heritable enhancement of drought tolerance of both intact plants and detached leaves. Engineering trehalose biosynthesis into other plant species may provide crucial protection against frost and salinity, as well as drought, and confer improved storage properties after harvest.

Kjell-Ove Holmström, Einar Mäntylä
Björn Welin, Abul Mandal
E. Tapio Palva*
Department of Molecular Genetics,
Uppsala Genetic Center,
Swedish University of Agricultural Sciences,
Box 7010, S-75007 Uppsala, Sweden
Outi E. Tunnela*, John Londesborough
Primalco Ltd, Biotec,
FIN-05200 Rajamäki, Finland

*Correspondence should be addressed to E.T.P.; present address of O.E.T.: The Technical Research Centre of Finland, FIN-02150 Helsinki, Finland.

Insect pheromone in elephants

SIR — (*Z*)-7-dodecen-1-yl acetate is used by the females of more than 126 species of insects, especially Lepidoptera, as part of their pheromone blends to attract insect males[1]. Female Asian elephants, *Elephas maximus*, also use a pheromone to signal to males their readiness to mate[2]. This pheromone is released in their urine during oestrus and before ovulation. We have now isolated this compound by bioassay-guided fractionation and purification. Remarkably, it is the same compound, (*Z*)-7-dodecen-1-yl acetate, used by insects including the cabbage looper, tomato looper, silver Y and turnip moths, the dingy cutworm, and the sugarcane stalk borer[1].

Female Asian elephants have a uniquely long oestrous cycle[3]. Urine from pre-ovulatory females elicits a high frequency of flehmen responses (a distinct truncal movement that facilitates chemosensation via the vomeronasal organ) from Asian bull elephants[4]. During this chemosensory response, liquids are transported by the trunk tip finger to the paired openings of the ducts of the vomeronasal organ in the anterior region of the hard palate. We have used this discrete motor pattern, which is exhibited specifically during the sensory evaluation of chemicals, to develop a specific and quantitative bioassay for isolating pheromone from oestrous urine[4].

Five sexually mature male Asian elephants (age 12–34 yr), four of whom were experienced breeders, were tested, principally at the Metro Washington Park Zoo, Portland, Oregon. Samples were dissolved in acetone in 500 ml of water. The number of check, place and flehmen responses in a defined period were recorded (see table). Physiological concentrations of the identified pheromones were estimated based on biological activity of oestrous urine aliquots, calculated bioassay units[4] and quantitative gas chromatography. Bioassay controls included non-oestrous female urine, solvents used for extraction and purification, and various synthetic compounds. When new compounds are tested, they occasionally elicit a moderately high frequency of flehmen responses which disappear in later assays. Multiple bioassays were conducted for each sample concentration to distinguish the sustained positive responses characteristic of the pheromone from such 'novel substance' responses.

BIOASSAY RESPONSES PER HOUR OF FIVE MALE ASIAN ELEPHANTS

Test elephants* (no. of trials)	Concentration (mM)†	Median (25–75 percentile) Check	Place	Flehmen
Male 2¶				
(8)	0.5 mM	6.0 (2.5–9.5)§	0 (0–0.75)	0 (0–15)
(7)	1.0 mM	8.0 (4.8–9.8)§	2.0 (0.25–2.0)§	1.0 (0.25–2.0)‖
(3)	2.0 mM	9.0 (2.3–21.0)	3.0 (0.75–3.0)§	1.0 (1.0–3.3)§‖
(2)	5.0 mM	6.5 (4.0–9.0)§	5.5 (0–11.0)§	1.5 (1.0–2.0)§
(5)	2.0 mM†	15.0 (3.0–21.0)§	15.0 (6.5–22.0)§	17.0 (2.8–25.0)§‖
Males 1–5	Pre-ovulatory urine	7.0 (5.0–8.0)§	5.0 (4.0–8.0)§	7.0 (1.4–9.2)§‖
Males 1–5	7-DDA	6.0 (4.0–9.0)§	6.0 (4.0–10.0)§	4.0 (2.0–6.0)§
Controls (10)				
Females 1–5	2.0 mM	0 (0–0.75)	0 (0–0.25)	0 (0–0)
Males 1–5	<0.5 M acetonitrile	0 (0–0)	0 (0–0)	0 (0–0)
Males 1–5	<0.5 M acetone	0 (0–0.25)	0 (0–0)	0 (0–0)
Males 1–5	<0.2 M dichloromethane	0 (0–0)	0 (0–0)	0 (0–0)
Males 1–5	<0.005 M hexanal	0 (0–0.75)	0 (0–0.25)	0 (0–0)
Males 1–5	Non-oestrous urine	0 (0–1.0)	0 (0–0.5)	0.5 (0–1.0)

'Check' responses occur when the dorsal trunk tip finger comes into direct contact with liquid samples. 'Place' responses occur when the entire trunk tip is placed for several seconds in liquid samples. These responses occur before and may be either independent or an integral part of the flehmen response. Samples eliciting check responses often elicit flehmen responses with increasing concentrations.

*Elephants participating in the assays live at Metro Washington Park Zoo, Portland, Oregon (3 males, 5 females); at Busch Gardens, Tampa, Florida (one male and 5 females, courtesy of R. Schmitt); and at Dickerson Park Zoo, Springfield, Missouri (one male).

†(*Z*)-7-dodeceny-1-yl acetate (7-DDA) with ~2% *E* isomer in 0.5 M acetone in 500 ml water.

‡In non-oestrous urine instead of water.

¶Similar results were obtained from all 5 test animals.

§Significant differences from controls. Data are expressed as medians (25–75 percentile). Analyses and tests for significant differences ($P<0.05$) included Kruskal–Wallis one-way ANOVA on ranks, Mann–Whitney rank sum tests, Dunn's test and Wilcoxon rank sum test.

‖Penile erections observed.

Urine was collected from nine mature female Asian elephants during pre-ovulatory days, determined from serum progesterone concentrations, assessment of cervical mucus, and daily monitoring of responses of males to females[3]. The purification procedure used a series of fractionation steps[4]. The active fraction finally obtained was identified as (*Z*)-7-dodecen-1-yl acetate (97%) and (*E*)-7-dodecen-1-yl acetate (3%) using gas chromatography/mass spectrometry, magnetic resonance spectrometry and appropriate reaction chemistry[5] (data not shown).

The biological activity of (*Z*)-7-dodecen-1-yl acetate was confirmed using commercially available synthetic material ((*Z*)-7-dodecen-1-yl acetate with about 2% *E* isomer; Sigma) using five bulls at three sites (see table). Robust positive responses were obtained with no decrease in the frequency of flehmen responses in successive bioassays (see table). The bioassay results of both elephant-derived and authentic (*Z*)-7-dodecen-l-yl acetate in water compared with urine suggests that additional components in urine, possibly proteinaceous, confer additional specificity.

That elephant and insect females use an identical molecule to signal males of their readiness to mate is an outstanding example of convergent evolution capitalizing on the chemical properties of such a compound, presumably its volatility. Future inter-species tests will include both the possible efficacy of this pheromone with male African elephants and the effect on local insect species populations known to utilize this pheromone. We now aim to define the source of the pheromone and its signal transduction function in the vomeronasal organ.

L. E. L. Rasmussen
Department of Chemistry, Biochemistry, and Molecular Biology,
Oregon Graduate Institute of Science & Technology,
Portland, Oregon 97291-1000, USA
Terry D. Lee
Division of Immunology,
Beckman Research Institute of the City of Hope, Duarte,
California 91010, USA
Wendell L. Roelofs, Aijun Zhang
Department of Entomology,
Cornell University, Geneva,
New York 14456, USA
G. Doyle Daves Jr
Department of Chemistry,
Rensselaer Polytechnic Institute,
Troy, New York 12180, USA

1. Arn, H., Toth, M. & Priesner, E. *List of Sex Pheromones of Lepidoptera and Related Attractants* (Int. Org. Biol. Control, Montfavet, France, 1992).
2. Rasmussen, L. E. *et al. Science* **217**, 159–162 (1982).
3. Hess, D. L. *et al. Biol. Reprod.* **28**, 767–773 (1983).
4. Rasmussen, L. E. L. *et al. J. chem. Ecol.* **19**, 2115–2128 (1993).
5. Zhang, A. J. *et al. J. chem. Ecol.* **20**, 2415–2427 (1994).