photoreceptor within the last abdominal ganglion [8] is involved in the response, and that inputs from the anterior part of the nervous system modulate the response.

At the initial stage of copulation behavior, the male opens the valva widely, hooks part of the female's genitalia with the dorsal hook, the superuncus [4], closes the valva, and then proceeds to the next step, insemination. Instead of proceeding to insemination immediately after the closing of the valva, the male usually repeats the opening and closing of bilateral valvae several times. This action probably stabilizes the copulatory posture. The photoreceptive site 1 (P1) in the male scaphium is covered during copulation, because the male pinches the female's genitalia tightly with the scaphium and the superuncus. If the male P1 is not covered, this means that the copulatory posture may not be perfect. The posture then needs to be corrected. Preliminary behavioral observations suggest that the male may on occasion release the female by opening the valva, apparently in an attempt to achieve the correct posture for proper insemination.

The photoreceptive area on the genitalia consists of only four photoreceptor cells [6]. These four photoreceptors essentially provide four separate eyespots, which, rather than being involved in resolving images, function simply to detect whether or not a particular domain of the body surface is covered. In the behavioral context described here, the eyespots, at least the P1s, may function as proprioceptors that monitor the copulatory posture.

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Aggregation Pheromones of Two Asian Palm Weevils, *Rhynchophorus ferrugineus* and *R. vulneratus*

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Two sympatric species of palm weevil, *Rhynchophorus ferrugineus* (Oliv.) and *R. vulneratus* (Panz.) (= *R. schach*) (Coleoptera: Curculionidae) [1], are important pests of coconut, oil palm, sago and aren palms in South and Southeast Asia [2,3]. Adults are attracted to wounded palms where eggs are laid [3–5]. The larvae tunnel into the terminal bud or trunk of the tree, leading directly to its death.

Aggregation pheromones have recently been identified for *R. palmarum* (L.) [2-methyl-5-(*E*)-hepten-4-ol] [6,7] and *R. phoenicis* F. (3-methyl-4-octanol) [8]. We report for the first time the identification, antennal perception, and behavioral activity of two male-produced pheromones for *R. ferrugineus* and *R. vulneratus*.

Twenty male and 20 female *R. ferrugineus* and 25 male and 25 female *R. vulneratus* were aerated separately for 1 week in modified Nalgene desiccators containing sugarcane [7]. A vacuum pump was used to draw charcoal-filtered air through the chambers and then through Porapak Q filters to capture insect- and host-produced volatiles. Volatiles were eluted from the Porapak Q with pentane and concentrated by distillation.

Analyses of volatile extracts with coupled gas chromatographic-electroantennographic detection (GC-EAD) [9] revealed in both species the presence of two male-specific compounds that elicited strong electrical potentials by male and female antennae (Fig. 1). The mass spectrum of the second EAD-active compound (*2,* Fig. 2) in *R. ferrugineus* and *R. vulneratus* was identical to that of a minor male-produced compound in the American palm weevil, *R. palmarum*. It

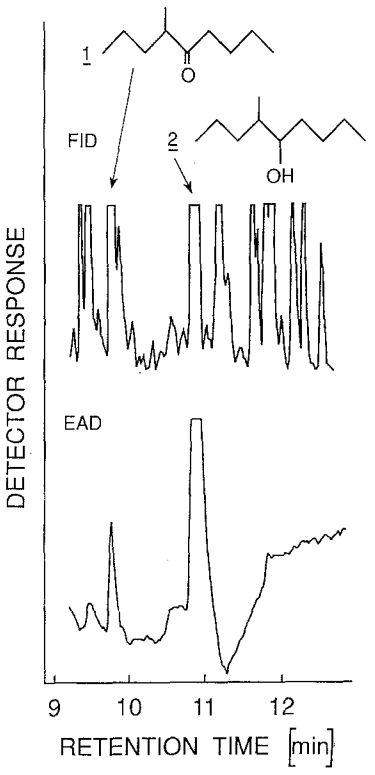

Fig. 1. Flame ionization detector (FID) and electroantennographic detector (EAD) responses to volatiles obtained from male *R. vulneratus* feeding on sugarcane. FID trace of 1 µl of extract where 1 ml = 1288 weevil hours of pheromone production. The antennal recording presented is that of a single male *R. vulneratus* antenna to 1 µl of extract diluted tenfold with hexane. Chromatography: Hewlett Packard 5890A gas chromatograph equipped with a DB-5 coated, fused silica column (30 m × 0.25 mm ID); 1 min at 70 °C, 5 °C/min to 240 °C

was hypothesized to be a secondary, methyl-branched aliphatic alcohol. Treatment of a male *R. palmarum* extract with Jones reagent [10] and subsequent mass spectroscopy of the oxidized compound gave a ketone with molecular weight 156, identical to that of the first EAD-active compound (*1*) in *R. ferrugineus* and *R. vulneratus* (Figs. 1, 2). Analysis of the ketone mass spectrum indicated the ketone group at C5. Mass and retention characteristics of authentic 4-methyl-5-nonanol, but not 3-methyl-5-nonanol, were identical with *2* in *R. ferrugineus, R. vulneratus*, and *R. palmarum*. In all three species, equivalent amounts of synthetic *2* and the male-produced compound elicited similar antennal responses by male and female weevils. 4-Methyl-5-nonanone (*1*) was present in male *R. ferrugineus* and *R. vulneratus*, but was not detected in male *R. palmarum*.

Racemic *2* was obtained by the slow addition of n-butyl lithium in hexane to freshly distilled 2-methyl-1-pentanal in hexane, cooled with an ice-water bath. Further oxidation with Jones reagent at room temperature yielded *1*. Both *1* and *2* were purified to >95 % by distillation under reduced pressure using a water aspirator.

Three field experiments were conducted at the Coconut Research Station (Balai Penelitian Kelapa), Pakuwon, Java from August to September 1992. White bucket traps (20 l) [11] were attached at 2 m height to coconut palms in randomized blocks with traps at 24-m intervals and blocks 70 m apart. All traps in each experiment contained 2 kg of 1-day-old coconut wood pieces (approx. 5×20 cm) treated with Basudin 60EC (diazinon; Ciba-Geigy), 0.24 % a.i. in water, to retain captured weevils. No significant differences were found between response patterns of female and male weevils of either species in any experiment, so pooled data for both sexes are presented here.

A 4-treatment, 10-replicate experiment tested the behavioral activity and optimal dose of *2*. At 3 mg/day, *2* was significantly more attractive to *R. ferrugineus* than all other stimuli (Fig. 3, Exp. 1), and even at a low release rate (0.3 mg/day), attraction exceeded that of the palm control. All doses of *2* were significantly and equally more attractive to *R. vulneratus* than palm alone (Fig. 3).

A second 10-replicate experiment compared the activity of *2* alone at 3 mg/day and in combination with increasing amounts of *1*. Significantly more *R. ferrugineus* were captured in traps with *2* alone or in a 10:1 ratio with *1*, than in other treatments (Fig. 3, Exp. 2). Increasing amounts of *1* significantly decreased attraction of *R. ferrugineus*, but not *R. vulneratus*.

Because the response patterns of *R. ferrugineus* and *R. vulneratus* were not significantly different (X^2 = 5.42, df=2, p > 0.05), data for both species were pooled to examine response patterns over time. The total number of weevils captured on day 5 was significantly higher than that on day 2 or day 7 (Fig. 4).

A final experiment with four treatments and five replicates compared the attraction of *2* and *1* in a 10:1 ratio to that of ten live males of either *R. ferrugineus* or *R. vulneratus*. Male *R. ferrugineus*, but not *R. vulneratus*, were as attractive as the synthetic pheromone blend to both *R. ferrugineus* and *R. vulneratus* (Fig. 3, Exp. 3).

4-Methyl-5-nonanol (*2*) is a pheromone of both *R. ferrugineus* and *R. vulneratus*, but is not behaviorally active in *R. palmarum* [12]. Since *2* is produced only by males but attracts both sexes, it can be classified as an aggregation pheromone [13] for both of these Asian palm weevils. Although 4-methyl-5-nonanone (*1*) was antennally active in both weevils, it induced observable behavioral responses only in *R. ferrugineus* (Fig. 3). On the

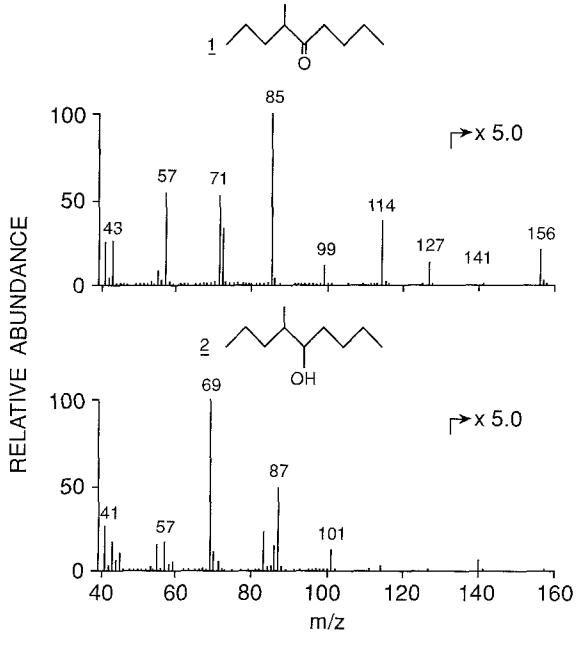

Fig. 2. Mass spectra of 4-methyl-5-nonanone (*1*), and 4-methyl-5-nonanol (*2*). Chromatography: Hewlett Packard 5885B gas chromatograph-mass spectrometer equipped with an SP-1000 coated, fused silica column (30 m × 0.25 mm ID); 1 min at 70 °C, 10 °C/min to 180 °C

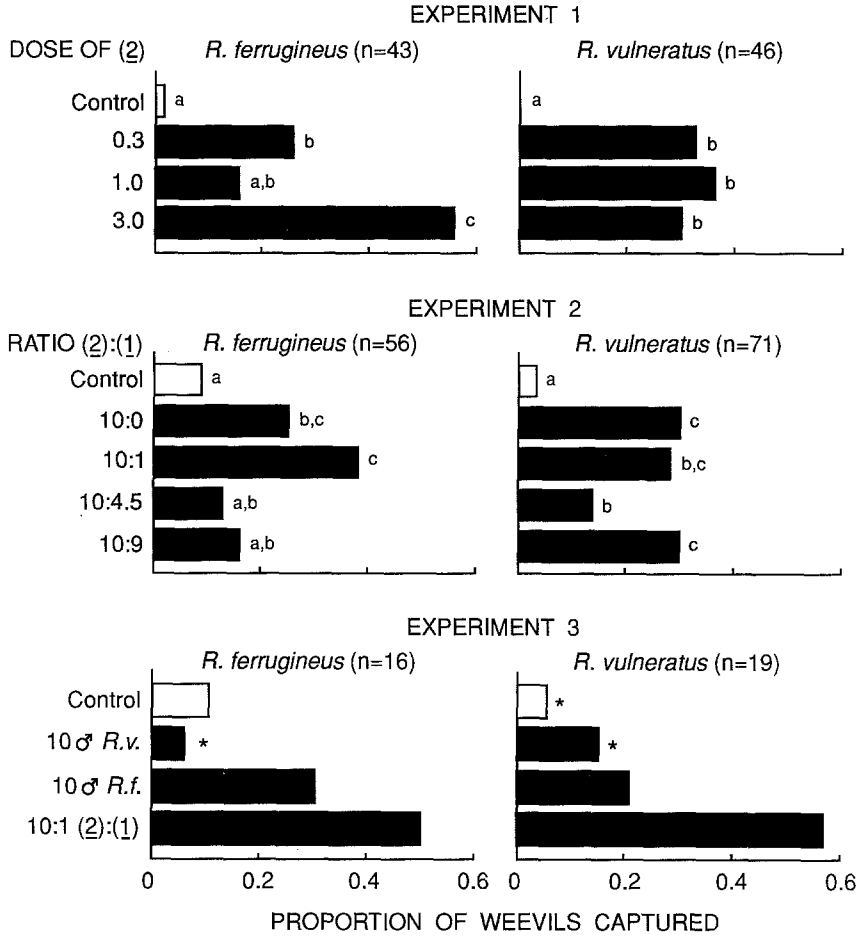

Fig. 3. Proportions of captured *R. ferrugineus* and *R. vulneratus* in three experiments using bucket traps, each containing 2 kg of freshly cut, diazinon-treated coconut wood, Coconut Research Station, Pakuwon, Java, Indonesia. Numbers in parentheses indicate total catch. Experiment 1: Traps baited with palm wood alone (Control) and in combination with *2* (4-methyl-5-nonanol) at three release rates; N= 10; 22–27 Aug. 1992. Experiment 2: Traps baited with palm wood alone (Control), in combination with *2* at 3 mg/day (10:0) or with *2* and *1* (4-methyl-5-nonanone) in three different ratios; N= 10; 28 Aug.–4 Sept. 1992. Experiment 3: Traps baited with palm wood alone (Control), in combination with a 10:1 ratio of (*2*):(*1*), or 10 live males of either *R. ferrugineus* or *R. vulneratus*; N= 5; 5–9 Sept. 1992. Males were placed in a glass jar half-filled with cut pieces of palm petiole. Twenty 3-mm holes in the jar lid provided ventilation and allowed weevil-produced pheromones to be released. In Exp. 1 and 2 species graphs, bars followed by the same letter are not significantly different, χ^2 test, $p<0.05$. In Exp. 3, bars followed by * are significantly different from 10:1 (*2*):(*1*), χ^2 test, $p<0.05$. Release rates of synthetic compounds from capillary tubes, placed in 400 µl Eppendorf tubes, and from 400 and 1500 µl Eppendorf tubes were estimated by calculating weight loss at approximately 27 °C and 50 % R. H. Release rates of *2* were 0.3 and 1.0 mg/day from 400 and 1500 µl tubes, respectively; and of *1* were 0.2 and 1.4 mg/day from capillaries and 400 µl Eppendorf tubes, respectively. Lures were suspended from the underside of the bucket lid, and all Eppendorf tubes had two 3-mm diameter holes drilled 1 cm from the top

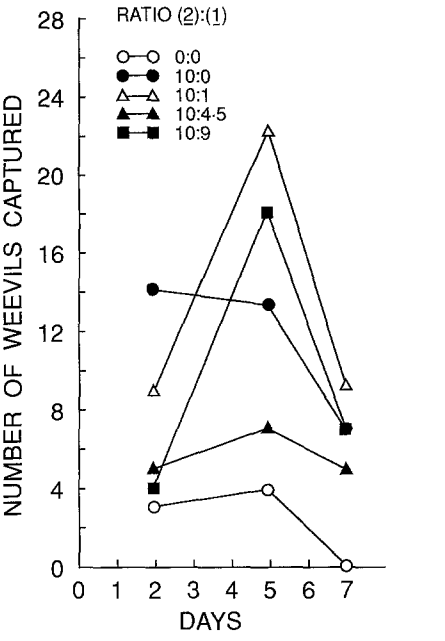

Fig. 4. Time-dependent attraction of both *R. ferrugineus* and *R. vulneratus* to coconut wood alone or in combination with *2* (4-methyl-5-nonanol) or *2* and *1* (4-methyl-5-nonanone). Significantly more weevils were captured on day 5 than on days 2 or 7 (χ^2 = 17.21, df= 2, $p<0.001$)

basis of these results, *1* can be classed as a pheromone only for this species. Additional research is required to elucidate the exact behavioral function of this compound. We propose the trivial names "ferrugineol" and "ferrugineone" for *2* and *1*, respectively.

As in many Coleoptera [13], one or both of these pheromones may interact synergistically with host volatiles. Palm weevils are known to be attracted to damaged or dying palms, split palm trunks, and to fermenting palm sap [4,5]; and split coconut petioles are used to trap *R. ferrugineus* in Sri Lanka [2]. Weevil catches peaked on day 5 and declined thereafter (Fig. 4). Similar patterns of response have been found in *R. palmarum* [11], *R. phoenicis* [8], and *R. cruentatus* (F.) [14], and are believed to be due to the peak production of attractive host volatiles several days after cutting. Although trap captures were low, attraction to palm wood alone (Fig. 4) peaked 4–6 days after cutting. The accentuation of this pattern by the addition of pheromone may be indicative of synergism between the insect- and host-produced compounds. However, direct comparison of the attractiveness of pheromone alone, palm wood alone, and the two in combination must be made before we can conclude that synergism is occurring. Synergism between a weevil aggregation pheromone and host compounds has also been shown for *Anthonomus grandis* Boh. [15].

Lack of pronounced differences in pheromonal production and response (Fig. 3) reopens the question as to whether *R. ferrugineus* and *R. vulneratus* are distinct species [1]. If they are distinct, reproductive isolation could be achieved by specificity of pheromone isomers [13]. While the reduced attraction of *R. ferrugineus* to treatments containing high amounts of *1* (Fig. 3) may have been a simple dose effect, the presence of repel-

lent chiral isomers in the racemic pheromone blends could have caused this effect. Production of and response to a chiral pheromone have been demonstrated for *R. palmarum* [7], and need to be investigated in *R. ferrugineus* and *R. vulneratus*. Other pre-mating mechanisms that could ensure reproductive isolation [16] include: differences in host compounds used as synergists, food preferences, specificity of stridulation or courtship rituals, and genital incompatibility. Although there are reports that *R. ferrugineus* more often attacks the trunk and *R. vulneratus* the terminal bud of the tree [2,5], both attack a variety of palm types, and coattacked trees have been observed [17]. Differences in food preferences or the use of specific host compounds as pheromone synergists, therefore, do not appear to be functioning to achieve reproductive isolation. Interspecific mating pairs of *R. ferrugineus* and *R. vulneratus* are often observed in caged populations, suggesting that incompatibilities in courtship rituals or genitalia may not exist or may not be sufficient on their own to maintain isolation.

In our experiments, 27 captured weevils (10.6%) had color markings intermediate between those of *R. ferrugineus* and *R. vulneratus*. These individuals had the black pronotum with a red stripe, characteristic of *R. vulneratus*, but their elytra and abdomens were reddish-brown, like *R. ferrugineus*. Such individuals were classed as *R. vulneratus*, in keeping with Wattanapongsiri's [1] taxonomic descriptions. The existence of these individuals and the lack of observed pheromonal differences to date necessitate investigation of other mechanisms of reproductive isolation.

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How Do Ants Acquire Their Celestial Ephemeris Function?

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Most recent work on celestial navigation in insects has focused on the spatial properties and neural representation of the skylight compass [1]. Temporal aspects related to the daily westward movement of the sun across the sky, i.e., to the rotation of the celestial (e-vector) pattern about the zenith, have received much less attention [2–5]. The question of how insects acquire the necessary information and to what extent innate components are involved still remains to be answered.

The problem the insect faces in accounting for the daily rotation of the celestial hemisphere is indeed formidable if it were solved *ab initio*. Whereas the sun moves along its arc with uniform speed (360°/24h = 15°/h), the horizontal (compass) component of the sun's position, the solar azimuth, does not. Its rate of movement depends on time of day, time of year, and geographical latitude (Fig. 1). Early experiments have shown that bees and ants are informed more or less accurately about the rates of these movements occurring during their foraging times [6–9], and that they use an internal circadian clock to correlate time-linked positions of the sun with an earthbound system of reference [10, 11], but how this knowledge is acquired has remained elusive.

Given the complexity of the task (Fig. 1), it seems unlikely that an individual forager comes innately programmed with a complete set of ephemeris functions (sun-azimuth/time-of-day functions) providing the animal with the exact set of data about the sun's daily movement for any particular time of year and any particular latitude at which the animal happens to be born. Instead,

