We have no evidence for the natural significance of a synergistic response to pheromone enantiomers, or of enantiomer-specific responses. However, such phenomena could provide means of ensuring reproductive isolation between species utilizing either pheromone enantiomer, or different enantiomeric ratios. Alternatively, as also proposed by Renwick *et al.* (*13*), the host range of an insect might be limited to those host species, races, or individuals that offer pheromone precursors of appropriate enantiomeric composition.

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Western Pine Beetle: Specificity Among Enantiomers of Male and Female Components of an Attractant Pheromone

Abstract. *The flight response of both sexes of* Dendroctonus brevicomis *to the mixture of myrcene, racemic frontalin, and (1*R,5S,7R*)-(+)-*exo*-brevicomin and to the mixture of myrcene, (1*S,5R*)-(−)-frontalin and racemic* exo*-brevicomin was significantly greater than the response to the same mixtures in which the antipodes were substituted. The flight response to these two mixtures was also greater than the response to the ternary mixture of myrcene, racemic frontalin, and racemic* exo*-brevicomin (MFE). The walking response of both sexes to the mixture of myrcene, racemic frontalin, and (+)-*exo*-brevicomin was not different from the response to MFE. Substitution of the antipode lowered the response when compared to that of MFE. When evaporated with ponderosa pine turpentine, (−)-frontalin was active in the field while its antipode was not.*

We have discovered that (1*R*,5*S*,7*R*)-(+)-*exo*-brevicomin and (1*S*,5*R*)-(−)-frontalin are active in the ternary mixtures containing the host-produced component, myrcene. The mixtures containing these compounds elicited both a walking and flight response from male and female *Dendroctonus brevicomis* Coleoptera: Scolytidae), while mixtures containing their antipodes were much less attractive.

Exo-Brevicomin (*exo*-7-ethyl-5-methyl-6,8-dioxabicyclo[3.2.1]octane) and myrcene, a component in the oleoresin of ponderosa pine, were isolated from frass produced by females boring in ponderosa pine (*1*, *2*), and frontalin (1,5-dimethyl-6,8-dioxabicyclo[3.2.1]octane) was isolated from hindguts of males (*3*). Subsequently, the combination of these two bicyclic ketal pheromones with ponderosa pine oleoresin and turpentine (*4*, *5*) and with myrcene (*6*) were shown to be highly attractive to both sexes in the field. Further, both of these ternary mixtures were shown to be much more attractive than any binary mixtures containing these or other compounds. Single compounds were either not attractive or they were much less attractive than the binary mixtures. Because these differences in response levels were not additive, the term synergism was used to describe this phenomenon. These evaluations (*5*, *6*) were made with racemic frontalin and *exo*-brevicomin. Recently Mori has synthesized the enantiomers of *exo*-brevicomin (*7*) and frontalin (*8*). Silverstein and co-workers (*9*) found (1*R*,5*S*,7*R*)-(+)-*exo*-brevicomin in cold-trap condensates from aerated ponderosa pine logs infested with females alone or with females and males. They also found (1*S*,5*R*)-(−)-frontalin in extracts of the hindguts of males (*9*). As a result of these studies, we sought to determine the attractant or interruptant activity of these optically active compounds (or both). The following notation is used throughout this report: Racemic *exo*-brevicomin (E), racemic frontalin (F), myrcene (M); F or E preceded by (+) or (−) refers to the enantiomer, and (±) refers to a mixture of both enantiomers in equal proportions.

In laboratory studies, the attractiveness of the enantiomers in various mixtures was evaluated in an open-arena olfactometer (*10*). A power-driven microsyringe was utilized to deliver the compounds (*11*). Each compound was dissolved in pentane (1 ng/μl) and delivered at the rate of 2 μl/min; a pentane check was also delivered at 2 μl/min. Beetles were released in groups of five or ten, and a positive response was recorded when an individual had traversed the 20 cm upwind to the attractant source.

Field tests were conducted at Grass Valley (Nevada County) and Bass Lake (Madera County), California. Both locations are at an elevation of approximate-

Table 1. Walking response of *D. brevicomis* to the combinations of *exo*-brevicomin (E) and frontalin (F) enantiomers, racemates, and myrcene (M). The data were obtained 4 to 11 June 1974.

Treatment	Males			Females		
	Number tested	Proportion responding	Confidence interval*	Number tested	Proportion responding	Confidence interval
Pentane	30	.066	.02–.23	10		
M,F	20	.10	.02–.32			
M,F,E	20	.65	.41–.84	10	.80	.44–.97
M,F,(±)E	20	.65	.41–.84	10	.50	.18–.81
M,F,(+)E	30	.50	.29–.78	20	.80	.56–.93
M,F,(−)E	30	.166	.06–.35	20	.15	.03–.37

*Confidence limits were estimated from a chart of binomial confidence limits (95 percent) for proportions. Differences between two proportions responding are highly significant if the intervals of the two proportions do not overlap.

ly 1000 m in a mixed-conifer forest with a large component of 60- to 80-year-old ponderosa pines. At Grass Valley the enantiomers of frontalin were evaporated in pure form from capillary tubes (inside diameter, 0.04 by 75 mm) at a rate of about 2 to 3 μl/hour, and a mixture of terpene hydrocarbons (*12*) was evaporated from a 50-ml Erlenmeyer flask at a rate of about 180 to 190 mg/hour. These delivery receptacles were placed inside the base of sleeve olfactometers (*13*) placed 10 m apart. These olfactometers were equipped with electric fans that forced air upward and around the receptacles and into a canvas sleeve. Insects were picked from the sleeve and placed in a solvent, and the sex was ascertained later. In one experiment, the attractiveness of (−)F and turpentine evaporated together was compared to that of (+)F and turpentine by exchanging treatment positions after ten beetles were trapped (23 May 1975, 1620 to 1910 hours, P.D.T.). In the second experiment, the attractiveness of (−)F and turpentine was compared to that of (−)F(+)F and turpentine by exchanging positions twice after 20 minutes and twice after 10 minutes. At Bass Lake, compounds were delivered from power-driven microsyringes (*14*) placed inside hardware cloth cylinders (20 cm diameter by 30 cm) coated with a sticky substance (Stikem Special), and elevated 1.5 m above the ground on pipe standards (*15*). Each treatment was some combination of enantiomers and racemic forms in mixtures containing equal proportions of *exo*-brevicomin, frontalin, and myrcene. The mixtures were delivered (without any solvent) at a rate of 1.25 μl/hour. Traps were placed in a line at intervals of about 40 m (*6*) to minimize trap interactions. In 1974, all four treatments were evaluated on four consecutive days and in four different trap locations. In 1975, five treatments were evaluated on five consecutive days in five different trap locations. Treatments were evaluated for about 5 hours on each test day. Beetles were picked from the traps daily and placed in the solvent, and sex was ascertained later.

Under laboratory conditions, the walking response of males to the mixture of MF(−)E was not different from that of the mixture of MF (Table 1). Further, the response to MF(+)E was not different from that of MFE, while the response to MF(−)E was lower than that to MFE. As with males, the walking response of females to MF(−)E was less than the response to MFE, while the mixture containing the antipode was not different from MFE. Further, the response to MF(−)E was lower than the response to MF(+)E. Results from our 1975 laboratory experiments with (−)F, (+)F, (−)E, and (+)E were too variable to draw statistically significant conclusions. However, the mean response to M(−)F(+)E and M(±)F(±)E was the highest when compared to the response to all mixtures that contained either (+)F or (−)E or both.

Table 2. Catch of in-flight *D. brevicomis* on traps baited with mixtures of enantiomers of *exo*-brevicomin and frontalin, racemic *exo*-brevicomin and frontalin, and myrcene. Bass Lake, California, 1974–1975.

Chemical treatment	Number trapped*	Sex ratio (M/F)
	June 1974	
M, F, E	117	0.92
M, F, (+)E	207	0.85
M, F, (−)E	52	0.58
M, F	15	0.67
	June 1975	
M, F, E	174	0.81
M, (±)F, E	248†	0.98
M, (−)F, E	278†	0.78
M, (+)F, E	6‡	5.00
M, E	9‡	1.33

*Differences between numbers trapped were tested for significance by a χ^2 test. The Latin square design implicity controls trap-location effects and trap-day effects, so that any differences are due to chemical treatment effects. Each set of data was analyzed on the basis of a multinomial distribution; use of the likelihood ratio technique led to a χ^2 test of equality between any two numbers. Except as indicated, $P \leq .01$ for all pairwise comparisons (highly significant differences). †$P \doteq .2$ (not significant difference). ‡$P \doteq .4$ (not significant difference).

In the Bass Lake field experiments, the ternary mixtures containing M(−)FE or MF(+)E were highly attractive (Table 2). Substitution of their antipodes in these mixtures greatly lowered the response. The slightly higher attraction to the ternary mixtures containing either the (+)E or the (−)F enantiomers compared to the response to MFE is probably explained by the doubled rate of release of the active (+)E or (−)F compounds. Unexpectedly, the response to the M(±)FE mixture was higher than the response to MFE. Also the response to MF(−)E was greater than to MF. This could be due to contamination of (−)E with (+)E. The response to MF increases rapidly with the addition of small amounts of E (*16*). In the latter two cases, further work must be done either to confirm or to refute these results. As with many field experiments where the activity of attractant mixtures is compared, high variability in trap catch is encountered as a result of trap location and total daily catch.

Further indication of the lesser activity of (−)E and (+)F is the similarity between sex ratios recorded for the ternary mixtures containing these enantiomers and the binary mixtures (Table 2). Although skewed sex ratios have been implicated as indicators of incomplete pheromone mixtures (*5*), the number responding to these binary mixtures in our experiments was too low for an adequate statistical analysis.

The Grass Valley studies show that the specificity observed for the ternary combinations is also expressed in binary combinations. Here, the (−)F enantiomer was active when delivered with the turpentine fraction from ponderosa pine oleoresin [response to (−)F and turpentine = 60, male/female sex ratio = 0.76; (+)F and turpentine = 0]. As was the case with the ternary combinations, we could find no evidence of response interruption when (+)F was delivered with its enantiomer and turpentine [response to (−)F and turpentine = 40, male/female sex ratio = 0.67; (−)F(+)F and turpentine = 28, sex ratio = 0.65]. However, the data in this experiment are still too scant to allow firm conclusions about response interruption. We could not test for interruption at higher release rates because of the small amounts of synthetic enantiomers available to us. As was the case with the mixture of M and F at Bass Lake, turpentine (24.9 percent myrcene) and (−)F evaporating together attracted more females than males (Bass Lake sex ratio = 0.67; Grass Valley sex ratio = 0.67).

Specificity of response to enantiomers of insect pheromones has been demonstrated for the alarm pheromone of the leaf-cutting ants *Atta texana* and *A. cephalotes* (*17*), the myrmicine ant *Pogonomyrmex barbatus* (*18*), and the sex pheromone of the female gypsy moth, *Porthetria dispar* (*19*). Only the (+) enantiomer of 4-methyl-3-heptanone is synthesized by *Atta* spp., while the optical activity of this compound from the mandibular gland of *P. barbatus* (*18*) and of the natural disparlure (*cis*-7,8-epoxy-2-methyloctadecane) (*19*) have not yet been established. In the ant studies, as in ours, a mixture of both enantiomers did not result in synergism or interruption of response.

Our studies demonstrate that males and females are attracted to ternary mixtures containing (+)-*exo*-brevicomin and (−)-frontalin and to the binary mixture containing (−)-frontalin. Further, the specific patterns for the ternary mixtures are the same for walking and flying insects, which indicates a very close relationship between these two modes of locomotion from sensory input to integration by the central nervous system. The synergism observed in previous studies (*1, 5, 6, 13, 20*) where racemic

frontalin and racemic *exo*-brevicomin were used, can now be ascribed to the (−) enantiomer of frontalin and the (+) enantiomer of *exo*-brevicomin. Recently, Borden (*21*) has observed a related phenomenon for the ambrosia beetle, *Gnathotrichus sulcatus*, but in this case both enantiomers of the same attractant pheromone compound [6-methyl-5-hepten-2-ol (*22*)] have low activity and together they evoke a synergistic response. With both bark beetle species, only the enantiomers that occur in nature are active, at least at the concentrations tested. Therefore we expect both a chiral synthesis and chiral olfactory receptor system in these species.

Note added in proof: Recently, (*S*)-(−)-ipsenol has been reported to be much more attractive to *Ips grandicollis* than its antipode (*23*).

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Characterization of the Androgen Receptor from a Syrian Hamster Ductus Deferens Tumor Cell Line (DDT_1)

Abstract. *The hamster ductus deferens cloned tumor cell line (DDT_1) contains a complex steroid receptor protein that binds ^{3}H-labeled 5α-dihydrotestosterone. Diethylaminoethyl (DEAE) chromatography of cytosol from these cells yields two major receptor peaks of activity. Identification of this steroid binding protein as a cytoplasmic receptor was confirmed by salt dissociation on sucrose gradients, stability of the hormone-receptor complex at 0°C, and the retention patterns on phosphocellulose and DEAE cellulose. Multiple forms of the receptor exist in a single homogeneous cell type. The data support the theory that steroid hormones bind to a cytoplasmic protein receptor composed of dissimilar subunits as the initial step in steroid hormone action.*

Steroid hormones are believed to exert their effects on target tissues by a series of events beginning with the combination of the steroid with a specific cytoplasmic receptor in the cell. This hormone-receptor complex is then translocated into the cell nucleus where it binds to the genetic material and initiates the appropriate responses to the steroid such as cell growth (*1*).

Model systems for studying growth responses to steroid hormones in cell culture have proved exceedingly difficult to establish. We have developed the DDT_1 cell line (*2*), from a leiomyosarcoma induced in the ductus deferens of a male Syrian hamster by long-term administration of testosterone propionate and diethylstilbestrol (*3*). The DDT_1 cell line contains androgen receptors that translocate to the nucleus and exhibits a growth response to androgens. The order of binding affinity is 5α-dihydrotestosterone (DHT) > testosterone (T) > 17β-estradiol > progesterone. Cortisol and the synthetic glucocorticoids dexamethasone and triamcinolone acetonide do not inhibit ^{3}H-labeled DHT binding to cytoplasmic receptor. We now report further characterization of the cytoplasmic androgen receptor from this homogeneous cloned cell population.

DDT_1 cells, clone MF-2, were grown at 37.5°C in 6-liter suspension culture vessels to a density of 1.5×10^5 cell/ml in a medium consisting of 93 percent Hams-F12, 5 percent fetal bovine serum, penicillin and streptomycin (100 μg/ml), and Fungizone (2.5 μg/ml) (all from Gib-

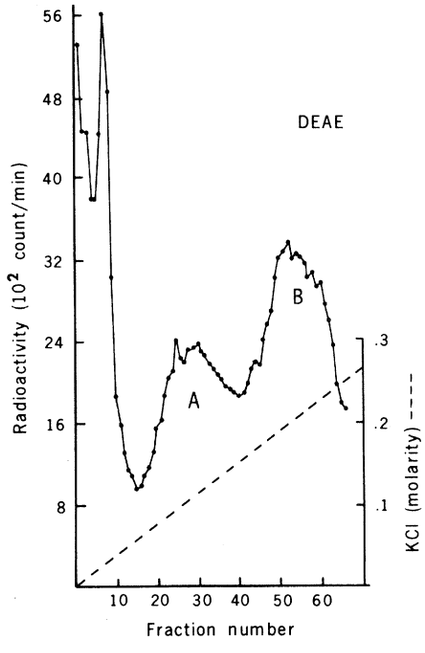

Fig. 1. DEAE-cellulose chromatography of 2 ml of DDT_1 cytosol labeled with [^{3}H]DHT and eluted with a 0 to 0.3*M* KCl gradient. Fractions (2 ml) were collected and the radioactivity in 0.5-ml samples were counted in 4 ml of Aquasol (New England Nuclear) at 25 percent efficiency (Beckman LS-233). Conductivities were determined with a radiometer conductivity cell and converted to KCl molarity. Two peaks of radioactivity were eluted: at 0.12*M* KCl (peak A) and at 0.23*M* KCl (peak B).