Tracking bee with harmonic radar

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Analgesic effects of myrrh

SIR — Myrrh is a natural compound secreted by shrubs of the genus *Commiphora* of the Burseraceae. It is common in northeast tropical Africa, and is composed of essential oils, water-soluble gums and alcohol-soluble resins[1]. In antiquity, myrrh was used by the Egyptians for embalming and by the Jews as anointing oil. Hippocrates recommended myrrh for sores, and the Romans used it for treating mouth and eye infections, coughs and worm infestations[2,3]. In St Mark's Gospel, "vinum murratum", wine with myrrh, was offered to Christ before crucifixion. In later centuries, myrrh was an essential component of the pharmacopoeia.

To investigate its medicinal properties, we administered a suspension of ground commercial myrrh by gavage to mice and measured the latency of their pain reaction (paw licking) when placed on a 52 °C metal plate. Mice given a 10% suspension of ground myrrh in saline (10 ml per kg) 15 min after the administration, had a licking latency time of 19.4±2.4 s, compared with controls administered saline (14.4±0.6 s, $P<0.01$). We then identified the constituents of myrrh with analgesic activity from *Commiphora molmol*. A hexane extract containing the analgesic activity was separated in different fractions by silica gel column chromatography, followed by semi-preparative high-pressure liquid chromatography. Using nuclear magnetic resonance and mass spectrometry, we identified three sesquiterpenes (see *a* in figure). The most abundant compound (>90%) was furanoeudesma-1,3-diene; the remaining compounds were curzarene and furanodiene. These sesquiterpenes had been previously identified[4,5] but their biological effects had not been described.

We injected the purified compounds intracerebroventricularly in mice at a dose of 1.25 mg per kg. Furanoeudesma-1,3-diene, given 30 min after administration, increased licking latency from the baseline value of 15±0.7 to 20.1±1.6 s ($P<0.01$), and curzarene increased it from 15.5±2.2 to 21±1.8 s ($P<0.01$), whereas furanodiene was ineffective (from 14.5±0.6 to 12.6±0.7 s).

For the more abundant furanoeudesma-1,3-diene, a dose of 50 mg per kg orally (p.o.) considerably reduced the number of writhes (abdominal muscle contractions) in mice after 0.6% acetic acid intraperitoneal (i.p.) administration. This effect was completely reversed by naloxone (*b* in the figure). Morphine (5 mg per kg, p.o.) had similar activity. Significant analgesia was also seen with the hot plate test after furanoeudesma-1,3-diene administration p.o. at the same dose (*c* in the figure).

We then tried to characterize the binding of furanoeudesma-1,3-diene to opioid receptors in brain membranes using a nonspecific radioactive ligand, [^{3}H]diprenorphine, and observed a dose-related [^{3}H]diprenorphine displacement, although with a high inhibition constant (pK_i, 5.7±0.8 M; ±s.d.).

In conclusion, two sesquiterpenes present in myrrh have analgesic effects blocked by naloxone, indicating an interaction with brain opioid mechanisms. This could explain the use of myrrh as a pain killer in ancient times. Its use for analgesia may later have been dropped and replaced by opium derivatives, given the presence in myrrh of other compounds with unknown or unfavourable pharmacological activity[6]. Furanoeudesma-1,3-diene could still have some medicinal applications, although its action on the central opioid pathways would limit its practical usefulness.

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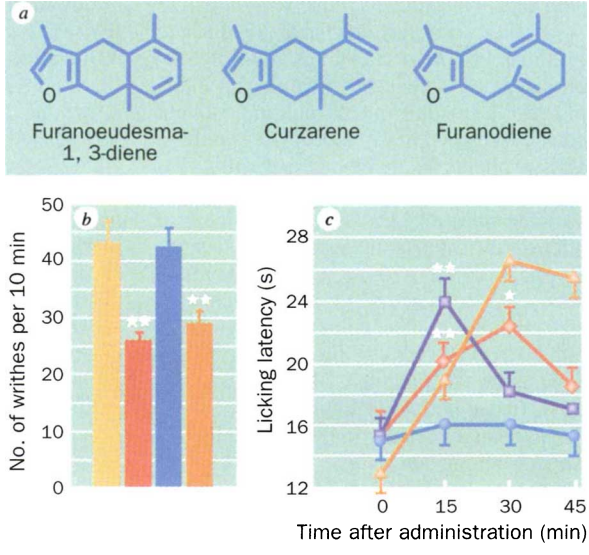

a, Structures of sesquiterpenes from myrrh tested for analgesic activity. *b*,*c*, Analgesic effect of furanoeudesma-1,3-diene and morphine in the hot plate and writhing test. The substances were administered by gavage or i.p. in a volume of 10 ml per kg of corn oil (for furanoeudesma-1,3-diene) or saline (for morphine and naloxone). Male Swiss–albino mice weighing 20–25 g were used. *b*, Writhing test: 25 min after test compound administration, 0.6% acetic acid (10 ml per kg) was injected i.p.; after an additional 5 min the number of abdominal muscle contractions (writhes) was scored for 10 min. Naloxone was administered 15 min after the test compounds. Yellow bar, corn oil (10 mg per kg, p.o.); red, furanoeudesma-1,3-diene (50 mg per kg, p.o.); blue, furanoeudesma-1,3-diene (50 mg per kg, p.o.) and naloxone (1 mg per kg, i.p.); orange, morphine (5 mg per kg, p.o.). *c*, Hot plate assay: furanoeudesma-1,3-diene was administered by gavage and morphine was administered subcutaneously. Naloxone was administered i.p immediately after gavage with furanoeudesma-1,3-diene. At 15, 30 and 45 min after the administration of the test compounds, mice were placed on an open aluminium chamber (diameter, 20 cm; height, 30 cm) maintained at 52 °C, observed for initial pain reactions (paw licking) and immediately pulled out of the chamber after the first pain reaction, the latency being recorded in seconds. Red curve, furanoeudesma-1,3-diene (50 mg per kg, p.o.); purple, furanoeudesma-1,3-diene (100 mg per kg, p.o.); blue, furanoeudesma-1,3-diene (100 mg per kg, p.o.) and naloxone (1 mg per kg, i.p.); orange, morphine (5 mg per kg, s.c.). Data are means ± s.e. ($n = 10$). **$P<0.01$; *$P<0.05$, by Student's two-tailed *t*-test.

Tracking bees with harmonic radar

SIR — Much of our knowledge of the high-altitude flight behaviour of insects has been derived from the use of pulse radars[1], and there are many instances where equivalent information about low-altitude flight would also be of considerable entomological value, host-finding behaviour by tsetse flies, foraging by bees and flight to pheromone sources by Lepidoptera to name but a few. Unfortunately, radar reflections from ground features (clutter) prevent this technique from being used to observe low-level flight, except where this occurs over extremely flat and bare terrain[2]. We have therefore used the harmonic radar principle[3,4] to develop a method of measuring the trajectories of low-flying insects over distances of hundreds of metres. We report here its first trial application, the observation of foraging flights by bumble bees and honey bees.

The technique requires that the target insect be 'tagged' with an electrically non-

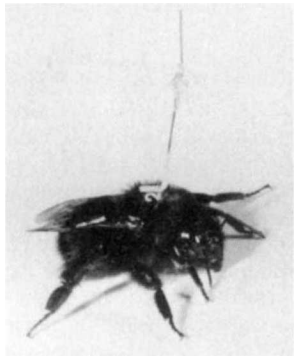

FIG. 1 Harmonic-generating tag mounted on a bumble bee (*Bombus terrestris*). The tag consists of a low-barrier Schottky detector diode mounted at the centre of a 16-mm dipole antenna, and in parallel with a 3-nH inductor. The complete assembly weighed ~3 mg, that is, 1.5% of the bee's weight. Free-fall experiments on the tag alone indicated that at a fall speed of 5 m s^{-1} its (broadside) aerodynamic drag was ~0.8 mg. By contrast, the body drag of a bumble bee flying at 5 m s^{-1} is 20–30 mg (ref. 5), so the tag seems unlikely to add substantially to the overall drag that the bee has to overcome. The upwards-turning moment that it induces may, however, perturb the bee's flight pitch angle, and this could perhaps result in a significant increase in drag.

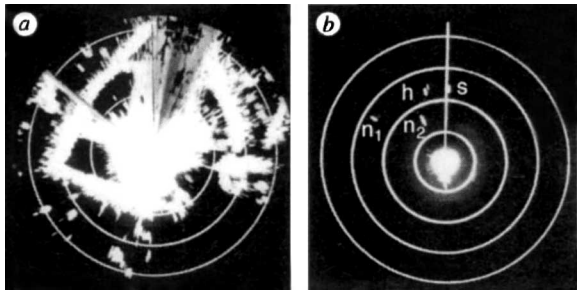

FIG. 2 *a*, The conventional radar mode display shows the 'clutter' from avenues of trees and ground features at our experimental site. *b*, The harmonic mode is activated, all ground clutter is eliminated, and the only fixed targets to appear are tags marking the two nests (n_1 and n_2) and the forage site (s). A honey bee is seen in flight (h), returning to n_2. The distance from the centre to the outermost ring represents 463 m, and the direction of north is indicated by the vertical line; radar resolution is approximately ±7 m in range and ±3.5 m in azimuth.

linear device designed to re-radiate an harmonic of the radar signal which can be detected against even strong ground clutter. The energy to operate the tag is delivered by the illuminating radar, so no 'on-board' battery is required and extreme miniaturization is therefore possible (Fig. 1). Our radar transmits 25-kW pulses of 0.1 μs duration and 3.2 cm wavelength from a 1.5-m diameter paraboloid, and receives 1.6-cm-wavelength harmonic returns in a second 0.7-m-diameter dish mounted above the first. Both paraboloids rotate in azimuth at 20 revolutions per minute. The transmitting and receiving beams are of equal angular width and are approximately co-linear; they provided coverage over an altitude range of about 3 m and a range of 700 m. In our experiments, the altitude coverage was normally set to begin a few centimetres above ground level.

Three bumble bee colonies (*Bombus* spp.) and a small hive of honey bees (*Apis mellifera*) were placed 70 m and 250 m WSW of a plot of *Phacelia tanacetifolia*, which provided the major attractive forage source within close range of the colonies. After a few days, electronic tags were glued to some regular foragers from each nest. Tagged bees were released from either one of the nest sites, or from the *Phacelia* plot. On release, they appeared as moving targets on the radar display (Fig. 2), and although they occasionally disappeared from the display when they landed or passed behind trees, round trips (nest to forage to nest) and one-way trips (from forage to nest) were satisfactorily tracked over distances of 50–250 m. Wind speed and direction were measured by a recording anemometer fixed at an altitude of 2 m, about 20 m north of position n_2 in Fig. 2. Subtraction of the wind vector from the bees' displacement vectors, measured by the radar, gave estimates of their air speeds. The mean value for air speed for all our tracks (n = 41) was 5.2 ± 0.3 m s^{-1}, which is very similar to the values of 4–5 m s^{-1} found for bumble bee flights in long greenhouses (T. Wolf, personal communication).

Evidence that tagged bees could forage was provided by one honey bee which made a round trip, beyond our experimental area, and returned with orange pollen loads (probably from *Tripleurospermum inodorum*), and by the direct observation that tagged honey bees and bumble bees foraged on *Phacelia*.

Further experiments are needed to establish whether the tags significantly modify bee behaviour, but it seems likely that the harmonic radar technique could be used to study many aspects of bee flight and ecology. It also has potential for examining the patrolling routes of male bumble bees, and the searching flights of queens for over-wintering and nest sites. Application of the technique to many other insect species seems feasible, especially if we succeed in our attempts to miniaturize the tag still further.

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Birth control for grey seals

SIR — Changing attitudes toward large marine and terrestrial mammals have fostered the need to develop new techniques for managing wild populations[1,2]. In particular, increased pinniped abundance in many parts of the world has led to renewed pressure to control their population growth[3]. During the 1980s, advances in our understanding of zona pellucida glycoproteins led to their use in immunocontraception of females to limit the productivity of mammalian populations[4]. The practicality of this approach for use in wild populations was limited by the need for multiple administrations. Here we report the development of a single-administration immunocontraceptive vaccine for grey seals (*Halichoerus grypus*).

In January 1992, 207 adult female grey seals (132 14-yr-olds, 35 20-yr-olds and 40 21-yr-olds) on Sable Island, Nova Scotia (43° 55′ N, 60° 00′ W), were randomly allocated to either a control group (given a placebo vaccine) or an immunized group (see table). Pregnant grey seal females show a high degree of site fidelity to a breeding colony, facilitating recapture to determine birth rates and monitor antibody titres[5]. We are confident that most returning females in our study were located during daily surveys of the colony throughout the breeding season.

One year post-immunization, fewer immunized females (49%) gave birth than those that were given the placebo (67%), although this difference was not significant (P = 0.1; see table). Significantly fewer immunized females returned to give birth than did females given the placebo in year 2, when the full effect of the vaccine should be realized (10.8% as opposed to 70.5%; P<0.001). Similarly, significantly fewer immunized females gave birth compared with the placebo group three years post-immunization (P<0.001). The fertility of the immunized group was reduced by about 90% in both years 2 and 3.

No anti-soluble intact zona pellucida (SIZP) antibodies could be detected in

NO. OF INJECTED FEMALE GREY SEALS RECAPTURED POST-IMMUNIZATION

	Post-immunization (yr)			
Vaccine	0	1	2	3
Placebo	105	70	74	66
Immuno-contraceptive	102	50	11	9
χ^2	–	2.7	45	41
P	–	0.1	<0.001	<0.001

Immunized females were given a single injection of SIZP (100 μg) encapsulated in multilamellar liposomes (M. M., US Patent 4,485,054) suspended in saline (0.5 ml) and emulsified in Freund's complete adjuvant (0.5 ml). Controls received a placebo vaccine lacking SIZP.