

they probably establish enough voltage across some ingressing cells—only 1 to 10 mV is needed—to redistribute charge components floating in their membranes and markedly affect their behavior (14); as they are pumped through the epiblast, they may establish significant transcytoplasmic gradients (15); and as they traverse the intraembryonic space, they may establish significant extracellular gradients. With this in mind, certain experiments involving incisions in the epiblast may now be tentatively reinterpreted. Long ago, Morita reported that a cut through the prestreak blastoderm can induce a whole extra embryo to form (16). More recently, Lipton and Jacobson reported that appropriate cuts through the stage 5 embryo permit somite and heart formation despite the absence of node and notochord (17). Perhaps these cuts act at least in part by artificially producing current leaks.

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Bees Have Rules

Abstract. Honey bees frequently dance with some view of the sky, orienting themselves to the sun or natural patterns of polarized skylight. Three new conventions have been discovered in the dance language which are used in these circumstances to eliminate potential ambiguity in the dance message.

Upon discovering a food source, a foraging honey bee can compute her distance and direction from the hive (1). This directional information can be encoded into a dance that specifies the location of the food (2). Normally, dances are performed on a vertical comb of the dark hive such that the dance angle with respect to vertical (up) is the same as the horizontal angle between the sun and the food (3) (the relative azimuth). The convention of defining "up" as the direction toward the sun permits recruits to decode and use the information. Likewise, distance is specified by the duration of the waggle phase of the dance (2). Since the communication system employs "abstract" conventions common to the members of a social group, Frisch and others refer to it as a dance language.

Dancing on vertical surfaces is a consequence of living in insulating cavities—a behavioral adaptation that permitted *Apis mellifera* to move out of the tropics and penetrate temperate latitudes (4). Tropical honey bees perform their dances on open clusters with a restricted view of the sky (5), and thus seem to lack the up-is-the-sun convention. On the surface of swarms (6) and at the hive entrance (3), temperate zone honey bees often dance on a horizontal surface

where the up-is-the-sun rule is useless. Since they are outdoors, the dancers orient directly by cues they see in the sky: the sun and extensive patterns of polarized skylight (1, 7). As long as dancers and dance attenders use the same reference system, the language works. However, bees frequently must dance on cloudy days or in locations where a view of the sky is restricted by trees or other landmarks. In these situations, it may not always be easy for them to agree among themselves whether what they can see is the sun or sky, and, if it is indeed part of the sky, which section it is. Nevertheless, dancers seem to resolve possible ambiguities, and successfully recruit other bees.

To study how bees do this, we turned an observation hive horizontally (so that the bees could not use the "up" rule) and provided an artificial light as the only cue for dance orientation. We could control its elevation, angular size, intensity, wavelength distribution, degree of polarization, and direction of polarization (E-vector orientation) (8). Bees were individually numbered at the food source, and danced for distances of 265 to 700 m. Where appropriate, these dances were videotaped and analyzed cycle by cycle.

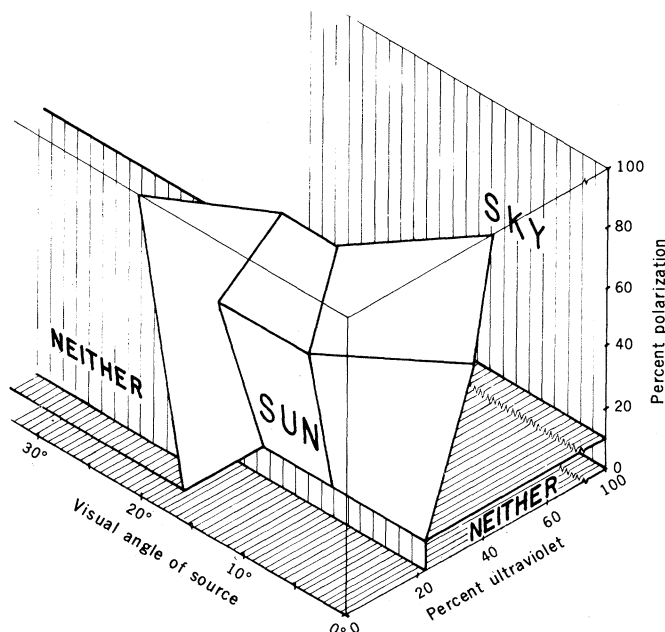


Fig. 1. Distinction between sun and sky. Dancing honey bees were shown one of more than 80 combinations of stimulus angle, spectral distribution, and percentage polarization (8). Subsequent dance orientations revealed whether the bees interpreted the pattern as sky or sun, or were disoriented ("neither" in the figure). Approximately 2450 dance cycles were recorded—at least five cycles from each of at least five dances. Near the boundary surface between sun and sky, a bee may exhibit both orientations. The 10 percent polarization boundary

is based upon the data of Frisch (2, p. 403). Unpolarized UV light often elicited dance orientations 180° from that predicted on the basis of sun orientation, as though this situation was taken as the "antisun."

For horizontal dances, three neural conventions serve to ensure that all bees respond to celestial cues consistently. The first rule is used to determine whether the observed cue is the sun or part of the sky, and therefore which of two very different dance directions to take. Bees use the size of a visual stimulus, its relative content of ultraviolet (UV) light, and its percentage of polarization to make this distinction (Fig. 1). The elevation is important only when the source is in the zenith (9). Although this rule results in certain patches of sky being identified as "sun," all bees apparently interpret the patch in the same way.

The sun-sky rule corresponds roughly to physical reality. Direct sunlight contains only about 8 percent UV on a photon basis (10), while skylight ranges from 20 to 35 percent UV. Sunlight is unpolarized and, depending on the wavelength considered and the prevailing atmospheric conditions, the sky within 15° of the sun generally has less than 5 percent polarization, while most of the rest of a clear sky ranges from 20 to 65 percent polarization for the UV (8, 11). That this

boundary based on polarization does not extend into the area of small spots with low amounts of UV is an arbitrary feature which, while leading to improper identification of the source of the stimulus, probably results in better dance orientation and recruitment. The two regions of the graph for which the rules fail ("neither" in Fig. 1) correspond to conditions that do not occur in the natural sky. The sun-sky rule corresponds to an ethological sign stimulus. It represents a diagnostic test based almost entirely on one stimulus parameter: the proportion of UV light.

The two other rules are used when the patch is judged to be part of the blue sky. In these cases, bees use the characteristics of the polarized light to identify which part of the sky they see, and then use the patch to orient their dances to the food. However, considering only the E-vector orientation, a given polarization pattern at a particular elevation generally exists in two places in the sky (Fig. 2). How do bees decide which of these they are actually seeing?

One possible solution would be to

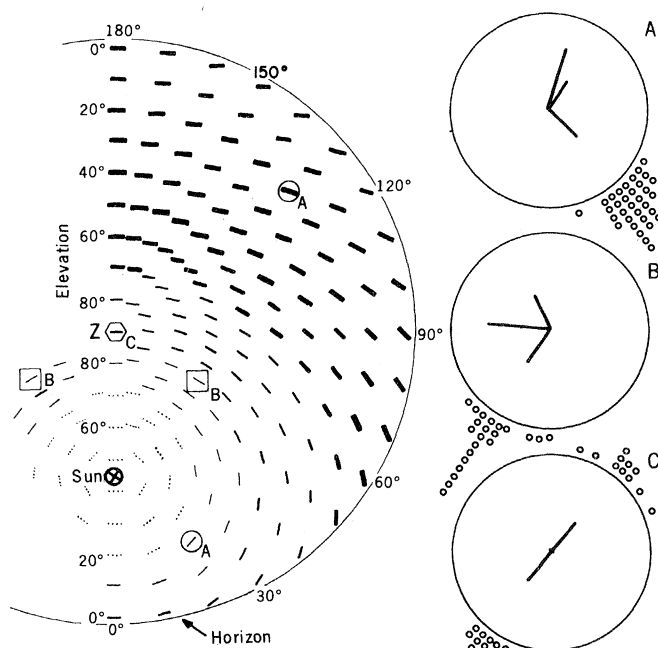
measure the degree of polarization in the patch, since theoretical Rayleigh scattering predicts that this will correlate with the distance of the patch from the sun (12) (Fig. 2). Our direct measurements of UV skylight polarization (8, 11), however, while indicating that actual E-vector orientations generally correspond fairly well with theory, demonstrate that the theoretical degree-of-polarization relationship is highly distorted at best for the natural sky, and behavioral experiments show that bees clearly ignore it. With a view of large areas of sky, bees might instead use gradients of percentage polarization, color, or E-vector orientation; but Frisch (2) has convincingly excluded these possibilities. In fact, bees consistently interpret a stimulus as being the further of the two possibilities from the sun (Fig. 2A), regardless of the direction of the food source. This second rule results in a stimulus being mistakenly identified nearly half of the time. Nevertheless, since the directions are referenced to the sun, mistakes are automatically eliminated during the outward flight of potential recruits.

A third rule is used when bees see one of two physically identical patterns that are located the same distance from the sun (Fig. 2). Although the "further-from-the-sun" rule fails, the bees are not confused. Regardless of the direction of the food source, the patch is always taken as the one that exists to the right of the sun (Fig. 2B). Although this rule identifies the patch incorrectly half the time, nevertheless it is essential because it eliminates another potential ambiguity. Again, the errors are systematic so that no mistakes occur during the flight out.

However, neither of these rules suffices for the zenith. Here, the two possible locations of the sun are the same distance away, and neither left nor right (Fig. 2). As a result, both rules fail. In fact, bees have no rule for this singular spot, and dance to both possible interpretations (Fig. 2C) (13).

These three new rules join three previously known dance-language conventions: the sun is the reference point, vertical is the direction toward the sun, and the number of waggles or sound bursts specifies the distance (3). The distance convention even differs between "cultures," so that each race of honey bees has its own private dialect (14). Each of these seemingly arbitrary rules is essential to the social communication of bees. They ensure that both sender and receiver are using the same reference system. The rules are presumably a consequence of a consistent and specialized system of neural wiring. They are not, however,

Fig. 2. Dance orientation to polarized light. (Left) Patterns of polarized light predicted by Rayleigh scattering for a sun elevation of 45 degrees are shown with the relative degree of polarization schematized by the thickness of the lines. The exact degree of polarization depends on wavelength and atmospheric conditions (8, 11). The dotted lines represent points for which the polarization of the natural sky is always below the perceptual threshold of honey bees. "Z" is the zenith. Only half of the sky is shown, since the other half is a mirror image. The direction of polarization (E-vector orientation) is plotted with respect to the horizon at each point's azimuth. (Right) Horizontally dancing honey bees were shown 5-degree patterns of 95 percent polarized, 100 percent UV light, and their subsequent dance orientations were measured. (Similar results are obtained with much lower percentage polarization and UV.) In these representative examples, each symbol corresponds to the orientation of a single waggle run. The long vector is the expected dance direction if the stimulus were interpreted as the sun, while the two short vectors correspond to the directions expected if the pattern were interpreted as part of the sky. There are two short vectors because the E-vector chosen exists at two points in the natural sky for the elevation from which it was projected. The circled points (left) correspond to one of many pairs at any particular elevation which have the same E-vector orientation, but which lie at different angular distances from the sun. When shown such a pattern (A), bees interpret it as being the one further from the sun. This convention has been confirmed recently by Rossel *et al.* (15). Each of the points in squares (left) is one of several pairs that exist at the same elevation and at equal distances from the sun. When shown such a pattern (B), bees interpret it as being the one to the right of the sun. (C) When the pattern is shown directly overhead (the zenith), the "further away" and "right hand" rules fail, and the individual dances are bimodal and hence ambiguous.



necessary or even desirable for any of the vast number of social and nonsocial animals that perform the same feats of navigation, but lack a symbolic language (16).

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9. Bees try to use any stimulus directly above them as if it is a part of the sky. A zenith sun has no azimuth, while a zenith sky pattern has only two possible interpretations. This explains the conflict between Frisch's report (2, p. 402) that bees treat small sources of white, polarized light as the sun, and the experiments of W. Edrich and O. von Helversen [*J. Comp. Physiol.* **109**, 309 (1976)], for which bees used white, polarized zenith stimuli as part of the sky. The difference between Frisch's finding (1) that bees need at least 10° to 15° of blue sky in order to orient to polarized light, and those of Edrich and Helversen in which bees oriented well to far smaller spots, is also explained. The natural sky used by Frisch made the data fall behind the 15° boundary of sky and sun in Fig. 1, while Edrich and Helversen's fall to the right of the 20 to 30 percent UV boundary. Bidirectional dances reported by others (1, 15) probably result from using stimuli near the boundary of sun and sky. Color-opponent interneurons with these characteristics have been found in bees by J. Kien and R. Menzel [*J. Comp. Physiol.* **133**, 35 (1977)], and could be the innate releasing mechanism.
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13. Bees also appear to use a fourth rule. When shown an E-vector orientation that does not exist at the elevation chosen (Fig. 2), they still perform consistently oriented dances. We do not yet know how this rule works since the dance orientation does not seem to be predicted by any geometrical theory [for example, K. Kirschfeld and M. Lindauer, *Z. Naturforsch.* **30c**, 88 (1975)] of polarization orientation in bees. Indeed, this ability on the part of bees suggests that they may not use the Rayleigh scattering relationships at all (8, 15).
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16. We thank R. Alexander, R. Dahl, K. Schenk, D. Thompson, E. Tyner, and H. Wildman for technical assistance and C. G. Gould, R. M. Shapley, W. G. Quinn, and especially D. R. Griffin, for valuable discussions. Supported in part by NSF grant BNS 76-01653 to J.L.G.

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Ethanol Embryotoxicity: Direct Effects on Mammalian Embryos in vitro

Abstract. *Exposure to ethanol retards growth and differentiation in cultured rat embryos during organogenesis. The development of untreated embryos is indistinguishable from growth in utero. These data suggest that the hypoplastic features of children born to chronically alcoholic mothers are due, at least in part, to a direct action of ethanol, which causes reduced embryonic cellular proliferation early in gestation.*

Excessive use of alcoholic beverages results in a variety of medical, psychological, and sociological disruptions that identify alcoholism as one of modern society's major problems. Since a characteristic pattern of congenital malformations associated with the offspring of alcoholic mothers was described (1), attention has been focused on the toxic effects of alcohol consumption in pregnancy (2). This fetal alcohol syndrome (FAS) has now been observed in more than 200 infants, and the relationship to chronic alcoholism is well established

(3). However, the means by which FAS is produced are uncertain at present. It is not known if the developmental anomalies are the result of a direct action of ethanol or its metabolites on embryonic tissue, or if they are the product of altered maternal function, or a combination of such factors. In addition, it is not known if there is a sensitive period of gestation during which alcohol may exert teratogenic effects or if prolonged heavy drinking before pregnancy is a prerequisite for the complete FAS.

We are currently evaluating the em-

bryotoxic potential of environmental agents in cultured rat embryos during a major portion of the organogenesis period. The culture system supports embryonic growth and differentiation indistinguishable from that in utero. Organogenesis is thought to be the interval of greatest embryonic sensitivity to environmental factors, and congenital malformations are most likely to be the result of teratogenic insult over this period. Our studies have shown that in embryos cultured in the presence of ethanol, both differentiation and growth were retarded as a function of dosage, but no gross alterations in morphogenesis were induced. To our knowledge, this is the first unequivocal demonstration of a direct action of ethanol on the developing mammalian embryo, without the confounding factors of altered maternal function, nutrition, or metabolism.

Our experiments were designed to investigate the development of embryos continuously exposed, during organogenesis, to ethanol at concentrations of 150 or 300 mg of ethanol per 100 ml of culture medium (4). Conceptuses were explanted from outbred rats (Charles River) during the afternoon of the tenth day of pregnancy (embryonic age, 9½ days) (5). All operations were carried out aseptically, and no antibiotics were used throughout the study. Embryos within the yolk sac and amnion were dissected free of maternal decidua and Reichert's membrane, the ectoplacental cone being left intact. Two conceptuses were cultured in 4 ml of medium (6) contained in 30-ml serum bottles. During culture, bottles were kept in gentle motion by use of a roller apparatus (6), and the temperature was maintained at 37°C for the 48-hour culture period. The oxygen concentration in the gas phase of the bottles was increased from an initial 5 percent O₂ to 20 percent O₂ at 17 hours, and 40 percent O₂ at 26 hours (5 percent CO₂ at all times, the balance N₂). At least two conceptuses from each rat were randomly assigned to 300 mg of alcohol per 100 ml, 150 mg of alcohol per 100 ml, and control bottles. Alcohol was added to the medium at the beginning of the culture from a stock solution of ethanol which was at a concentration such that the osmolarity of the serum (305 mosmole/liter) was maintained (7). Control bottles received the same volumes of Hanks basic buffered salt solution isosmolar to the serum.

At the end of the culture, embryos and their associated membranes were examined, measured, photographed, and frozen for subsequent biochemical analysis. To estimate differentiation and abnormal organogenesis, we have devised a com-