974, Grant et al. 1975, Sanders et al. 1977, Klun and Mani 1979). Careful quantifications of pheromone components from pooled samples of tortricid pheromone glands suggest narrow-variance regulation in production. Ratios of geometrically isometric components vary no more than a few percent from sample to sample. Nevertheless, accurate assessments of the variability in pheromone signals within a population must be obtained from numerous quantifications conducted on individual moths. Here we report such a study conducted on wild and laboratory populations of female redbanded leafroller moths, *Argyrotaenia velutinana*. Based on pooled abdominal tip extracts, the pheromone of this tortricid moth has previously been reported to be a 91:9 ratio of Z/E-11-14:Ac, along with excess dodecyl acetate in female effluvium (Roelofs et al. 1975).

Materials and Methods

Sources of Insects

First- and 2nd- generation pupae were collected from commercial vineyards in Fredonia, NY, during mid-June and late Aug., 1976, respectively. Most of the pupae were found in leaves of English plantain (*Plantago lanceolata* L.), which was the most abundant weed in these vineyards. Additionally, numerous pupae were found among grape leaves and a few were collected from other assorted weeds.

The laboratory population of *A. velutinana* was drawn from the Geneva, NY, colony (Glass and Hervey 1962), which had been maintained for over 30 yr in greenhouses on fava bean plants, *Vicia fava*.

Collected female pupae were held at ca. 25°C in 19 *X* 14 *X* 11-cm plastic boxes provided with dental wicking soaked with water. A 15-h photophase was maintained throughout the study. Eclosed adults were collected daily and transferred to 12x9x6-cm cm plastic boxes containing wicks wetted with 10% sucrose solution.

Collection of Pheromone Extracts

Harvesting of pheromone from individual *A. velutinana* females was designed to maximize yields (Miller and Roelofs 1977). Hence, pheromone collections were restricted to 4 to 5-day-old moths and

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were carried out during early to mid-scotophase. Candidate moths were immobilized by rapid chilling. In rapid succession, individual moths were weighed and then their abdominal tips were excised between segments VIII and IX and transferred into vials containing 100 μl of redistilled dichloromethane (CH_2Cl_2). Ten μl of an internal standard [(*Z*)-11-pentadecenyl acetate] solution (7.5 ng/μl CH_2Cl_2 were then added to each extract. Excised tips were allowed to soak for 14–16 h after which time the solvent bathing each tip was removed and stored separately in another vial at -10°C until analyzed.

The quantity and relative proportions of E11- and Z11-14:Ac's in each pheromone extract were assessed with a Packard Series 7400 gas chromatograph. As reported by Miller and Roelofs (1977), chromatography of *A. velutinana* tip extracts on a polar 10% XF-1150 (50% cyanoethyl, methyl silicone) column yields a series of well-resolved peaks, 2 of which correspond to E11- and Z11-14:Ac's (Roelofs et al. 1975). Such a one-step separation of constituents would suffice for establishing pheromone component ratios, provided no additional compounds were co-chromatographing with the acetates of interest. When *A. velutinana* tip extracts were chromatographed on a 4 m column having the lst 1.5 m packed with non-polar 3% OV-1 (methylsilicone) and the remainder packed with 10% XF-1150, no further peaks were resolved and the ratios of E11- to Z11-14:Ac did not change significantly from those determined by XF-1150 analysis alone. Analyses on an OV-1 column showed that there were no corresponding alcohols to complicate the analyses on XF-1150. Ozonolysis of the E11- and Z11-14:Ac peaks collected separately from XF-1150 yielded no evidence for other positional isomers or unozonized impurities (limit of detection = ca. 1% of each Δ-11-14:Ac).

A routine analytical procedure was then adopted. The CH_2Cl_2 was evaporated from each sample via a gentle stream of N_2. Without delay, 1 drop of redistilled CS_2 was added and the vial was shaken briefly to insure redissolution of the entire sample. CS_2 was the solvent of choice for injection on GLC because it produces a comparatively small solvent peak as detected by F.I.D. The extract was then reduced under N_2 to <10 μl and a major portion was injected onto a 2 m *X* 2 mm ID glass column packed with 10% XF-1150 on 100/200 mesh Chromosorb W-AW-DMCS. The column oven was held at 150°C and the N_2 carrier flow was 30 ml/min. Samples were injected every 20–25 min with negligible interference from the preceding injection. As a check on the precision of the system, a small aliquot of pooled *A. velutinana* reference tip extract was injected at the beginning and end of each series as well as after ca. every 10th sample.

The quantities and ratios of E11- and Z11-14:Ac's were computed from the GLC tracings using relative peak areas for these acetates and the internal standard. Peak height *X* retention time was taken as the measure of relative area.

Results

As shown by the GLC Tracing in Fig. 1, the CH_2Cl_2 extracts of individual *A. velutinana* abdominal tips yielded sufficient material for quantifying ratios and amounts of pheromone components. Even in those cases where certain females contained as little as 10 ng of pheromone, the extract was clean enough and the GLC was sensitive anough to obtain the data.

The precision of the analytical system was excellent. Across all analyses, the standard deviations for measurements of ratio and quantity of the 11-14:Ac's for the reference extract were 0.4% and 1.5 ng, respectively. Precision at the end of a day's analyses was as good as at the start.

After it was determined that there were no significant differences in any of the measured parameters between the field populations of moths collected in June (N=68) and Aug. (N=160), data for the field-collected moths were pooled for all other statistical analyses. The pheromone content of the individually analyzed moths is summarized in Table 1. Significant differences were discovered between the field and laboratory populations. Although body weights were very similar, field insects contained 30% more pheromone than the lab-reared insects.

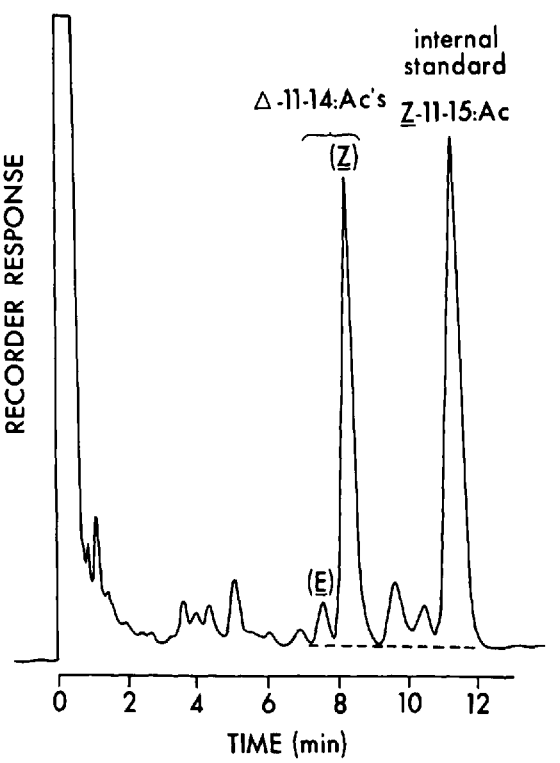

FIG. 1— GLC separation of the components in the abdominal tip extract of a single redbanded leafroller moth. See the text for conditions.

The variability of the ratios of pheromone components for the 2 populations is evident in Fig. 2. The ranges for both distributions were similar, ca. 4–15% E11-14:Ac, although the mean ratio of E/Z-11-14:Ac was significantly lower for the lab moths (Table 1). When the data were untransformed, each distribution proved to be significantly skewed toward the higher ratios. A $\sin^{-1}(X^{1/2})$ transformation restored an excellent fit to a normal distribution. The transformed mean and standard deviation for the field and lab populations was 17.5 ± 1.7 and $26.7 \pm 2.6\%$, respectively. The coefficient of variance (computed from the transformed data) for both populations was 9.7%.

Correlation analyses failed to establish any significant relationships between moth body weight, pheromone component ratio, and quantity of pheromone. In fact, 2-way plots of these parameters yielded a highly random scattering of data points. For any combination of parameters, R^2 values for regression lines were < 0.02.

Discussion

The data collected in this study add further proof for precise regulation in ratios of geometrical isomers comprising the sex pheromones of tortricid moths. If anything, the present data may slightly overestimate the variability in ratios because random error in the analytical procedure would tend to spread the data points. A possible source of such error was uncovered with a correlation analysis of pheromone quantity against deviation of each ratio datum from the mean for the population. Although the resultant data points were very highly scattered ($R^2 = 0.10$), a slight but significant ($P < 0.05$) correlation did exist, indicating that ratio deviation from the mean increased slightly as the quantity of pheromone in the samples decreased. This effect can with good reason be assigned to the diminishing signal to noise ratio; however, a biological basis for this effect was not ruled out.

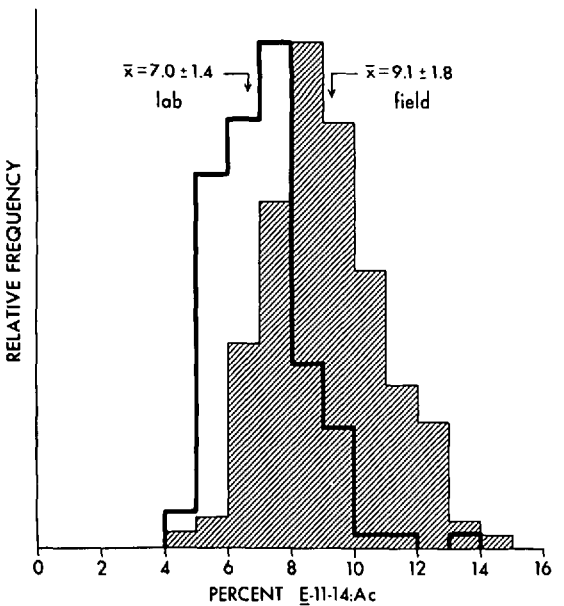

FIG. 2—Variability in ratios of E11- and Z11-14:Ac pheromone components produced by individual females in field and laboratory populations of redbanded leafroller moths.

How might the measured disparity in pheromone component ratios for the lab vs. field populations of *A. velutinana* have arisen? Among other possibilities, certain unknown selection pressures in the lab may have lowered the ratio as the colony passed through more than 250 generations. We would not yet know whether the pheromone ratio has stabilized at a 7% mean or whether it might presently be shifting either lower or higher.

Alternatively, and in our opinion somewhat less likely, the pheromonal disparity may have arisen from the founder effect. This 30-yr-old moth colony was established and is propagated by rearing larvae from egg masses collected from those females that mate and oviposit in small mating cages. The original females brought into the lab or those at some point during colony propagation may, by chance, have favored the comparatively rarer lower ratios. With different natural selection pressures than those that existed in the field, the pheromone component ratios may never have been restored to the frequency distribution having a 9% mean that is presently found in the field insects. The assumption, of course, is that the lab population has shifted relative to the field population and not vice versa.

Despite the significant difference in ratio means for the *A. velutinana* populations, the variances remained equally narrow (CV = 9.7%). Apparently, relaxation in blend regulation did not occur under laboratory conditions where requirements for a long-range sex attractant were decreased and where stabilizing selective forces such as maladaptive interspecific contacts were removed. These narrow-variance pheromonal systems might be regulated by negative feedback inherent in closely co-adapted signaler and receiver.

The present data on variability in female emissions of pheromone foster a more accurate assessment of the co-adaptations of male receivers. Although redbanded leafroller females produce pheromone blends within a narrow range, it is possible to elicit redbanded leafroller male responses through a broad range of component ratios. In laboratory box olfactometer bioassays (Baker et al. 1976), male wing-fanning responses were elicited with all mixtures from pure Z11-14:Ac to pure E11-14:Ac, but a close approximation to the natural blend (8% E) was active at the lowest load rates. Pure Z11-14:Ac and a mixture of 70:30 Z11/E11 required 100 times more material than the

Table 1—Pheromone content of individually analyzed redbanded leafroller moths.

	Field collected	Lab colony
No. analyzed	228	153
$\bar{x}$ body weight, mg	14.0± 2.6	13.8± 3.5
$\bar{x}$ % E11-14:Ac	9.1± 1.8	7.0± 1.4*
$\bar{x}$ 11-14:Ac's, ng	139.0±81.0	107.0±58.0*

*Indicates a highly significant difference $P<0.01$ in a *t*-test.

natural blend to effect a 50% response, and pure E11-14:Ac required a 10,000-fold increase. Specificity of male activation responses to the natural isomer mixture appears to be produced by the lower response threshold for these mixtures at low concentrations, rather than by a sensory system that is activated only by the correct isomers. Trapping studies (Roelofs et al. 1975) showed that males can be captured by blends containing 2-20% E-isomer, although the best catches are with lures containing ca. 6-10% E-isomer. The present studies show that trap catch with unnatural blends is seemingly not correlated to the presence of corresponding females in the population that produce these blends. None of the females were found to produce blends with 2% or 20% E-isomer, although these blends did elicit capture of some males in the field at the concentrations used. These data indicate the importance of tesing signals with a narrow coefficient of variance at the naturally occurring concentrations.

A field test with Oriental fruit moth (OFM) *Grapholitha molesta* (Busck) (Cardé et al. 1976) also showed that male moths can be captured in unnatural ratios, but that they are still optimally responsive to the natural blend if given a choice. In trapping studies, many males are ensnared before they have a choice of all treatments in the test. In the OFM test, an attraction-marking-reattraction technique with wild males was used. Males were attracted by blends of 3, 8, or 11% (*E*)- in (*Z*)-8-dodecenyl acetate in dishes containing differently colored dyes. The marked males were trapped back in succeeding nights in trap tests utilizing the same 3 blends. If there were disparate phenotypes using slightly altered blends, then males marked at one blend should have been preferentially recaptured in traps containing that blend. The test revealed, however, that the males were reattracted to all blends in the same relative proportions as they were marked. This supports the redbanded leafroller data in that the males can be attracted in the field to a wide range of blend ratios at certain release rates, but they are optimally attuned to the specific ratio produced by a population of conspecific female moths.

In conclusion, our data suggest that significant shifts can occur in the narrowly variant pheromonal signals produced by *A. velutinana* subpopulations experiencing differing selection pressures. Occurring in isolation over evolutionary time, such shifts might eventually lead to divergence in the mate recognition systems between subpopulations and ultimately to speciation (Paterson 1978).

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Footnotes

[1] Lepidoptera: Tortricidae. Received for publication Nov. 20, 1979.
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