Chemistry of male dominance in the house mouse, *Mus domesticus*

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Summary. Two terpenic constituents, E,E,-α-farnesene and E-β-farnesene, were found to be elevated in dominant male urine when compared to subordinate or control males. These two urinary compounds were absent in the bladder urine of males; however, they were the most prominent constituents of the preputial gland's aliquots. The results of a two-choice preference test, conducted on ICR/Alb subordinate males, gave a strong indication that these two terpenic constituents introduced into the previously attractive stimulus significantly discouraged prolonged investigations by male mice. The compounds, whether present in the urine matrix or water, rendered the stimulus with a quality behaviorally similar to the urine of dominant males. It appears that they may be synonymous with the previously described aversion signal produced by dominant males. We suggest that these compounds may play a wide-ranging role in the territorial marking behavior of male mice.
Key words. Preputial gland; aversive stimulus; submission-dominance; mice; olfactory preference; farnesene; chemosignals.

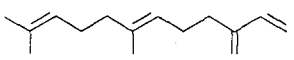
Dominance-associated traits in male mammals, such as aggression and territoriality, have long been viewed as ecologically significant. It is generally agreed that dominant male mice tend to sire more litters than subordinate males[1,2]. The reproductive success of dominant males can be partially explained by the fact that dominant males are known to deter subordinates from approaching receptive females[3]. The subordinate males, occupying less desirable habitats, have a lower survival rate as well as a lower reproductive potential. There are numerous physiological attributes of subordination, such as poor spermatogenesis, decreased gonadal activity, and abnormal adrenal function[4–7].

Since social subordination suppresses gonadal function, emission of male-specific chemical signals (pheromones), which are under testosterone control[8], is expected to be altered as compared to dominant males. Indeed, both the male-to-male and male-to-female signals appear to be affected; urine from dominant males is far more effective in promoting puberty acceleration in young female mice than urine from subordinate males[9], while the androgen-dependent 'aversion pheromone' shows greater potency in the urine of dominant males as compared to subordinate animals[10,11]. The urine from castrated males is ineffective; however, testosterone treatment restores the aversive efficacy with a five-day latency[12].

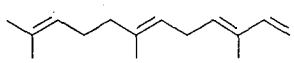
While physiological, social, and biological attributes of dominance/subordination have been the subject of numerous studies, no chemical information was reported on the signals responsible for such observed phenomena. Encouraged by the reports that dominant males tend to have higher levels of testosterone than subordinates[13,14] and that male mouse urine has an odor indicative of social status[10,11], we investigated differences in the urinary volatile profiles of trained fighters and subordinate males.

Chromatographic comparisons clearly indicated quantitative differences in the urinary profiles between dominant and submissive males[15]. The known male mouse pheromone, 2-(*sec*-butyl)-4,5-dihydrothiazole[16–18], and two terpenic constituents (α- and β-farnesene) were elevated in dominant male urine several days after dominance had been established when compared to subordinate or control males[15]. These two urinary compounds, α- and β-farnesene, were conspicuously absent in the bladder urine[15].

We now report that α- and β-farnesene (structures given below)[19] are linked to the preputial gland, a long-suspected source of androgen-dependent, sex-related pheromones[20]. We further demonstrate that the pure farnesenes dissolved in an appropriate amount of water or mouse urine (mimicking their natural concentrations in mouse urine) significantly discouraged prolonged investigations by subordinate male mice.



E-β-farnesene



E, E-α-farnesene

The founders of our ICR/Alb albino mouse colony were purchased from Ward's Natural Science (Rochester, NY). All animals were maintained at 21 ± 0.2 °C, 50–70% humidity, and a 12 h light/12 h dark regime (lights on at 06.00 h). Purina mouse chow and water were supplied ad libitum throughout the experiments. Bedding was changed weekly.

Mature, virgin female mice serving as urine donors were kept alone in their home cage at least 4 weeks before urine collection. The social-rank test for the males was performed as described previously by Harvey et al.[15]. Dominant (N = 11) and subordinate (N = 11) males were kept alone in their home cage for one week after the social-test was completed, after which period the excreted urine and bladder urine were collected. These socially experienced animals (dominants and subordinates) together with control males (inexperienced, singly-caged, at

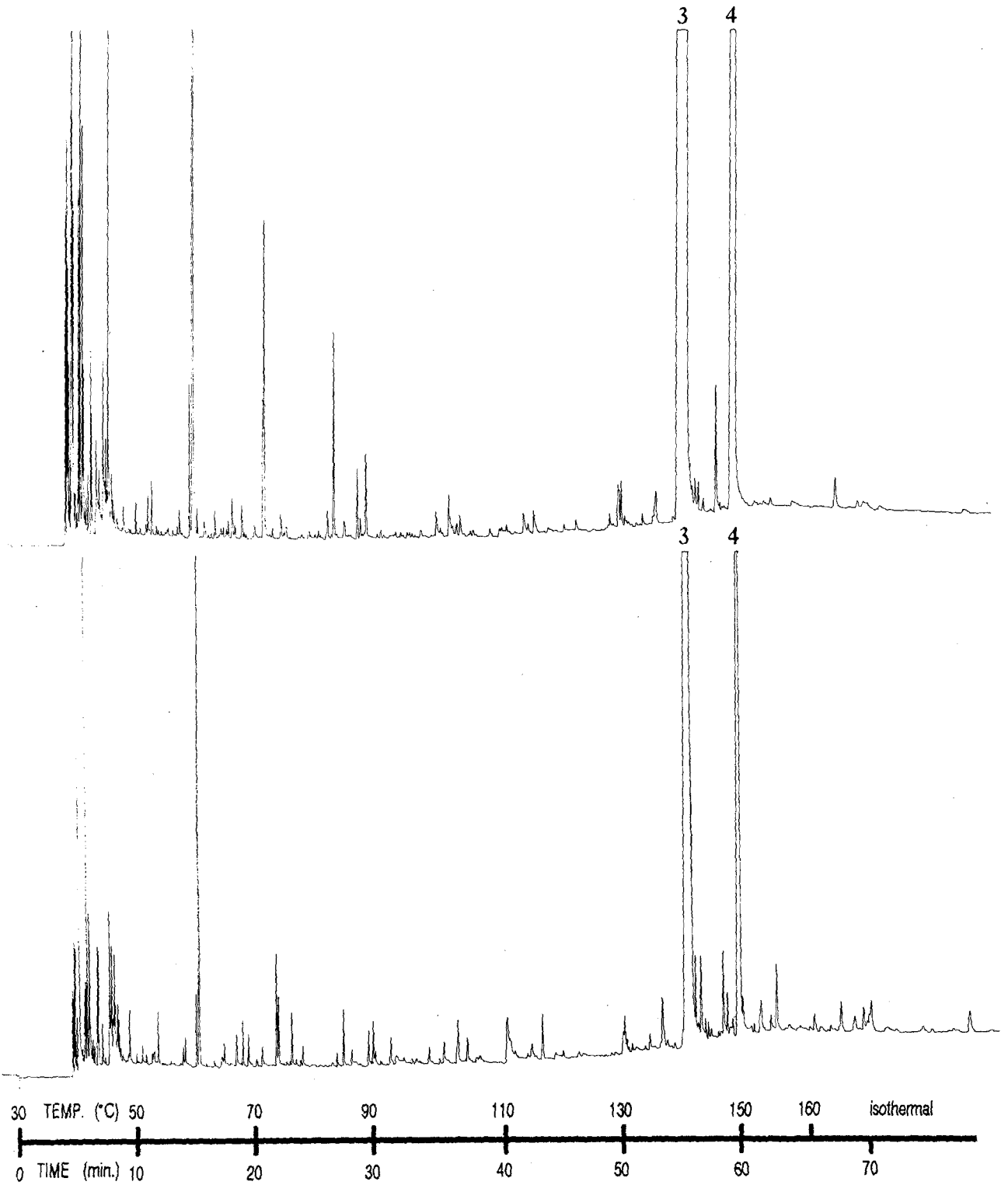


Figure 1. Representative chromatograms of the volatiles from dominant (upper) and subordinate (lower) preputial gland.

age of 6 months, N = 10) were then sacrificed and the preputial glands were dissected out, cleared of fat tissue, and weighed. In agreement with the observations of other authors[9], dominant males from our experiment were found to exhibit larger preputial glands than subordinates and controls (dominant: 163 ± 8.3 mg > subordinate: 125.9 ± 9.9 mg > control: 116.0 ± 5.1 mg; $p < 0.02$, Student's t-test).

The volatile constituents of 0.1 g aliquots of the preputial glands were preconcentrated in a Tenax GC absorbent by

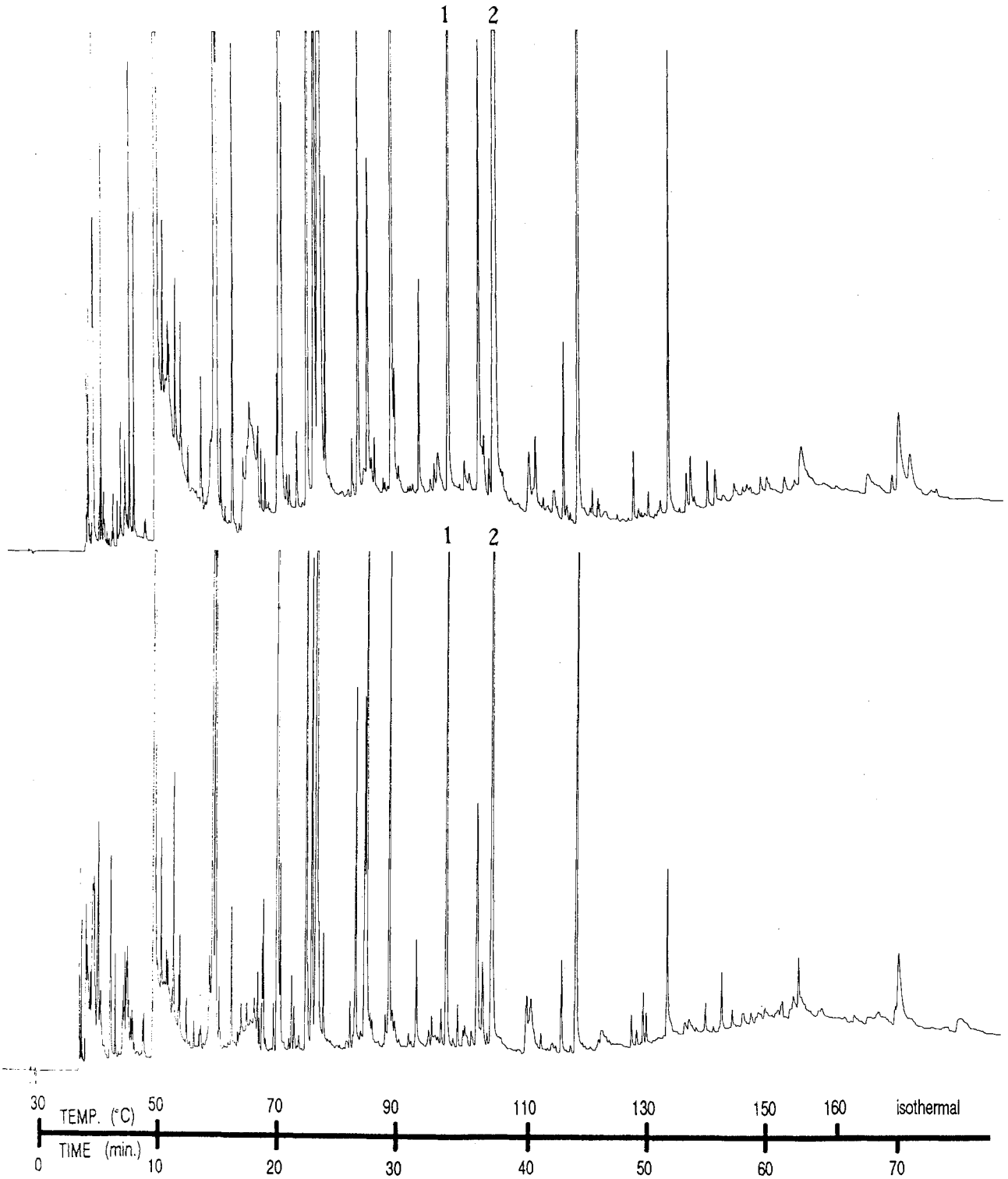


Figure 2. Representative chromatograms of the volatiles from dominant (upper) and subordinate (lower) bladder urine.

headspace sampling prior to chromatographic analysis according to the previously described procedure[21,22]. The amounts of α- and β-farnesene were substantially greater in an aliquot of the glandular tissue from dominant males compared to subordinate and control males (dominant vs. control: 255% and 127% of increase for α- and β-farnesene, respectively; dominant vs subordinate: 156% and 80% of increase for α- and β-farnesene, respectively; number of samples per each group was 3). Subordinate and control males did not show significant

differences in the amounts of α- and β-farnesene in an aliquot of the glandular tissue of the preputial gland (subordinate vs control: 28% and 21% of increase for α- and β-farnesene, respectively). Under similar chromatographic conditions, the relative amounts of α- and β-farnesene can be compared for the samples of urine and glandular source (figs 1 and 2). As seen in figure 1, α- and β-farnesene (peaks 3 and 4), are the most prominent constituents of the preputial gland, and they are totally missing from the bladder urine (fig. 2). The previously identified male pheromones[16-18], dehydro-exo-brevicomin (peak 1) and 2-(*sec*-butyl)-4,5-dihydrothiazole (peak 2) are not contained in the preputial gland headspace even in trace quantities. However, they are present in the bladder urine (fig. 2, above).

Based on the above findings, potential behavioral responses of these compounds must now be assessed. Since the androgen-dependent aversive signal was previously shown to be absent in bladder urine[10,11], dehydro-exo-brevicomin and 2-(*sec*-butyl)-4,5-dihydrothiazole are unlikely candidates for this pheromone, while the farnesenes are strongly suspected. Since testosterone restores the aversive efficacy in castrated males with a five-day latency, involvement of an androgen-dependent tissue rather than an androgen-excreted metabolite is suggested[12].

Based on our previous results[15] showing that a) concentration of α- and β-farnesene increased significantly only in dominant male urine some time after dominance status was established and b) α- and β-farnesene appear testosterone-dependent, we suspect that the preputial gland is a target tissue for testosterone to produce the aversion chemosignal. The preputial glands have long been considered a possible site for sex-related signals[23-24], while territorial marking is of paramount importance for the establishment of social and/or sexual dominance. The two androgen-dependent chemosignals[16] present in bladder and excreted urine, dehydro-exo-brevicomin and 2-(*sec*-butyl)-4,5-dihydrothiazole, are sufficient to elicit increased aggressiveness in trained fighters. Interestingly, bladder urine alone is known to induce inter-male aggression, but not the aversive action of subordinate males; however, the combination of bladder urine and preputial gland secretion has been reported to be more effective than bladder urine alone in eliciting aggression in trained fighters[11]. It is thus entirely possible that α- and β-farnesene are the constituents potentiating the known effectiveness[16] of these two compounds, and that they can also act as the aversive signal.

In order to elucidate the possible role of α- and β-farnesene as the aversive signal, we designed a series of two-choice preference tests involving the investigatory behavior of male ICR/Alb albino mice.

The test males, 4–6 months old, were housed in groups of four per cage. The dominant male of each cage was identified and excluded from testing to limit behavioral variation. Each remaining animal was tested in a cylindrical arena (30 × 16 cm) with a replaceable floor. Two circular ports (1 cm in diameter), located 3.5 cm above the base of the wall and 180° apart, were used for odor presentation. A glass vial (1 × 3.5 cm), containing 0.2 ml of a stimulus solution, was fitted into each port so that an animal could sniff the opening without contacting the liquid. Each male (n = 9) was tested daily for a 5-min

Response of male mice to the odor of various urine types and water presented with or without synthetic compounds: α- and β-farnesene.

Experiment	Total investigatory time (s)	Total activity (s)	Decrease of activity (%)	# of males preferring urine over water
A. 1. Female urine	1917 a**			9
Water	860 b	2777	0.0	0
2. Female urine and α- + β-farnesene	755 b*			3
Water	567 c	1322	52.4	0
B. 3. Dominant urine	355 a**		26.8	0
Water	641 b	966		9
4. Subordinate urine	868 b**			8
Water	453 a	1321	0.0	0
C. 5. Bladder urine	1000 a**			8
Water	573 b	1573	0.0	0
6. Bladder urine and α- and β-farnesene	288 d**			1
Water	636 b	924	41.3	8
7. Bladder urine	930 a*			6
Water and α- + β-farnesene	427 c	1357	13.7	0
8. Bladder urine and α- + β-farnesene	393 c*			2
Water and α- + β-farnesene	247 d	640	59.3	0

Statistical comparisons were made using t-test for small samples (Paired t-test)[30].
Those values not marked with the same letters (a,b,c,d) within the experimental group A,B or C, are significantly different at the 0.05 level. Asterisks are related to two-choice test comparison within group 1–8; * significant at the level 0.01; ** significant at the level 0.001.

period, following a 5-min period of habituation. Five trials were performed for each male in eight different (see below) experimental situations. The investigatory time was determined as the amount of time the animal spent with a part of its snout in the sample port. The following urines were tested relative to water: [1] female urine; [2] female urine containing α- and β-farnesene; [3] male dominant urine; [4] male subordinate urine; [5] subordinate male bladder urine; and [6] subordinate bladder urine containing α- and β-farnesene. Additional experiments tested bladder urine [7] and bladder urine containing both sesquiterpenes [8] relative to water containing α- and β-farnesene. The synthetic compounds were present in the samples at concentrations simulating their content in dominant male urine (about 5 p.p.m., v/v, each).

The table shows the total time that the animals spent investigating the sample ports during the 5-min test for each of the eight experimental conditions. The results clearly indicate the male's preference for female urine, subordinate urine, and male bladder urine (conditions 1, 4, and 5) when compared to water. Additionally, the tested animals exhibited significantly lower sniffing activity in the presence of dominant male urine when compared to urine from submissive males. The most dramatic decrease in investigative activity was observed when any sample contained α- and β-farnesene. Both female urine and male bladder urine, when spiked with these compounds (conditions 2 and 6) are less preferable to tested males when compared to corresponding trials with unspiked urine (conditions 1 and 5). Addition of the sesquiterpenes into these samples decreased the total motor activity of animals in the test chamber by approximately 50%. An extremely low motor activity was observed when the test animals were simultaneously exposed to two samples containing the farnesenes; in condition 8, the sniffing activity of animals dropped 62.5% compared to the testing condition (5) employing the bladder urine and water.

We have shown here that addition of α- and β-farnesene into the previously preferred olfactory stimuli significantly discourages prolonged investigations by subordinate male mice. The chemosignal so created appears behaviorally similar to the aversion pheromone contained in the urine of dominant males. The fact that the farnesenes originate in the preputial gland further supports the notion. We suggest that these compounds may play a wide-ranging role in the territorial marking behavior of male mice.

The farnesyl pyrophosphate is a well-known key intermediate in the biosynthesis of steroids[25]. Interestingly, farnesenes have also been implicated previously as insect pheromones[26–29].

1 DeFries, J. C., and McClearn, G. E., Am. Nat. *104* (1970) 408.
2 Oakeshott, J. G., Oecologia *15* (1974) 143.
3 Huck, U. W., Banks, E. M., and Wang, S. C., Behav. Neural Biol. *33* (1981) 364.
4 Christian, J. J., Lloyd, J. A., and Davis, D. E., Rec. Progr. Horm. Res. *21* (1965) 501.
5 Bronson, F. H., Bull. ecol. Soc. Am. *47* (1966) 182.
6 Bronson, F. H., Stetson, M. H., and Stiff, M. E., Physiol. Behav. *10* (1973) 369.
7 Chapman, V. H., Desjardins, C., and Bronson, F. H., Proc. Soc. exp. Biol. Med. *130* (1969) 624.
8 Bronson, F. H., Q. Rev. Biol. *54* (1979) 265.
9 Lombardi, J. R., and Vandenbergh, J. G., Science *196* (1977) 545.
10 Jones, R. B., and Nowell, N. W., Physiol. Behav. *11* (1973) 35.
11 Jones, R. B., and Nowell, N. W., J. Endocr. *59* (1973) 302.
12 Mugford, R. A., and Nowell, N. W., Nature *226* (1970) 967.
13 Rose, R. N., Bernstein, I. S., and Gordon, T. P., Psychosom. Med. *37* (1975) 50.
14 McKinney, T. D., and Desjardins, C., Biol. Reprod. *9* (1973) 370.
15 Harvey, S., Jemiolo, B., and Novotny, M., J. chem. Ecol. *15* (1989) 2061.
16 Novotny, M., Harvey, S., Jemiolo, B., and Alberts, J., Proc. natl Acad. Sci. USA *82* (1985) 2059.
17 Jemiolo, B., Alberts, J., Sochinski-Wiggins, S., Harvey, S., and Novotny, M., Anim. Behav. *33* (1985) 1114.
18 Jemiolo, B., Harvey, S., and Novotny, M., Proc. natl Acad. Sci. USA *83* (1986) 4576.
19 E,E-α-farnesene and E-β-farnesene were identified from their mass spectra and retention of authentic compounds through capillary gas chromatography. E,E-α-farnesene was synthesized (Negishi and Matushita, Org. Synth. *62* (1984) 31–36) and E-β-farnesene was isolated from the oil of chamomile (Murray, K. E., Aust. J. Chem. *22* (1969) 197–204; Sorn, F. et al., Coll. Trav. Chim. Tchec. *14* (1959) 699–715; Sorn, F. et al. Coll. Czech. Chem. Commun. *16* (1951) 626–638.
20 Jones, J. B., and Nowell, N. W., J. Endocr. *60* (1974) 19.
21 Novotny, M., Lee, M. L., and Bartle, K. D., Chromatographia *7* (1974) 333.
22 Schwende, F. J., Wiesler, D., Jorgenson, J. W., Carmack, M., and Novotny, M., J. chem. Ecol. *12* (1986) 277.
23 Caroom, D., and Bronson, F. H., Physiol. Behav. *7* (1971) 659.
24 Aron, C., Physiol. Rev. *59* (1976) 229.
25 Lehninger, A. L., Biochemistry, 2nd edn, pp. 679–684. Worth Publishers, Inc., New York 1975.
26 Cavill, G. W. K., Williams, P. J., and Whitfield, F. B., Tetrahedron Lett. *23* (1967) 2201.
27 Bowers, W. S., Nault, L. R., Webb, R. E., and Dutky, S. R., Science *177* (1972) 1121.
28 Pickett, J. A., and Griffiths, D. C., J. chem. Ecol. *6* (1980) 349.
29 Gibson, R. W., and Pickett, J. A., Nature *302* (1983) 608.
30 Bailey, N. T. J., Statistical Methods in Biology, 2nd edn. John Wiley and Sons, New York 1981.

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