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16. The data for samples of doped H_2O and D_2O in Figs. 3 and 4 serve as a check of the accuracy of the experiments. The amount of water in each sample was adjusted so that its signal intensity was comparable to those of the muscle samples. The data were plotted on a scale ten times larger than the scale for the muscle data for the sake of clarity. The angular dependence for both the ^1H and ^2H echoes and the fluctuation for the ^2H echoes of the muscle samples were observed for several different frogs and were well within experimental uncertainty. The fact that the deuterium spin echoes for doped D_2O (curve A, Fig. 4) did not depend on pulse spacing but those for muscle did (curves B and C, Fig. 4) further indicates that the spin echo train of D_2O in muscle is modulated by nonzero static quadrupolar interaction.
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20. The author is the recipient of an NIH research career development award. This work was supported by NSF grant NP S72-04771 A01. The Fourier transform accessories for the XL-100 spectrometer were purchased through grants from the National Science Foundation (GP-38410) and Phillips Petroleum Company.

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Direction Finding by Hornets Under Gravitational and Centrifugal Forces

Abstract. *The effect of centrifugal and gravitational forces whose resultant ranged between 26° and 45° on comb construction by hornet workers was assessed experimentally. Comb construction by hornets exposed to centrifugation at 1 to 2 days of age differed from that of hornets similarly exposed at 3 to 7 days of age. Juvenile hornets built their cells in the direction of the resultant force, whereas adults resisted the centrifugal force and tried to build in the direction of the gravitational force. Juveniles started their comb from the side walls, whereas adults started from the roof, as did nonspinning, control hornets. The findings suggest that hornets rapidly learn the gravitational force during the first days of life, and that they are aided by geometric cues of the breeding box to build in the direction of the force to which they had become habituated.*

Ordinarily the first comb in the nest of Vespinae is built by the queen in the spring, following the hibernation period. The queen initially constructs a stem or pedicle which is attached to the roof of the nest and from which a comb made up of 15 to 30 cells is suspended. The queen oviposits one egg within each cell. The eggs are glued to an inner wall of the cell to prevent their falling out, and the queen attends the brood until eclosion of the first worker hornets from their pupal cases (*1*). Thereafter, the worker hornets assume the construction duties while the queen engages primarily in oviposition. Ishay (*2*) observed that young workers of *Vespa orientalis* which are kept in groups of 5 to 40 individuals without a queen also build a comb similar to the queen's vernal one. In previous studies (*3, 4*) it was suggested that hornet comb construction is influenced by gravitational cues.

The aim of the study reported here was to observe how hornets build the comb and cells under the influence of gravitational and centrifugal forces. For this purpose a four-armed centrifuge was designed to accommodate regular artificial breeding boxes (ABB's) (*5*). Five such boxes were attached firmly to each arm at different distances from the center of rotation, with the top and bottom walls of the ABB vertical to the direction of the earth's gravity. When the centrifuge rotated at 25 rev/min, a centrifugal force of $0.325g$ acted on the first box, placed 75 cm from the center. The direction of the resultant force acting on this box was 26° from the normal; the angle is given by

$$\tan \phi = (2\pi f)^2 r/g = 4\pi^2 (25/60)^2 r/980$$

where $2\pi f$ is angular frequency, r is the radial distance from the center, and g is the constant of gravity. The corresponding angles for the second to fifth boxes were 32°, 35°, 41°, and 45° (the fifth box was attached 144 cm from the center).

Concomitantly two controls were used: (i) a rotating control consisting of two ABB's placed in opposition at a distance of only 5 cm from the center of the centrifuge and (ii) a stationary control consisting of two ABB's placed immediately adjacent to but not in contact with the centrifuge.

Twenty hornet workers were introduced into each ABB. These were either juvenile (1 to 2 days old) or adult (3 to 7 days old) and were provided with a clump of clay soil as building material and with a 200-cm^3 cotton-plugged bottle of 30 percent sucrose solution, fastened to one side of the ABB, which served as a liquid food source. Once every 2 days, the centrifuge was stopped for 5 to 10 minutes to introduce solid protein food (honey bees or hornet pupae) into each ABB and to service the machine. To prevent possible hornet adaption to the rhythm of activation or interruption of the centrifuge (*6*), these stops were made at different hours of the day or night. Acceleration of the centrifuge to constant speed required 7 seconds, and deceleration 6 seconds. Each experiment continued for 3 weeks, a period sufficient for most vesparium-reared hornets to build a comb and rear brood up to the pupal stage. In the vesparium, the hornets ordinarily start building on the third to fourth day of life and continue intensive construction for 2 to 3 weeks, and then the building activities diminish (*7*).

In two experiments, the centrifuge was exposed to direct sunlight and the temperature in the attached ABB's ranged from 22°C at night to 30°C in the daytime. In another two experiments, the centrifuge was activated within a room with a dome-shaped ceiling, under fluorescent illumination and at a constant temperature of 27°C. All the hornets used in these experiments were laboratory-eclosed specimens derived equally from queenright colonies (colonies in which a living queen is present) and queenless colonies (orphan colonies whose queen was lost or died), and were fully supervised from the moment of eclosion from the egg. Juvenile hornets were maintained for several days in ABB's at 27°C before being transferred to the centrifuge on the third to seventh day of life. In all instances, the experimental hornets were placed in ABB's in which there was no prior comb construction.

The direction of the cells with respect to the normal was measured with a goniometer within ½° (although such precision was not necessary). It was found that in an undisturbed environment—that is, when building in nature or in the ABB's of a vesparium—hornets build their cells toward the center of the earth, but there is a scatter of ± 3° in the hornets' determination of this direction. Such a scatter was also observed for the direction of the comb stalk. We found that the stalk was, in general, orientated in the direction of the central cell. Around the central cell, an initial ring of three to six cells is usually built, whose direction is 6° to 8° away from the central cell, but the average orientation is the same as that of the central cell. A second circle of cells is then built, comprising 8 to 12 cells orientated 12° to 14° away from the normal, but again the average orientation is the same as for cells of the first ring. Only in the subsequent outermost ring are cells again orientated in the normal direction.

Guided by these observations, we adopted the following procedure for measuring the direction in which the hornets build within the centrifuge. Three different measurements were made: (i) the direction of the stalk with respect to the normal, (ii)

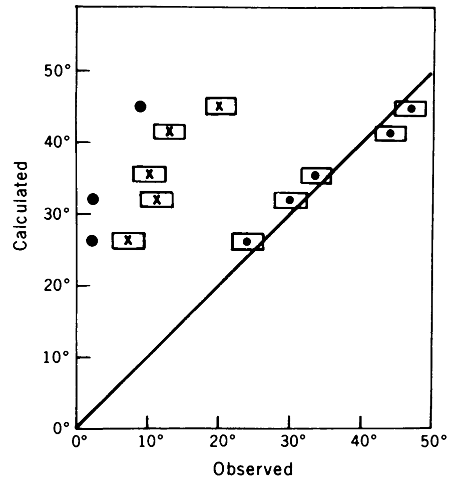

Fig. 1. Observed versus calculated angles for two experiments. The average results for all juvenile hornets (●) fall close to the calculated line. (**X**) Results for the adult hornets in the centrifuge. (●) Results for adult hornets kept in total darkness (only three of the five groups kept in ABB's in darkness built). Boxes represent error bars. There is marked tendency of the adult hornets to resist the centrifugal force and keep building normally.

the direction of the central cell, and (iii) the direction of each cell of the first and second rings. Within an error of ± 3° these three types of measurement yielded the same results in all the experiments; that is, the direction of the stalk, the direction of the central cell, and the average direction of cells of the first and second ring were identical. The results are discussed below.

1) *Hornet behavior in the centrifuge.* Observations of hornets within spinning ABB's showed that after a period of adaptation of about 1 hour for the adults and about 12 hours for the juveniles, the hornets apparently resume normal behavior, collecting solid food material, imbibing sugar solution, and later gathering building materials and constructing combs, laying eggs, and nursing the eclosing larvae—but flying activities are infrequent. During the intervals when the centrifuge is stopped, most of the hornets in the ABB's commence intermittent flying and imbibe sugar solution but rarely engage in construction.

Throughout each experiment, a comb was constructed in the majority (90 percent) of the ABB's, and in some of them two separate combs were constructed. Within each comb cell an egg was laid and glued, as customary, to an inner wall. The eclosing brood was nursed normally to the pupal stage. Complete hornet development exceeded the duration of the experiments. However, when some of the ABB's containing brood at the pupal stage were removed from the centrifuge and set aside, the pupae metamorphosed to male hornets, which were indistinguishable from ones eclosing in control ABB's.

Hornet mortality under centrifugation was up to 32 percent, exceeding that of control hornets by about 16 percent. Since there were no significant differences in hornet mortality at various distances along the centrifuge arm, we believe that the excess mortality was produced by the rotatory movement and not by the size of the force exerted on the hornets.

2) *Building.* In the course of the experiments, attention was paid to (i) the site of attachment of the comb to the substrate, (ii) the angle of the comb construction, and (iii) the aftereffects of centrifugation—that is, the behavior of the hornets, particularly from the standpoint of building activities, after their removal from the centrifuge.

Following are the results of two separate runs with the centrifuge, one in daylight in the open and the other under fluorescent illumination in a closed room. In both, one arm (with five ABB's) harbored juvenile hornets, while the remaining three arms harbored adults. The average angles for both experiments are shown in Fig. 1. For combs of the juvenile hornets, the direction of the constructed cells coincides with the direction of the resultant of the gravitational and centrifugal forces—which, incidentally, is also the direction in which the roots of plants lengthen (*8*).

This result is expected because, according to Einstein's equivalence principle (*9*), there is an equivalence between the gravitational and centrifugal forces and no instrument or sense organ can distinguish between the two. Thus, whatever the hornet's mechanism for direction detection, it does not distinguish between the two forces and builds in the direction of the resultant. Invariably, the comb of the young hornets, with or without a stalk, was attached to one side of the ABB and not the roof—that is, laterally rather than vertically.

The direction of building by the adult hornets, however, was more problematic. The adults spent several days in stationary ABB's before being transferred to the centrifuge. As shown in Fig. 1, they did not build in the direction of the resultant force, but tried to persist building in the direction of the gravitational force. Not only the comb but also the stalk affixing the comb to the substrate (roof) was built vertically, invariably depending from the roof of the ABB, just as in stationary control ABB's. There were no differences in this respect between adults from queenright and queenless colonies, which suggests that, at least with regard to gravitation, queen pheromone is not essential for proper comb orientation. This is interesting as previous studies (*2*) have shown that queen pheromone helps hornets with amputated wings to build correctly within stationary ABB's. In these studies, juvenile workers from queenless colonies attached the comb to one of the walls of the ABB, whereas juvenile workers from queenright colonies fed on or exposed to the natural or synthetic queen pheromone attached the comb to the roof of the ABB.

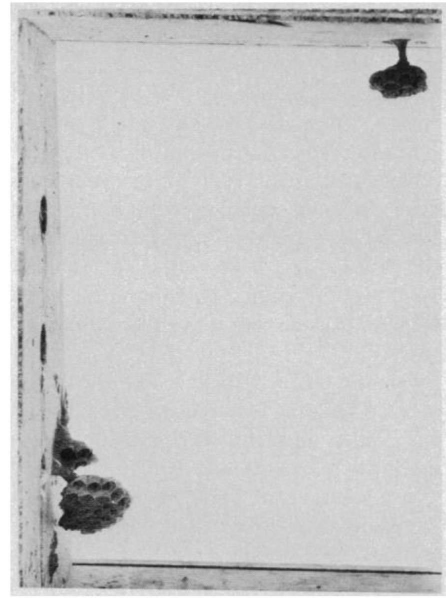

Fig. 2. Artificial breeding box with side walls removed to reveal the combs built by a mixed population of juvenile and adult hornets. The comb on the top was built by the adults and the one on the side was built concurrently by the juveniles.

In further experiments we found that comb construction in the dark (in ABB's wrapped in tinfoil) was identical to that in light, that the building in the light was the same under sunlight as under fluorescent illumination, and that hornets (in five ABB's) who had been placed under ether anesthesia for 10 minutes or immobilized by cooling (to 4°C for 3 hours) before centrifugation built in the same way as hornets that did not undergo this treatment.

At first glance, the building behavior of the adult hornets seems to contradict the equivalence principle. However, whereas the juvenile hornets were exposed from the start to the two forces concurrently, the adult hornets were first—during the few days before their introduction into the centrifuge—under gravitational force alone, and were only subsequently under the influence of both forces. Presumably, when under gravitational force alone, they may have marked its direction or become accustomed to it, so that by the time centrifugal force was applied they were already marked for gravitation and able to resist a competing force. Old hornets were probably aided by the perpendicular walls of the ABB's in determining direction. In any case, the results do not contradict the equivalence principle.

From a previous study (*4*) we know that building hornets destroy or abandon cells whose direction they did not determine and construct new cells according to their own judgment. So far as thickness of cell walls is concerned, there is no difference in construction by juvenile and adult hornets. In fact, by the time they start building, juvenile hornets are of the same age as adult hornets and continue building in the same direction for the rest of their building period.

Consequently, we believe that the differences in building between the juveniles and the adults must be attributable to dissimilarity of orientation. To check this and to test whether the building orientation of the adults could affect that of the juveniles or vice versa, we spun together ten adults and ten juveniles in each of the five ABB's attached to one arm of the centrifuge. The outcome of this experiment was that two combs were built in each ABB—one built laterally by the young hornets (which had been marked by a number on the back) and one built normally (vertically) by the adults (Fig. 2).

The results of all these experiments lead us to believe that there is a critical phase in the life of the hornet during which the "feel" for gravitation is rapidly acquired. The evidence suggests that this takes place during the first 2 days of life.

3) *Aftereffects of centrifugation*. Hornets removed from the centrifuge after a fortnight but allowed to remain in the same ABB (now stationary) continued to build new cells on combs started under centrifugation. Hornets introduced into the centrifuge as adults added cells of the same volume and in the same direction as in the centrifuge, whereas hornets spun as juveniles first built cells twice as large as in the centrifuge but gradually reduced the size so that the sixth or seventh cell built under stationary conditions was actually smaller than in the centrifuge (0.85 compared to 1.01 cm^3) (*10*). Furthermore, the new cells built by the juveniles were all directed toward the center of the earth.

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Host Tree Location Behavior of a Tropical Vine (*Monstera gigantea*) by Skototropism

Abstract. *Seedlings of the arboreal, ground-germinating, tropical vine* Monstera gigantea *(Engler) are shown to grow directly toward potential host trees; they do not find hosts by haphazard growth or random searching. Our experiments show that these vines are attracted to the darkest sector of the horizon. In nature trees provide these dark sectors. We term this response skototropism (growth toward darkness). Skototropism is probably produced by a modification of the molecular and cellular mechanisms that produce negative phototropism. We introduce the new term to emphasize the adaptive nature of the response; whereas the term "negative phototropism" can imply either growth away from light or growth toward darkness, only growth toward darkness can lead the vine directly to a host. This is because, in nature, hosts will not be aligned 180° from the lightest sector of the horizon relative to the vine.*

Arboreal vines are a mainly tropical phenomenon; species diversity of this group is much higher at low latitudes (*1*) where perennially warm and moist conditions allow the high surface-to-volume ratio of their elongate form. This form allows vines the unique advantage of lateral movement across the canopy, to occupy diverse areas not available to trees anchored at a single site. The capacity for lateral canopy movement, however, costs vines their self-supportive ability and presents arboreal species with the problem of finding a host tree to climb. Many biologists have been concerned with the mechanism of vine host location. Darwin interpreted his experiments with the vine *Bignonia capreolata* to show that hosts are found by

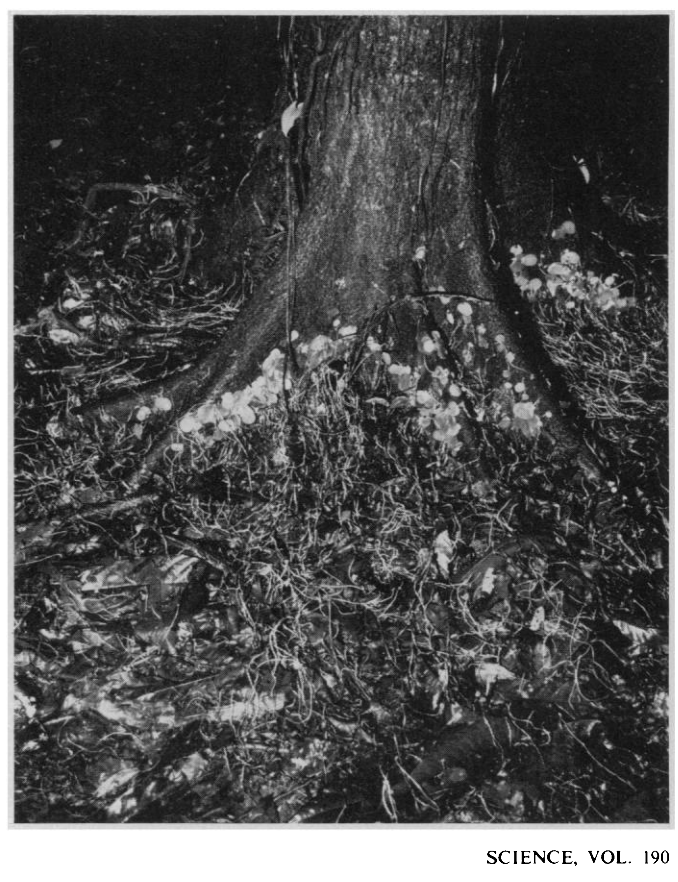

Fig. 1. Seedlings of *Monstera gigantea* converging upon a will-be host tree, at Finca La Selva, Costa Rica. Notice erect shoot tips, which serve to constantly apprise of host location relative to the minor diversions of forest floor debris.