

## Flower petal microtexture is a tactile cue for bees

(insect learning/behavior/plant taxonomy/pollination/coadaptation)

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Communicated by G. E. Hutchinson, March 13, 1985

**ABSTRACT** Honeybees are shown to be able to detect, learn, and discriminate between microsculptured epidermes of flower petals. The sensilla trichodea at the tips of the bees' antennae are in the same size range as the microsculptural features of the petals (ca. 10  $\mu\text{m}$ ), which presumably deflect these mechanoreceptive sensilla in characteristic ways. Honeybees were trained to associate reward with one floral texture and to choose that over another. Further, the bees also recognized differences in textures at different ends of petals of the same species. The phenomenon is significant in that it suggests another way in which insect pollinators can discriminate between the flowers of different plant species and so act as species isolators. Also, the microsculptural patterns differ from one end of a petal to the other and, therefore, can be used as nectar-guides by foraging bees. This study presents a previously unreported conditioned response to texture by insects and shows the functional significance of a floral character used in plant taxonomy.

Surface microsculpturing of flower petals is taxon-specific in the Compositae and other plant families such as the Leguminosae (1–4). As viewed with the scanning electron microscope, the adaxial corolla surfaces of ray florets of Compositae may be longitudinally ridged, as in *Heterotheca* (Fig. 1A), or papillate, as in *Helianthus* (Fig. 1B). The former type is composed of rectangular cells (5), each with a longitudinally thickened outer wall that is ridged orthogonally to the long axis of the cell (Fig. 1A and D). The latter has thin-walled isodiametric cells (6–8). Other pattern types, such as that of *Xylorhiza wrightii* (A. Gray) E. Greene (Fig. 1C), are also known (1–5). The functional significance to the plant of such taxonomically diagnostic microsculpture has been attributed to differential refraction of incident light by thin-walled cells, which would contribute to the color of the flower as perceived by insect pollinators (5, 6). However, this hypothesis can account only for the properties of the papillate and *Xylorhiza* epidermis types. We show that petal epidermes are perceived as textured surfaces and distinguished as different among plant species by honeybees.

Tactile perception by insects is accomplished by mechanoreceptive sensilla trichodea, which are concentrated on various parts of the body including the tips of the antennae (9, 10). These sensilla typically exhibit phasic-tonic nervous discharge so that simple deflection results in an initial rapid discharge rate and a continuing rate as long as stimulation continues; some sensilla may respond also to direction, degree, and velocity of bending (11, 12). The size and spacing of the major (ca. 5–7  $\mu\text{m}$  high and 10  $\mu\text{m}$  apart) and minor (ca. 0.5–1  $\mu\text{m}$  high and the same apart) components of the epidermal micropatterns on petals of flowers are such that the antennal sensilla trichodea of bees (ca. 7–10  $\mu\text{m}$  long by 0.5–1

$\mu\text{m}$  in diameter and spaced by ca. 10  $\mu\text{m}$ ), when brought into contact with the petal surface, would be deflected by the elements of the microtexture in a manner characteristic of the particular texture (Fig. 1D and E). Mechanoreception is important to insects in general, and in honeybees the sensilla on the tip of the antennae are involved in the determination of the thickness and smoothness of the wax walls of the comb (13). However, the role of texture sensitivity in pollinating insects for (i) location of intrafloral food resources and (ii) food plant discrimination was hitherto unknown and unsuspected.

### METHODS

The hypothesis that insects are able to detect and discriminate between microtextures of flower petals was tested on honeybees in two ways. The test textures of dried corollas were mounted on aluminum tabs and coated with gold (as if for scanning electron microscopy) to eliminate color and odor cues, which would otherwise emanate from raw corolla surfaces. In the first method, over 600 individual honeybees were trained in a Y maze to associate reward (30% sugar syrup in a capillary tube) with the texture of the adaxial (upper) surface of *Helianthus annuus* L. corollas (Fig. 1B) in natural orientation (i.e., reward at the proximal end). During testing, the bees were admitted to the Y maze one at a time. At the juncture of the Y, the bees emerged from a narrow tunnel and immediately encountered the familiar texture associated with reward diverging at 45° in one direction and another texture diverging at 45° in the other direction. To minimize the effects of the bees learning the direction to the reward, the experiments were repeated with the familiar texture in both left and right sides of the maze. The reward source was ca. 1.5 cm distant from the point of choice. The experimental textures were (i) the abaxial (under) surface of *H. annuus* corollas, which is almost flat relative to the adaxial surface, (ii) corollas of *H. annuus* reversed so that the bees encountered the end of the corolla that normally would be reached last in natural foraging, and (iii) corollas of *X. wrightii* in natural orientation. *Xylorhiza* was chosen because the corollas are about the same size as those of *H. annuus*, but the two textures are quite different (Fig. 1B and C). For experimental control, both sides of the maze had the training texture. Attempts to minimize the effects of insect odor or scent marking ("footprint" pheromone) (14, 15) influencing the bees' choices consisted of washing the surface textures with absolute ethanol between trials.

To overcome the possible effects of scent marking (14, 15) influencing the results of the experiments using the Y maze, an apparatus was devised to isolate the bees completely from the textured test objects except during experiments and then to allow the bees' contact with the texture to be only by their antennal tips. The test textures of dried, gold-coated ligules of *H. annuus* and *X. wrightii* were mounted on the apparatus and could be moved up and down, from side to side, and back and forth relative to the bees. The bees were mounted on the

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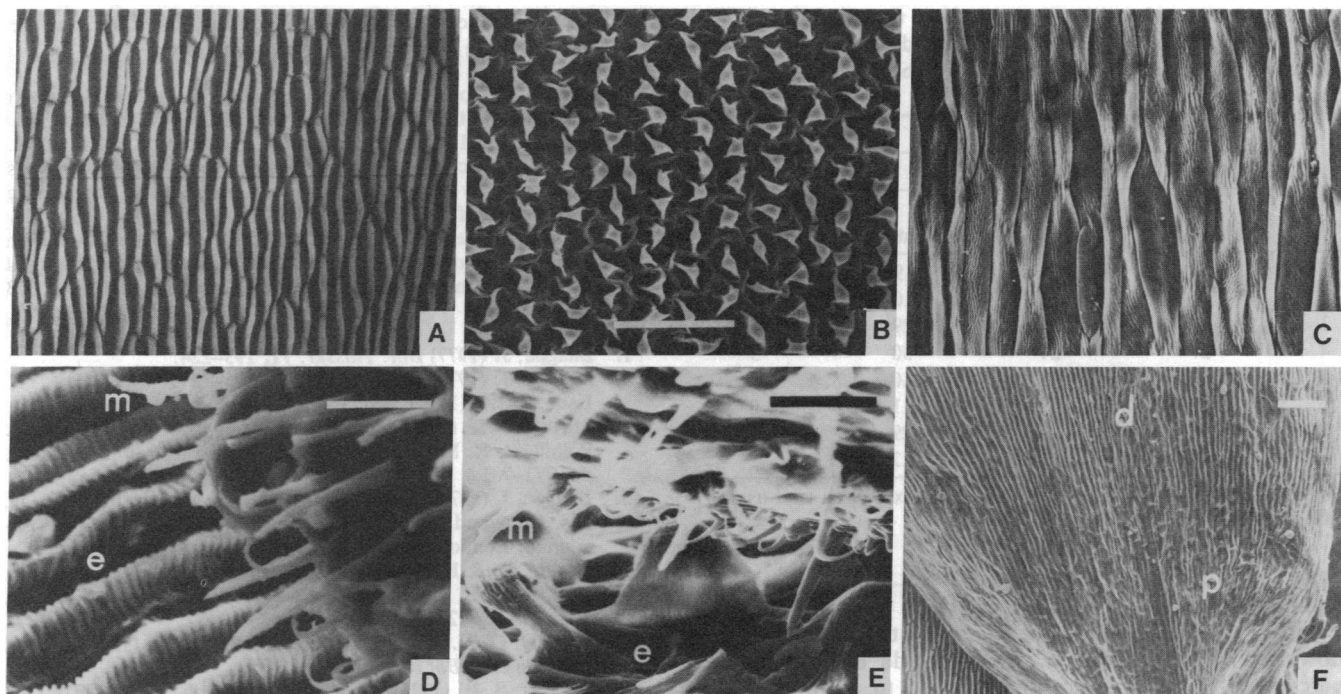


FIG. 1. (A–C) Scanning electron micrographs of the adaxial surfaces of Compositae ray corollas. (A) *Heterotheca inuloides*. Note the ridges and grooves of the major textural pattern and the orthogonal minor pattern. (B) *H. annuus*. Note the thin-walled papillate cells. (C) *X. wrightii*. Note the long-rectangular cells and difference in overall pattern from *Heterotheca* and *Helianthus*. ( $\times 525$ ; bar = 50  $\mu\text{m}$ .) (D and E) Antennae in contact with corollas. (D) *Apis mellifera* antenna in contact with *H. inuloides* corolla. (E) Megachilidae antenna in contact with *H. annuus* corolla. Note the correspondence of size and spacing of the mechanoreceptive sensilla (m) with the components of the corolla epidermal texture (e). ( $\times 2000$ ; bar = 10  $\mu\text{m}$ .) (F) *Bradburia hirtella* corolla, showing the longitudinal direction of the major pattern from the distal (d) to the proximal (p) portion of the corolla, where pollinator reward is located. ( $\times 100$ ; bar = 100  $\mu\text{m}$ .)

apparatus by first restraining them in segments of drinking straws with crossed pins. The straw, with its restrained bee, was mounted on the apparatus horizontally so that the bee's mouthparts were oriented downwards. The apparatus permitted the test objects to be brought delicately into contact with the bees' antennal tips. When *H. annuus* was presented, the bees were offered a drop of 30% sucrose solution, which stimulated the restrained bees to extend their mouthparts to feed. When *X. wrightii* was presented, no reward was offered. After an initial training period, followed by five positive responses (i.e., tongue extensions) to presentations of *H. annuus* and no response to *X. wrightii*, the behavior of the bees was recorded. The sequence of presentation of textures was random, with a maximum of four consecutive presentations of the same object. The experiments were terminated after the bee failed to respond positively to *H. annuus* three times in succession, at which time it was probably satiated.

## RESULTS

In the Y maze, bees consistently distinguished between the adaxial and abaxial surfaces of *H. annuus* petals and between corollas of *H. annuus* and *X. wrightii*, but they accepted either of the two *H. annuus* corollas when offered simultaneously and in their natural orientation (Table 1). The bees did distinguish *H. annuus* corollas placed in their natural orientation from reversed corollas (distal end towards the reward), but some individuals were observed to have difficulty doing so; this is reflected by the lower  $\chi^2$  and higher  $P$  value for this experiment (Table 1). This difficulty may be explained on two bases: (i) when bees were feeding on the naturally oriented corollas during training, the textures of both the distal and proximal ends would have been sensed and, thus, possibly confused by the bees during the experi-

ments, and (ii) the textural differences between the distal and proximal ends of the corollas are much less than between the surfaces offered in other experiments.

The results obtained from the experiments on restrained bees (Table 2), involving eight replicated experiments, each using a different bee, show that the bees discriminated between the test objects, which differed only in texture, and were free of pheromone.

From statistical analysis (after Anscombe's arcsine transformation of the proportions) by paired  $t$  test,  $t = 7.44$  for  $P = 0.000014$ ; we conclude that the responses of the bees to each texture were highly significantly different, indicating that bees have a keen level of tactile discrimination.

## CONCLUSIONS

Texture sensitivity in insects has been studied little. We report here a much finer level of textural discrimination than has been documented previously and demonstrate a condi-

Table 1. Numbers of honeybees choosing different corolla textures presented in a Y maze after they had been trained to associate reward with a single familiar (F) texture

Experimental texture*	No. honeybees selecting		$\chi^2$	$P$
	Familiar texture	Experimental texture		
F	41	41	0	1.0
X	178	64	54.6	<0.001
D	86	31	24.9	<0.001
R	94	66	4.9	<0.025

\*F = *H. annuus* corolla in natural orientation, D = *H. annuus* corolla adaxial side down, R = *H. annuus* corolla reversed end-for-end, and X = *X. wrightii* corolla in natural orientation.

Table 2. Number of positive responses (i.e., tongue extensions) from the presentations of test textures of dried, gold-coated corollas of *H. annuus* or *X. wrightii* to honeybees trained to associate reward only with the former

Trial	Texture			
	<i>H. annuus</i>		<i>X. wrightii</i>	
	Presentations	Positive responses	Presentations	Positive responses
1	8	8	9	2
2	9	9	10	0
3	27	24	29	5
4	27	24	30	5
5	8	8	10	2
6	9	9	11	0
7	29	27	29	2
8	11	11	12	2

Apparatus and methods are explained in text. Each trial used a different individual bee.

tioned response to texture by an insect. We relate these findings to the life of the insect and point out the functional significance of the textures they sense on the surfaces of the flowers that they pollinate. We conclude that the tactile perception of bees is sensitive enough to allow them to discriminate between the epidermal microsculptural patterns of different species of Compositae. The textures of corollas of Compositae, which differ (e.g., Fig. 1 A-C) among co-occurring and synchronously blooming taxa (1-5), may function in concert with color and odor as another kind of marker for host-plant identity, thereby contributing to optimal foraging by the pollinators and minimizing interspecific pollen flow (16). Because the pattern of microsculpturing is always oriented towards the source of floral reward (Fig. 1F),

it possibly provides a microtextural nectar-guide directing the pollinators to the center of the blossom.

We are grateful to N. Swanson, W. Punchedewa, and R. Gadawski for their help with the training of the bees and to W. Barthlott, M. Breed, J. Estes, M. Grant, V. Grant, J. Hanken, Y. Linhart, R. Menzel, and C. Michener for discussing our results. This work was supported in part by grants from the Natural Science and Engineering Research Council of Canada (A-9098) to P.G.K., from the University of Colorado (Biomedical Research Committee and the Council on Research and Creative Work) to M.A.L., and from the National Science Foundation to P.G.K. (DEB 79-10786) and M.A.L. (DEB 78-60083).

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