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BEE MEGACHILE ROTUNDATA (FABRICIUS)

Abstract approved [REDACTED]

The leaf-cutter bee Megachile rotundata (Fabricius) is an important alfalfa pollinator in western North America.

This study was designed to provide information on methods of orientation used by M. rotundata in order to make recommendations regarding the parameters within which adult nesting populations may be relocated.

Preliminary studies suggest that this bee species may possess a sun compass although they depend predominantly on landmarks for orientation.

Color discrimination experiments with bees conditioned to various colors (i. e., red, orange, yellow, green, blue and purple) showed that they could distinguish all colors except red from 22 shades of gray, black and white. Tests with yellow indicate that it was a much stronger stimulus for homing bees than other colors

tested. Bees appeared to be unable to distinguish red from black and therefore are thought to be color blind to red.

Figure studies indicated that bees could discriminate between a variety of configurations including squares, circles, triangles, crosses, stars, I's and Y's. In these studies, bees were conditioned to a cross-shaped figure and tested for their ability to discriminate between it and triangles, squares and circles, all of three-inch maximum diameters. The data indicate that bees are quite able to make the distinction.

Adult relocation studies indicated that population losses were high (50 per cent or more) when bees were moved from nesting sites possessing many landmarks (e. g., buildings, rows of trees) into alfalfa fields with few prominent landmarks; from field situations providing an abundance of foraging blossoms into fields with little bloom; and when strong winds prevailed.

Conditions favoring minimum relocation population losses were moving bees with their original shelters into areas similar in landmark composition to original nesting sites; moving them in progressive steps of 20-100 yards when relocating populations over short distances; color conditioning bees to shelters painted various colors before short distance moves; moving bees into areas with sufficient bloom to maintain themselves; and conducting moves when strong winds did not exist.

General orientation was discussed with respect to flight range, landmark importance, nest entrance location and orientation inside the nest.

With the advent of commercial nesting boards containing hundreds of similar nesting tunnels, bees experience major orientation difficulties. Recommendations are outlined in this study regarding methods to ameliorate proximate orientation problems using combinations of figures and colors applied to the surface of nesting boards.

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Megachile rotundata (Fabricius)

by

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MECHANISMS OF ORIENTATION IN THE LEAF-CUTTER
BEE MEGACHILE ROTUNDATA (FABRICIUS)

INTRODUCTION

The leaf-cutter bee Megachile rotundata (Fabricius)¹ is an efficient alfalfa pollinator. Semi-domestication of this species and the alkali bee, Nomia melanderi Cockerell, has helped Oregon alfalfa seed growers average approximately two and one half times more seed per acre (449 pounds) than the national average (184 pounds) for the years 1953-67. (U. S. Department of Agriculture Statistical Reporting Service. 1961, 1966) In several areas of North America where these bees are abundant, yields of 1000-1800 pounds of clean seed per acre have been reported (Stephen, 1965).

During the seven years in which the leaf-cutter bee has been used as an alfalfa pollinator, research efforts have been directed at methods of increasing its numbers and effectiveness for maximum seed production. One of the several unique problems that have

¹The status of this species name is currently in question (Hurd, 1967). Hurd's examination of the original specimens used by Fabricius in describing Megachile rotundata showed them to be distinctly different from the species designated by that name in this country. In addition, he discovered that the lectotype of M. rotundata agreed in description with that of Megachile (Megachile) centuncularis (Linnaeus) and the type of the latter species was in turn found to be a female of M. ligniseca (Kirby). As a result of these findings a revision of the genus Megachile is currently being conducted to ameliorate this situation, but until its completion M. rotundata as we know it is without a name.

arisen is the perfection of techniques for the management of adult foraging populations.

Unlike the honeybee this bee normally restricts its flight to areas near its nesting site. It would be desirable for growers to be able to move adult populations as alfalfa bloom is exhausted at a given location in order to utilize his bees more efficiently. However, seed growers generally have been unable to move adult bees without losing great numbers of them. It appears that bees become disoriented when moved into a new area and fail to return to their relocated nesting domiciles.

Several attempts were made to relocate adults of M. rotundata in 1962 and 1963 (Osgood, 1964). Of the 1111 bees moved in 1962, only 108 bees remained after the last introduction, and in 1963, only 210 of 1521 bees reestablished at the new site.

Tests were undertaken to break the conditioned orientation response of adults of M. rotundata by refrigerating them for periods up to three days prior to a move. Refrigeration apparently did not affect site conditioning in the species, for the per cent of reestablished bees was no greater than that which resulted from direct transfer (Osgood, 1964).

This study was designed to provide information on the methods of orientation used by M. rotundata. The fundamental information on orientation mechanisms may provide a basis for recommendations

that will prescribe the parameters within which adult bees may be moved. The ease with which this leaf-cutter bee can be manipulated, its gregariousness, its ready acceptance of artificial domiciles and the fact that it rarely stings, make it a suitable test insect for studies of this type.

LITERATURE REVIEW

Most of the research in bee behavior has been directed towards the social species, in particular the honeybee. Stephen et al. (1968) list approximately 3,500 bee species in America north of Mexico of which 70-80 per cent are solitary nesters.² Behavior studies in this group have been grossly neglected, and although several species appear well adapted to orientation studies no one is presently working on the problem. Possible explanations for this neglect are more evident when one examines the nature of solitary bees. Stephen (1966) points out that most species of solitary bees have only limited periods of activity and are more geographically limited than social species. Moreover, they are usually found in low population numbers, are rapid fliers and tend to be small in size, making them more inconspicuous than some of their social counterparts (e. g., bumblebees and honeybees). While they exhibit great diversity in nesting habitats, few nest in proximity to man and his dwellings as do bumblebees and honeybees further limiting their detection. Lastly, until recently, solitary bees have not been considered to be of economic importance as they do not produce commercial commodities such as honey and beeswax nor have they contributed largely to commercial pollination.

²It is also reasonable to assume this proportion to be accurate for the estimated 19,000 (Michener, 1955) bee species of the world.

Color Vision

Color vision was first demonstrated in the honeybee by Lubbock in 1882 and greatly elaborated by von Frisch (1915, 1950). Von Frisch's studies involved training bees to collect sugar water from watch glasses placed on colored cards interspaced with cards of various shades of gray. The fidelity of trained bees to the color-associated watch glasses led von Frisch to conclude that honeybees could distinguish orange, yellow, violet, purple and blue from all shades of gray but that they were unable to distinguish between red and black. An extension of these studies indicated that when bees were similarly trained but with all cards at the feeding site colored rather than gray, they had difficulty distinguishing blue from violet and yellow from orange and green.

Kühn (1927) modified von Frisch's experiments by using prisms for training bees to food associated with definitive portions of the light spectrum. In addition to confirming von Frisch's results he also demonstrated that honeybees were highly sensitive to spectral regions from 500-480 $m\mu$ (blue-green region) and from 400-310 $m\mu$ (ultraviolet region).

Daumer (1956) using more sensitive spectral emitters and analyzers found that violet (440 $m\mu$) and "bees purple," a mixture of yellow (588 $m\mu$) and ultraviolet (360 $m\mu$, 2-50 per cent) were also

recognized as distinct hues by bees.

Goldsmith (1961) suggests that although honeybees can be trained to several spectral regions they exhibit minimum confusion in: red-yellow-green (650-500 m μ ; blue-green (500-480 m μ); blue (480-400 m μ ; and near ultraviolet (400-300 m μ).

There are several excellent reviews on color perception in insects. Those of Weiss (1943), Goldsmith (1961), Burkhardt (1964) and von Frisch (1967) are of particular interest in discussing the large volume of literature concerning honeybee color vision.

Recently, von Frisch (1950, 1967) conditioned honeybees to colored tin plates affixed to the entrance of their hives. Using a series of adjacent hives and transposing the colored plates he showed that bees returned to the conditioned color irrespective of the hive with which it was associated. Therefore, von Frisch suggested painting hives to assist honeybees in orienting to their domiciles. The following colors were recommended: pure red; black (but not proximate to red); yellow; true light blue (without ultraviolet reflection); cobalt blue 660 (ultraviolet reflecting); zinc white; and "Satolith White" (strong ultraviolet reflection).

A few reports on color vision in aculeate Hymenoptera other than the honeybee have appeared in the literature. Mazochin-Proschnjakov (as cited by von Frisch, 1967) demonstrated electrophysiologically that bumblebees have color sensitivity similar to the

honeybee but extending further into the long-wavelengths.

Color vision in the solitary wasp Odynernus frauenfeldi Saussure has been recently studied by Tsuneki (1961). The tests involved conditioning wasps to colored discs associated with their nest entrances, moving these discs to various positions on a background board to which were affixed many similar circles painted gray or colors, and observing the return behavior of the wasps. His results indicated that O. frauenfeldi can perceive and distinguish at least two color regions, yellow and blue. The yellow region was defined as consisting of hues red, yellow and green, and the blue region as including green-blue-blue, blue and violet.

Sun and Polarized Light Orientation

Wolf (1927) discovered that honeybees were capable of maintaining a given direction in relation to the sun while returning to their hive from a feeding source. Von Frisch (1950, 1967) greatly elaborated on these findings and showed that in addition to using the sun as a compass, honeybees are capable of conveying distance and directional information to their hivemates based on the sun's position in relation to the hive.

Tsuneki (1950) showed that pompilid wasps maintain flight direction using the sun, and Evans (1966) suggests that further work will reveal that many other wasps possess the capacity for sun

orientation.

Von Frisch (1948, 1949) demonstrated the sensitivity of the honeybee to polarized sky light. He was able to change the direction or completely disorient the bee dance by rotating a polarization filter in situ mounted above them.

Jacobs-Jessen (1959) studied orientation to plane polarized light in a number of social and solitary Hymenoptera including representatives of the solitary bee genera, Andrena and Halictus. She reported that under a polarizing filter, Andrena sp. oriented at a 45° angle while Halictus sp. oriented parallel, perpendicularly and at a 45° angle with respect to the plane of polarized light. In contrast Bombus terrestris (Linnaeus), B. agrorum Fabricius, and the honeybee oriented only perpendicular to the polarization plane.

Jander (1963) repeated Jacobs-Jessen's work and obtained similar results when using bees recently captured outdoors. However, if he allowed honeybees to feed on sugar water before testing, they oriented parallel, at a 45° angle and perpendicularly to the plane of polarized light.

Von Frisch (1967) suggests that there is no evident biological significance for orientation to polarized light unless one supposes that by using it an animal is able to maintain a given direction between two points.

Landmark Orientation

Several studies have appeared in the literature dealing with form discrimination in bees and wasps.

Von Frisch (1915, 1950, 1967) demonstrated that honeybees could be trained to discriminate between certain floral simulating patterns affixed to cardboard boxes containing sugar water. However, he found that they could not distinguish between geometric figures such as triangles, squares and circles. He concluded that since these figures were not found in flowers "perhaps they failed to learn the new patterns because they had never before encountered triangular or square colored patches."

Hertz (1929) continued these studies and demonstrated that honeybees distinguished between certain figures by the degree of brokenness or dissectedness of their outlines. For example, she found that figures such as a solid painted circle, square, or triangle could be distinguished from an X, Y or hollow square, but members of either of these figure groups could not be distinguished from others of the same group. From her work she concluded that the properties of figures which made them suitable orientation markers were articulation, richness of contours, "closedness," and contrast with background.

Wolf and Zerrahn-Wolf (1936) challenged Hertz's conclusions

and stated that instead of honeybees distinguishing figures on the bases of form or pattern alone they did so through flicker perception. In other words, more dissected figure outlines produce more frequent alternations between darkness and lightness in relation to the background and thus produced higher flicker frequencies to which bees respond. Von Frisch (1967) suggests that this is not the entire answer and quotes from Kunze (1961) that bees still see in the patterns "more than the sum of the different frequency components."

Tsuneki (1961) examined figure discrimination in the solitary wasp Odynerus frauenfeldi Saussure. He found that when these wasps were conditioned to a disc associated with their nest entrance and subsequently subjected to choose between it and a similar disc of greater diameter, they chose the latter with significantly greater frequency. Tsuneki relates that Hertz (1929) observed the opposite to be true for the honeybee, which decidedly preferred the smaller of two adjacent circles. Tsuneki was unable to adequately explain these differences.

Tsuneki concluded, from meager data, that the figure discrimination capacity of this wasp was poorly developed when compared with the honeybee. He attributed this inability to natural selection and suggested that these wasps were not intimately associated with floral designs, thus did not evolve the figural acuteness exhibited by the honeybee.

Of the literature dealing with the more gross, Gestalt or "stimulus complex" aspects of landmark orientation, the following works are considered most pertinent to the present study.

Although honeybees seem to rely most heavily on their sun compass for orientation, von Frisch and Lindauer (1954) demonstrated that prominent landmarks were at times used without regard to the sun's position. They trained bees to a feeding table south of the hive with a flight path 60 meters from and parallel to a forest edge. When the hive was moved to a new area in which the experimental situation differed only in that the forest border was oriented in an east-west direction, most of the foraging bees searched for food along the forest edge disregarding the new sun angle.

Similar experiments, but with the flight path 210 meters from the forest edge, showed that when the hive was moved into the new area bees oriented to the correct compass direction and disregarded the forest boundary. Apparently the forest border was too far away to provide the strong orientation stimulus observed in the first series of experiments.

Displacement experiments further demonstrated that regardless of their apparent visual conspicuousness in open fields, individual or small groups of trees were rejected in favor of the sun compass for orientation (von Frisch, 1967).

The role of landmarks in orientation has been studied in a few

species of solitary wasps. The most extensive of these have dealt with the characterization of orientation in Philanthus triangulum Fabricius (Tinbergen, 1932, 1935; Tinbergen and Kruyt, 1938; Tinbergen and van der Linde, 1938; and van Beusekom, 1948). These authors conclude that P. triangulum learns the configuration of landmarks associated with its nesting sites through visual means alone and uses these in orienting to the nest entrance.

Certain objects (e. g., stick, clump of grass or fir cone) were removed or displaced from the area of the nesting site with little effect on orientation (Tinbergen and Kruyt, 1938; van Beusekom, 1948). For example, van Beusekom (1948) demonstrated that when a nest of P. triangulum was encircled with a ring of 16 fir cones, as many as six could be removed before the wasp detected a difference in the arrangement. It was apparent from these studies that the stimulus-complex, "circle of cones," rather than the precise number and shape of the cones themselves served to direct the wasp to its nest.

Tinbergen and Kruyt (1938) analyzed the characteristics necessary for an object to be considered a good orientation marker. They found articulation in plane and space, contrast with the background, and height to be of prime importance.

Van Iersel (1952) and van Iersel and van den Assem (1964) studied nesting orientation in the diggerwasp, Bembix rostrata L,

and found it to be similar to that of P. triangulum. They observed that if a female of B. rostrata returned to her nest and was temporarily disoriented by an unfamiliar object near it, she greatly increased her reorientation time upon leaving the nest.

Within certain dimensions the effect of these disturbing objects resulted in an increase in reorientation time upon leaving the nest proportional to the time of disorientation before entering the nest. It appeared that a box measuring 18 centimeters in width by nine centimeters high was optimal for causing the greatest disorientation. If the object was greater in width than 18 centimeters (e. g., a sheet of paper) the wasp would alight in search of its nest but remained in flight much longer if the object height was greater than nine inches. Both situations resulted in long searching periods before nest entry but much shorter reorientation times than would be expected. Through further testing van Iersel and van den Assem concluded that when the disturbing objects exceeded critical proportions, as described above, wasps were unable to recognize the immediate nest surroundings and shifted their orientation to known cues outside the area and on the horizon in order to find their nest. Therefore on subsequent reorientation flights wasps demonstrated shorter flight times because they used undisturbed distant objects rather than attempting to incorporate disturbing objects near the nest into their orientation memory.

Schricker and Stephen (1968) examined factors influencing orientation of the wasp Stenioloa scolopacea albicantia Parker to its sleeping plant. They reported, as did Tinbergen (1932) for P. triangulum regarding nesting site objects, that no olfactory stimuli are associated with locating the sleeping plant. Within a radius of 10-15 meters about their sleeping plants, wasps were well oriented and found the displaced plant without difficulty. However, if the plant was displaced 15-20 meters wasps became disoriented and accepted other plants for sleeping. Plant displacement distances greater than 20 meters resulted in reorientation by the wasp on the following morning. The most important characteristic of the sleeping plant was found to be height. Form was demonstrated to have little importance in orientation.

Chmurzynski (1963, 1964) proposed a classification for homing behavior in flying Hymenoptera in which he distinguished five successive stages: (a) distant orientation (recognition of the general nest area), (b) proximate orientation (recognizing the nest vicinity), (c) immediate orientation (locating the nest entrance), (d) perceiving the nest itself, (e) orientation inside the nest. Employing this classification he characterized the homing behavior of Bembix rostrata (L.) as follows (Chmurzynski, 1963): distant orientation extends to a radius of 0.5-0.7 kilometers; proximate to four meters; and two areas within immediate orientation, one at 18 centimeters and one

at three centimeters about the nest entrance. As the wasp returns from the field, each area provides sequential homing clues which tend to become smaller in size and gain in orientation significance as the nest is approached.

Baerends (1941) found that the outer limits of distance orientation for Ammophila pubescens Curtis were about 40 m. while Tinbergen and van der Linde (1938) reported that P. triangulum would return to the nest from as far away as one kilometer.

Several reviews dealing with aspects of orientation in Hymenoptera have appeared of which Baerends (1959), Jander (1963), Chmurzynski (1964), Evans (1966) and von Frisch (1967) are of special interest.

Management

All of the literature dealing with the propagation and management of M. rotundata has appeared within the past ten years. Those papers by Stephen (1962), Bohart (1963) and Hobbs (1967) are most complete and deal with aspects of biology, nesting media, shelter construction, overwinter and insecticide protection.

MATERIALS AND METHODS

Most of the data presented here were collected through observations at bee nesting and foraging sites in or near the towns of Boardman and Ontario, Oregon, during the summers of 1964-1967. The town of Boardman is surrounded by a small, isolated irrigation district which borders the Columbia River on one side and is sharply delimited by an arid sagebrush desert on the other sides. Farms in the area are small and diversified, many producing alfalfa for hay. A large number of old buildings and an abundance of dead Lombardy poplar provide ideal nesting sites for M. rotundata. Studies at Ontario were conducted principally at the Malheur Branch Experiment Station where large populations of M. rotundata were maintained for pollination of alfalfa clones.

All bees used in these studies were individually marked. Since females sleep with their abdomens facing the entrance of their tunnels, marking was readily accomplished by applying a small amount of quick-drying lacquer paint to the fifth and sixth abdominal tergites.

Sun Orientation Studies

Sun orientation experiments were conducted in a vast, flat desert area located 10 miles southwest of Boardman, Oregon, on the Boardman Bombing Range. The native vegetation consisted mainly

of sagebrush, Artemisia sp; rabbitbrush, Chrysothamnus sp. and various grasses. This area extended for several square miles and was considered optimal for solar orientation studies since it was practically devoid of prominent landmarks. In addition, the slightly rolling terrain proved ideal for these studies because it was possible to position alfalfa plots so that they could not be seen by the bees from their nesting domiciles.

Domiciles employed in these studies consisted of pint sized milk cartons each containing approximately 250, 4 inch long 7/32 inch diameter soda straws. These domiciles were housed in pole-mounted wooden shelters (20 x 11 x 10 inches) positioned so that domiciles were three feet above the ground (Figure 1).

In these tests, straw domiciles containing pupal bees were placed in a shelter box positioned beside alfalfa plot one (Figures 2, 3, 4). After as many females had emerged as could be supported by the available bloom, the shelter and domicile were moved progressively on successive nights to a final position 70 yards NW of plot one. The progressive move technique, used extensively in honeybee training experiments was employed to condition bees to gradually increasing distances between their domiciles and alfalfa plot one. All moves were conducted at night since females generally spend nights in their nesting domiciles. These procedures were employed in all solar orientation experiments.

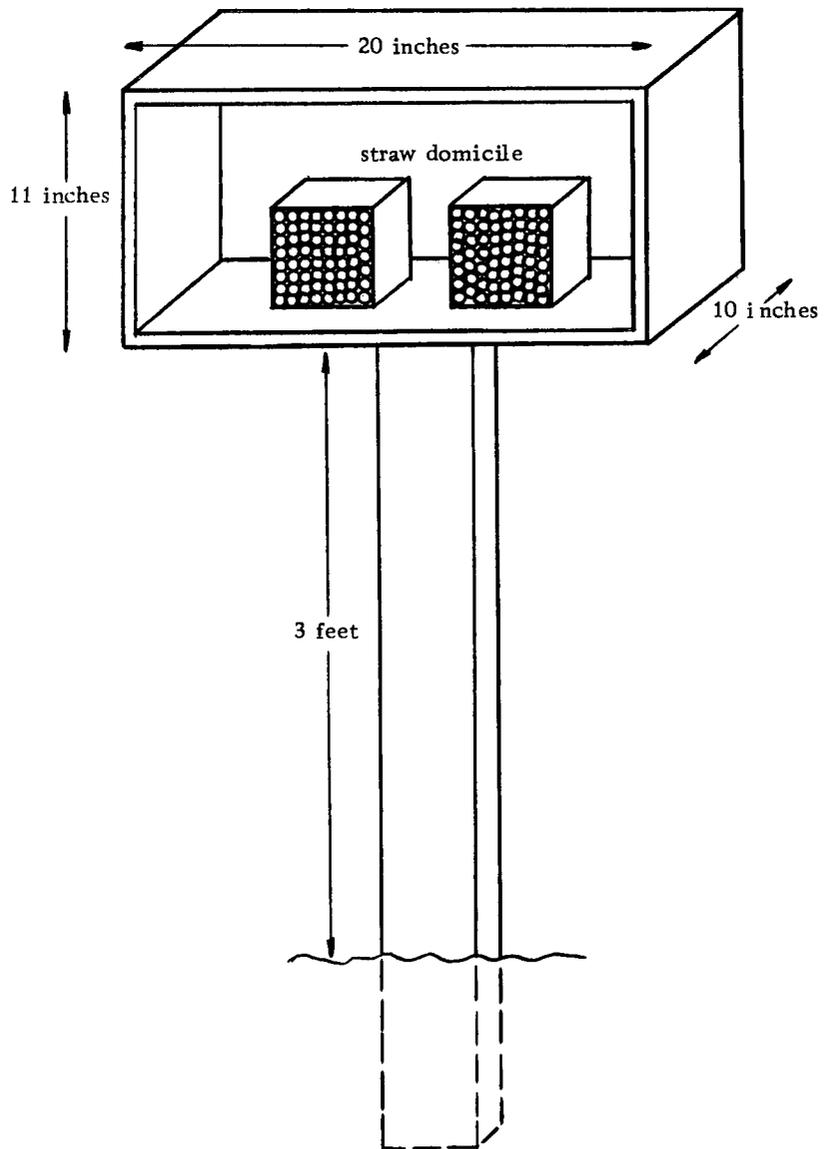


Figure 1. Shelter box with drinking straw domiciles.

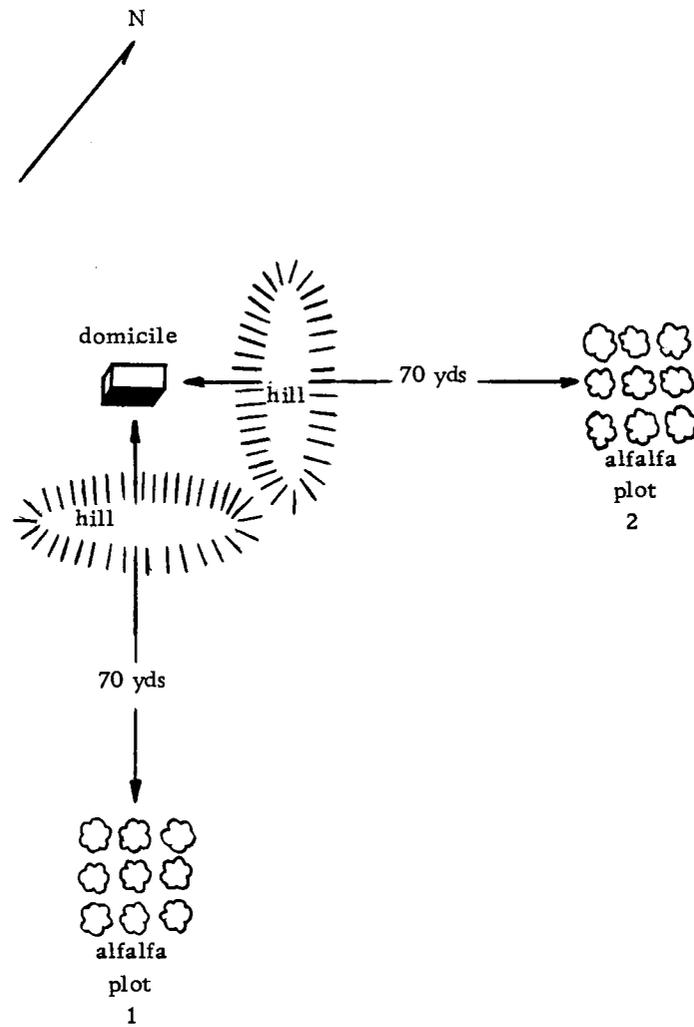


Figure 2. Diagram of sun orientation experiment I.

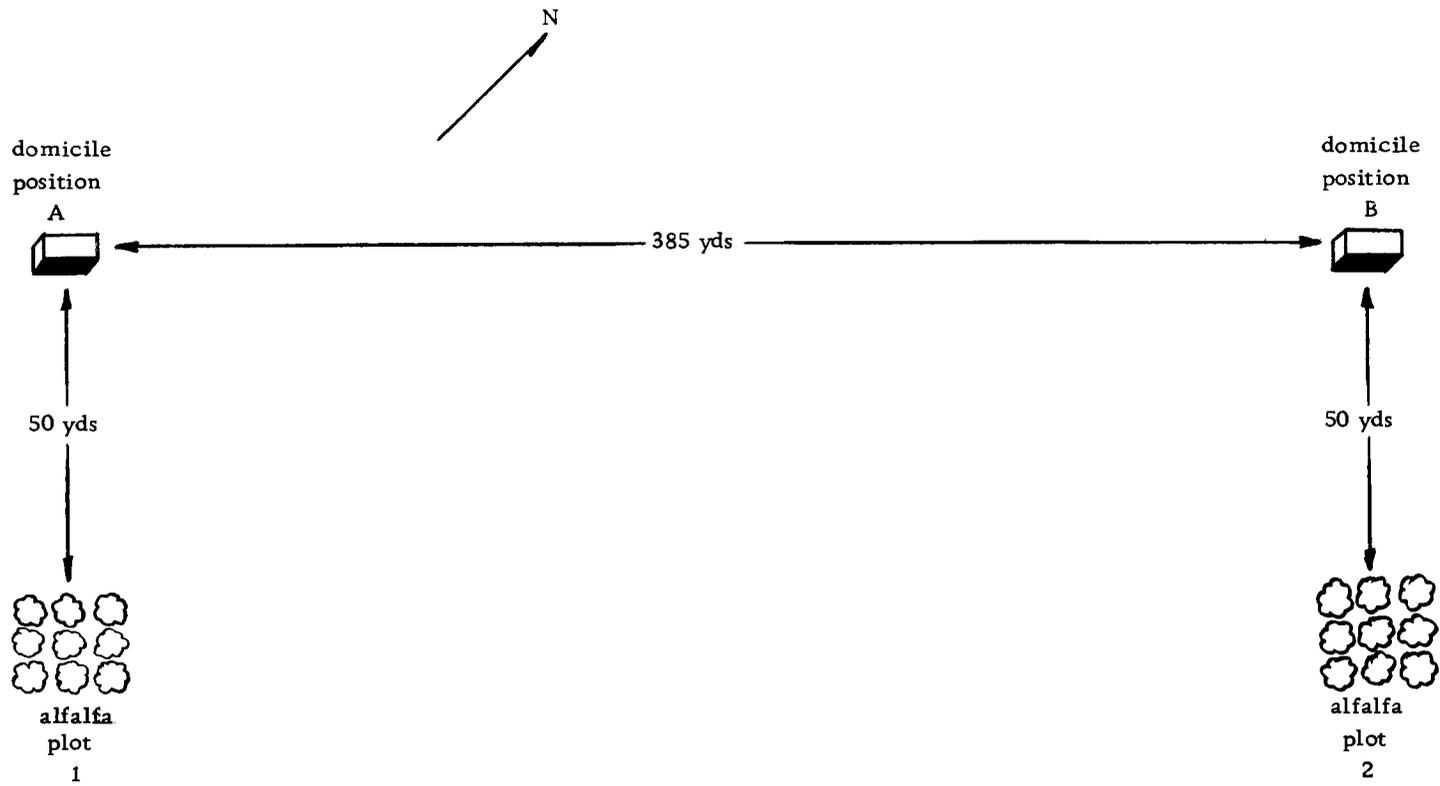


Figure 3. Diagram of sun orientation experiment II.

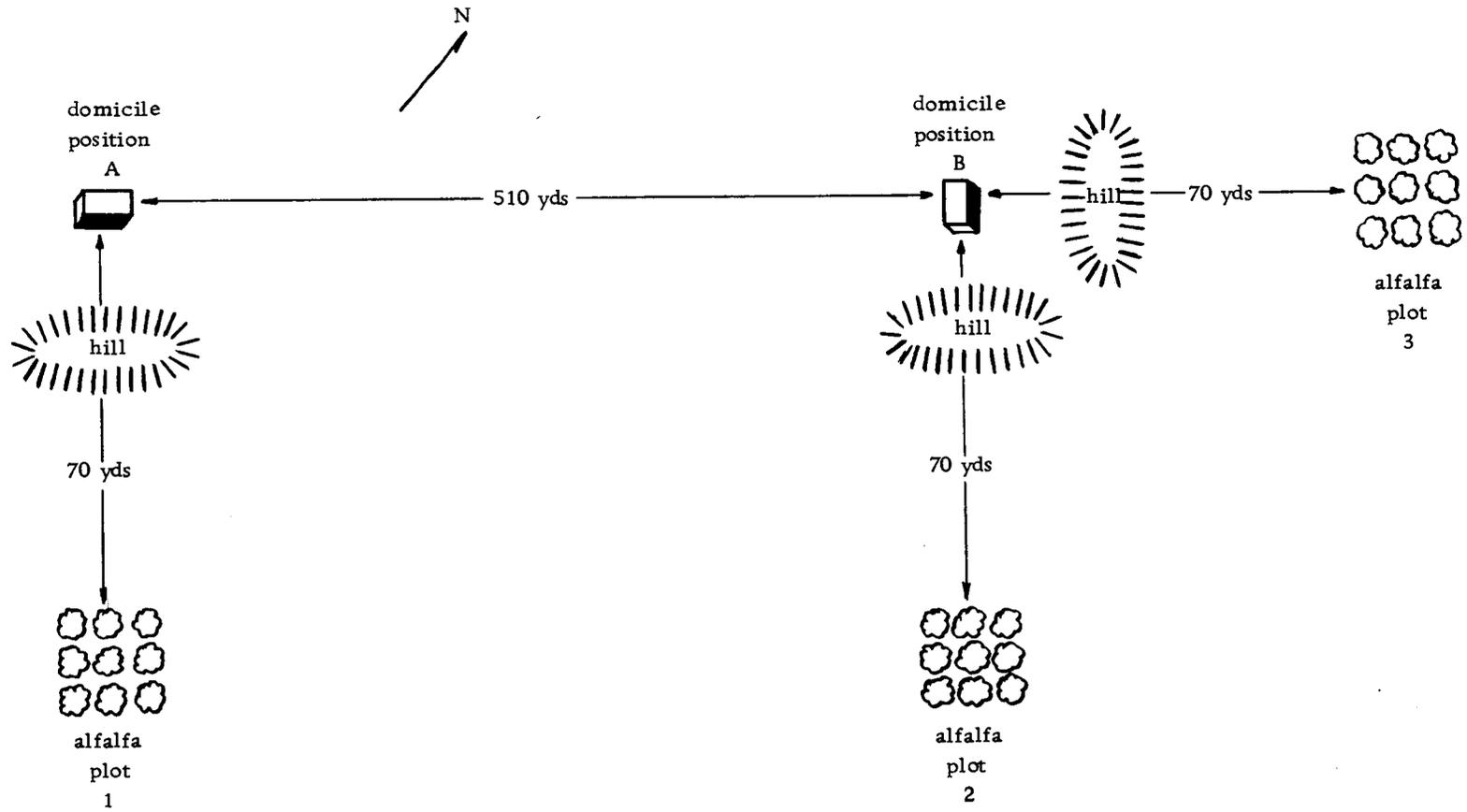


Figure 4. Diagram of sun orientation experiment III.

After completion of training to plot one, experimental procedures varied in individual tests described below. Two types of alfalfa plots were used, one temporary and the other permanent. Permanent plots were among several established in 1961 and consisted of approximately 40 plants each enclosed by a wire fence to prevent rabbit damage. Plot one of experiments I, II, and III, and plot two of experiment II were permanent. (Figures 2, 3, 4)

Temporary plots consisted of alfalfa plants individually planted in plastic buckets and cultured to maintain peak bloom during the testing period. Plots consisted of twenty plants each in which the buckets were buried so that only the plant foliage was visible above ground. These plots were always assembled during the night or early morning before a test so that bees had no previous knowledge of their existence. Plot two of experiments I and III, and plot three of experiment III were temporary.

Experimental bees were marked during the night before a test in order to distinguish them from wild individuals in the area.

Experiment I. The shelter domicile and bees were moved progressively 70 yards northwest of plot one which then was not visible from the new domicile position (Figure 2). The shelter was rotated 90 degrees so it faced the site at which plot two, also hidden from the view of the bees, was to be established. Plot two,

consisting of potted alfalfa plants was placed 70 yards northeast of the domicile on the same night.

On the following morning observers were stationed at each alfalfa plot and the effects of rotating the domicile were evaluated by recording the arrival times of marked bees at the plots. Because of individual differences in the time of commencing flight, observations were continued until it was apparent all bees had foraged that were going to do so. During the night following testing a count was made of the bees spending the night in the domicile.

Experiment II. To exclude the possibility that bees are familiar with the terrain immediately about the nesting site, a second group of bees was trained to and subsequently moved a distance of 50 yards from plot one (Figure 3).

During the night before testing, the shelter, domicile and bees were moved from the training position (domicile position A) to domicile position B, 385 yards away. Here they were located in precisely the same direction and distance from a second permanent alfalfa plot (plot two) of the same size and composition as plot one. A new similar shelter and domicile were erected at domicile position A. On the following morning one observer was stationed at each of the domiciles and plots. Records similar to those for experiment I were kept.

Experiment III. Bees were trained to a distance of 70 yards from alfalfa plot one (Figure 4). Temporary plots two and three were established on the same night that the shelter was moved 540 yards from position A to position B (Figure 4). The test domicile at position B was again rotated 90 degrees and faced alfalfa plot three. A new empty domicile and shelter were placed at position A. Due to the normally short flight range and previous absence of foraging plants within a radius of over 500 yards around position B, it was improbable that test bees had become familiar with the surrounding area.

On the following morning observers were placed at each of the domicile positions and plots. Data similar to experiments I and II above were recorded.

Color Discrimination Studies

Two experimental procedures were used in these studies. The first involved the use of a block of styrofoam containing 25 holes, spaced three-quarters of an inch apart. This block fitted tightly into a 3 3/4 inch square cardboard box three inches deep. A soda-straw was placed in each hole and a painted 3/4 inch posterboard square was centered over each straw (Figure 5). Each domicile was prepared so that a test bee could be exposed to as many as 25 different colors upon its return.

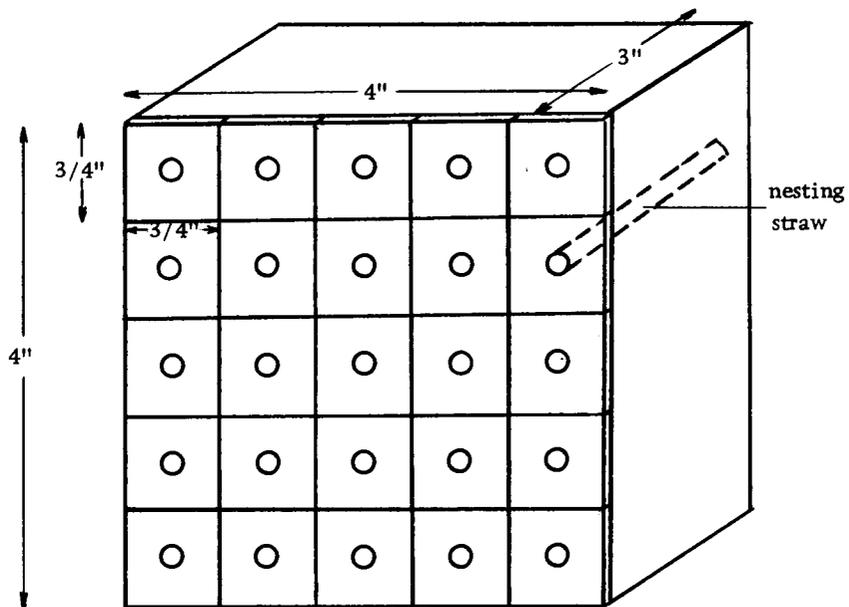


Figure 5. Domicile used for testing colors with 22 shades of gray, black and white.

A second procedure was subsequently employed to reduce the number of variables and simplify the acquisition of data. Four pint milk cartons, each containing a styrofoam block with a single hole drilled into its center, were affixed to the back of a piece of 12 x 24 inch plywood. The cartons were attached so that the center hole of each lay directly behind one of four holes spaced 4 1/2 inches apart in the plywood. Drinking straws were placed in each hole so that the end of each straw protruded 1/16 inch beyond the surface of the plywood. Posterboard cards measuring 3 x 3 inches and painted as individual tests demanded (Figure 6) were centered over each straw.

"Flo-paque" paints manufactured by Flowquil Products, Inc., Cobleskill, New York, were selected because they yielded non-gloss finishes and could be applied easily to the surfaces used. Thirty milliliter quantities of 23 shades of gray were prepared using white and black paint stocks.

The following blends were made by adding enough black paint to the quantity of white cited below to bring each sample to 30 milliliters.

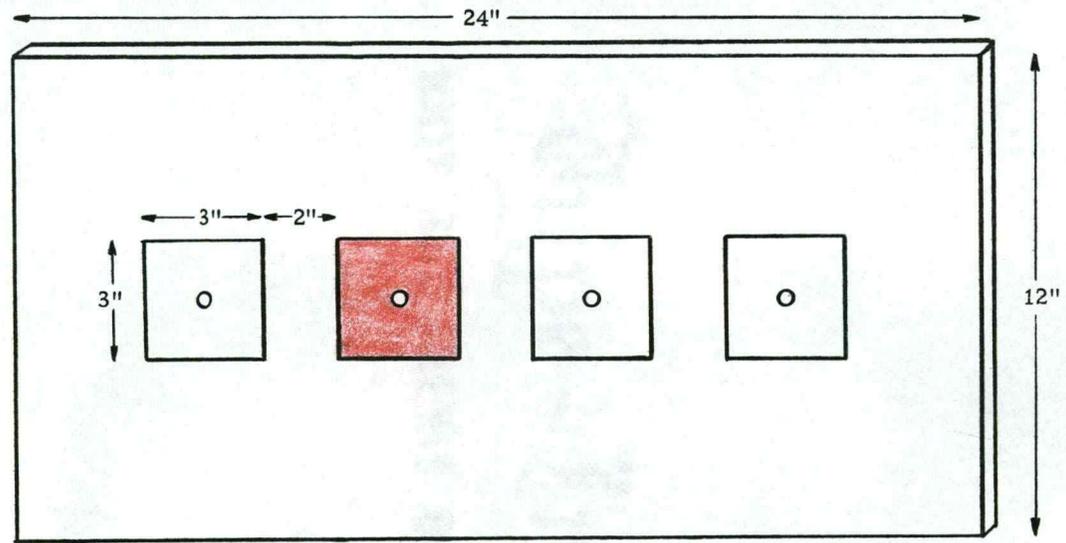


Figure 6. Domicile used for testing colors with one shade of gray or black.

<u>milliliters of white stock used</u>	<u>milliliters of white stock used</u>
30.0 = white	24.0
*29.9 = gray 1	23.0
29.75	22.0
29.65	*20.5 = gray 3
29.5	20.0
29.4	18.0
29.0	17.0
28.5	15.0
*28.0 = gray 2	13.0
27.5	12.0
27.0	* 0.0 = black
26.0	
25.0	

*These dilutions and black were the only ones used for color tests conducted using the second procedure described above (Figure 6).

Several chromatic colors were used which correspond to the standards illustrated in Reinhold's Color Atlas:

*blue = 21E8 (deep blue)
 blue = 23B6 (grayish blue)
 green = 26F8 (dark green)
 orange = 6C8 (brownish orange)
 purple = 18E8 (deep violet)
 *red = 10C8 (blood red, deep red)
 yellow = 4A8 (flame yellow, dark yellow)

*Only chromatic colors used in color tests conducted with the second procedure described above (Figure 6).

Color studies using the first type of domicile described above (Figure 5) were conducted in the following manner. The test surface of a domicile was fitted with 22 shades of gray, black, white and one chromatic color (from the list above) and randomized for each position except that for the chromatic color. Randomization was

accomplished by drawing painted cards from a box and affixing them in rows on the domicile surface. Each chromatic card to be tested was placed for training in a more central position, thus providing achromatic cards on all sides. This technique was considered most desirable to assure scanning of the entire test surface by the homing bee.

All straws, except that associated with the chromatic colored card, were plugged with black cotton yarn, inserted one inch deep to prevent nesting. Each domicile was placed on the east side of a barn to permit a bee from nearby populations to nest in the straw to which the test color was affixed. Once the straw was accepted, the bee was permitted to nest in it for at least three days to become conditioned to the color and its position within the domicile.

The night before testing the bee for color response she was marked to ensure rapid identification. In the morning the bee was observed for two consecutive foraging trips following which the plugs were removed from all of the straws. The bee again was kept under observation and its homing behavior observed. If no noticeable deviations in behavior were noted, the nesting straw was replaced by a new one and the domicile rotated in situ or the color square interchanged randomly with a gray square in the domicile while the bee was foraging. The responses of the female upon return to the domicile were recorded in regards to how well she was able to locate her

displaced colored square. Data rating included:

- + = bee found and entered conditioning color rapidly
- = bee entered incorrect color
- ± = bee uncertain, experienced great difficulty in locating conditioning color

Using the second color procedure described above, domiciles were constructed and placed on the east side of a barn at the Malheur Branch Experiment Station, Ontario, Oregon. For each domicile a color card to be tested was centered over the soda straw at position II (Figure 6) and a bee permitted to nest in the straw. The other three straws were plugged with black cotton yarn and remained without color cards until the start of testing. A bee was permitted to nest for a minimum of three days to condition it to the color and position of its nesting straw before commencement of tests.

Tests for color recognition were conducted in the following manner. Several changes were effected at the test domicile while a test bee was foraging: black yarn plugs in straws at positions I, III and IV were removed; the nesting straw at position II was replaced by an unused one; the color card was moved from position II to position III; three identical black or gray cards were centered over straws at the remaining three positions. These procedures are referred to as arrangement one in the following text.

The following actions of the returning bee to the experimental situation were recorded: (a) hovering - flight characterized by

oscillations in front of a given domicile position, (b) alighting - landing of the bee at one of the domicile positions with or without attempting to enter the straw located there, (c) entrance - the bee's body passes further into the straw than the anterior portion of the thorax.

When a bee entered a straw and found it to be the incorrect one, she backed out and continued her searching. At this time the colored card was removed from position III and replaced by a gray or black card similar to those already present at the three other positions on the test board. This manipulation is referred to as arrangement two in the following text. Data similar to that obtained for arrangement one were recorded.

After the bee had responded to arrangement two by entering a straw, withdrawing and subsequently continuing her search, the gray or black card and associated straw were removed from position II and replaced by the nesting straw and the colored card to which the bee had been conditioned. This manipulation is referred to as arrangement three in the following text. The same type of data obtained after arrangements one and two were taken.

Tests were conducted to determine if female bees could discriminate red and blue from three shades of gray and black. The formulation of the gray shades is outlined in the materials section above. Using arrangements one, two and three described above,

the responses of a test bee were recorded for each shade of gray and black with reference to the training color (red or blue). The sequences in which the grays and blacks were used were randomized in each test. In addition, the order of arrangements one and two was alternated to eliminate possible conditioning to their order when repeated with the same bee.

Figure Discrimination Studies

Initial figure discrimination studies were conducted using a four by eight foot, vertically mounted sheet of plywood with four, twelve inch diameter figures affixed to it (Figure 7).

The above test board proved to be inadequate, so another was constructed using two adjacent, vertically mounted sheets of plywood (Figure 8). Nine, 2 3/4 inch square holes, spaced approximately 19 inches apart, were cut into the plywood permitting figure domiciles (Figure 9) to pass through the surface so that the attached figure adjoined the plywood surface. The surface of the plywood was painted with a mat gray lacquer to prevent the possibility of bees orienting on the grain of the wood.

Domiciles used in these studies were of the following construction. A styrofoam block with one hole in the center was placed in a pint milk container and affixed to the back of a cardboard figure. A nesting straw was then inserted through the center of the figure

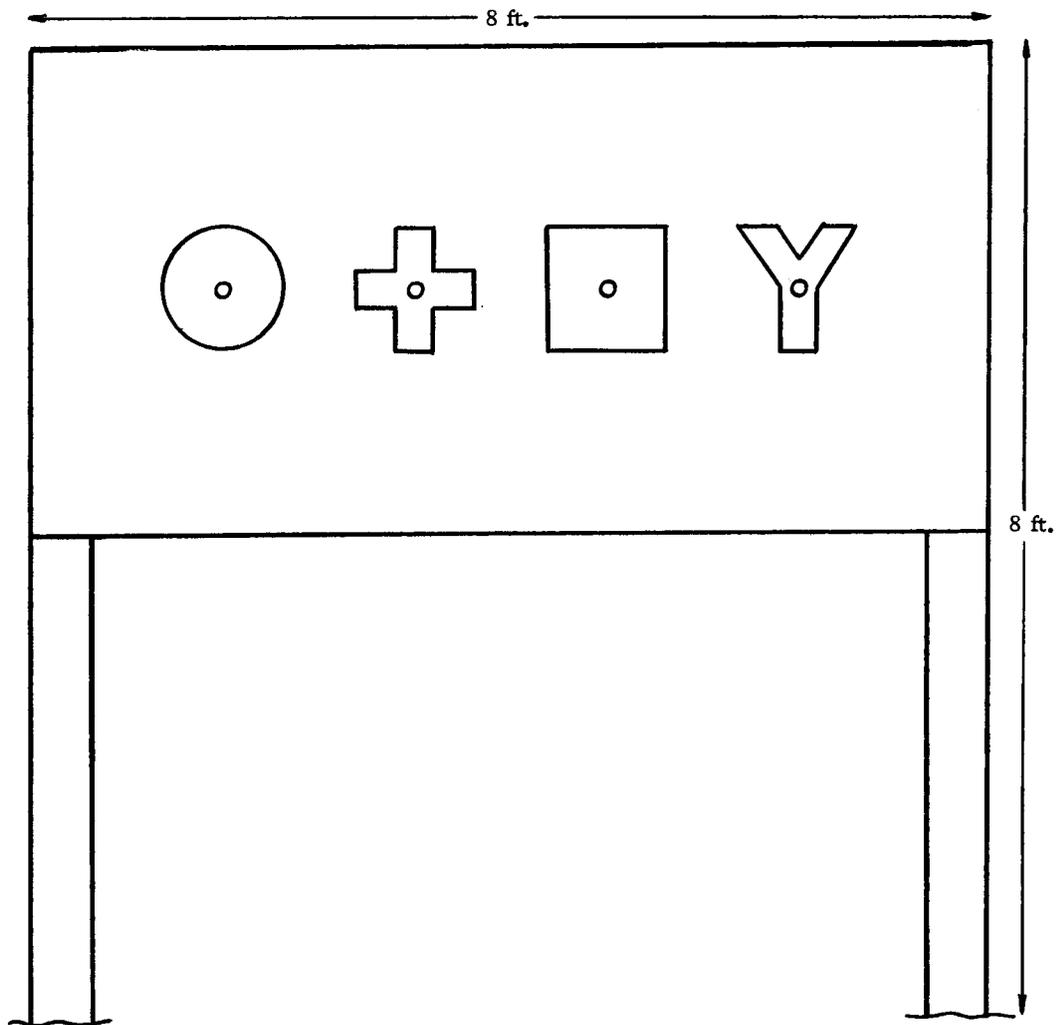


Figure 7. Experimental board for four position figure studies.

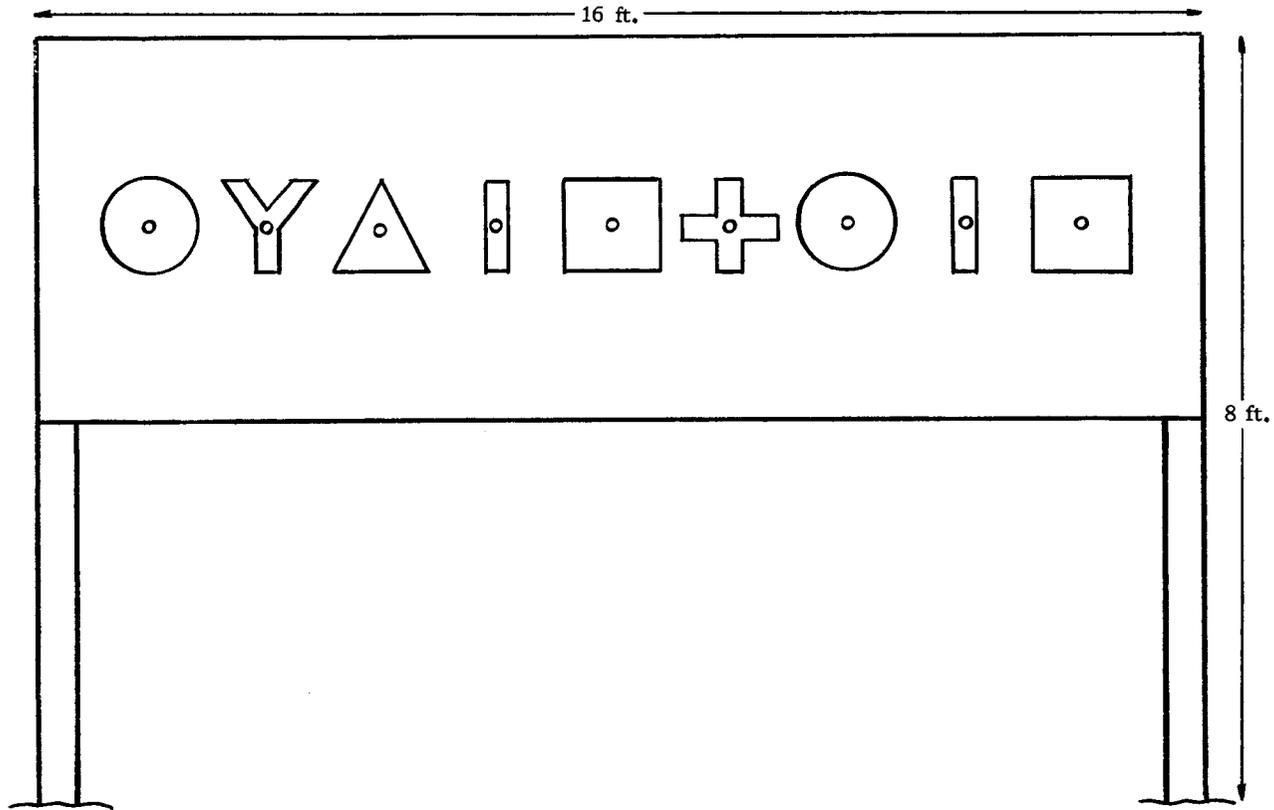


Figure 8. Experimental board for nine position figure studies.

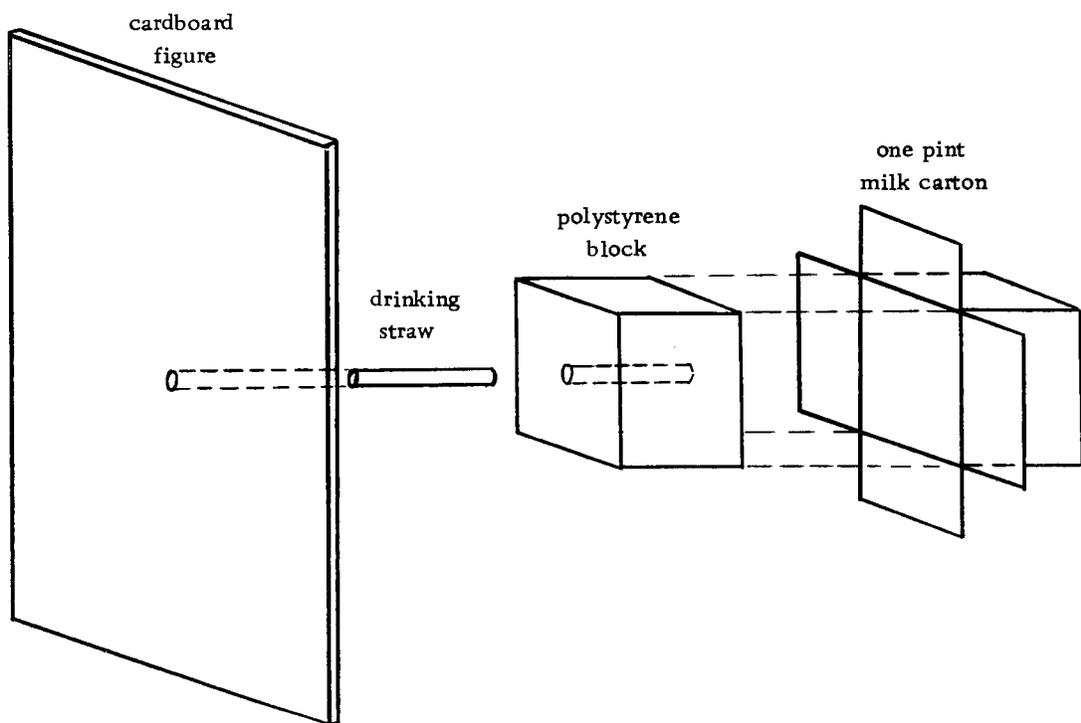


Figure 9. Structural diagram of a figure domicile.

into the styrofoam block behind (Figure 9).

Figures of several shapes were tested using the apparatus described above including squares, circles, triangles, crosses, Y's and I's. Each figure was cut from cardboard and painted yellow. Two series of figures were constructed, one with a six inch and the other with a twelve inch maximum diameter.

A second series of figure experiments was conducted using the same methods and type of test domicile described for those color studies in which a color was tested against a single shade of gray (Figure 6). Figure tests differed from the color tests only to the extent that figures were used in place of color and gray cards. In these studies only figures with cross, triangle, square and circle configurations were tested.

All figures of the second series were painted with flat black spray paint, on 3 x 3 inch poster cards. The background area not covered by a given figure was painted the same shade of gray as the plywood background. All figures were constructed to their greatest dimensions on the cards and centered over the hole through which the nesting straw was positioned. Training crosses were constructed with "arms" measuring one-half inch in width. Triangles were of an equilateral configuration and of the maximum size possible on three inch square cards.

After figures and domiciles had been positioned on the plywood

sheets (Figures 7, 8), bees from the local population were permitted to nest in all figures for three or four days before testing their ability to discriminate between figures. During the night before experimentation each bee to be observed was marked using a small amount of lacquer paint. On the following morning while nesting bees foraged, two of the figures were interchanged and bee homing responses recorded in regards to their accuracy in locating their displaced conditioning figure. Similar tests were conducted with a number of bees using each of the figures cited above. Data were recorded using the following rating system:

positive = bee entered conditioning figure on first approach

negative = bee entered incorrect figure

Experiments were conducted only in the morning when the board and its figures were well illuminated to prevent possibilities of behavioral misinterpretation when the figures were shadowed.

Bees used in the second type of apparatus described above were trained to position II on test boards over which cross-shaped figures had been placed. Crosses were the only figures used for conditioning bees in these studies. Training consisted of permitting bees to nest in straws associated with cross figures for several days to allow adequate time for conditioning to occur. During this period the other three positions were without figure cards.

After training, manipulations (arrangements one, two and three) were conducted to compare the responses of the bees to the training crosses and each of three other figures: a triangle, a circle and a square. These manipulations were conducted in the manner described under color discrimination studies.

Displacement Studies

Several farms near Boardman and Ontario were used for displacement studies. These areas were chosen for their apparent similarities, each having large fields of blooming alfalfa proximal to nesting domiciles with few prominent landmarks.

Experiments consisted of moving domiciles containing nesting females and comparing population losses associated with these relocations. Bee populations were housed in painted and unpainted shelters (Figure 1) as the individual tests demanded.

Adult population displacement experiments were of three types: 1) moving domiciles with their bees from the sides of various dwellings (e. g., barns, trailerhouse) to field shelters located 3.5 to 25 miles from the original nesting site; 2) transferring bees and their field stations from one field to another over distances from four to ten miles; 3) moving bees and their associated field stations to different places within a single field, where moves consisted of

progressive displacements of 20 yards each (Figure 10), and discontinuous displacements of 100 yards from the original nesting site.

In the progressive displacement studies, two bee populations were permitted to nest in two shelters positioned 100 yards apart. At the start of testing each shelter was moved 20 yards closer to the other on successive nights until they were positioned adjacent to one another.

All bee populations used for these displacement studies were permitted to nest in their respective domiciles for at least one week before testing commenced. As in previous experiments, moves were made at night while the bees were inactive in their domiciles. Each bee at the nesting site was marked and when two populations of test bees were nesting near one another each group was painted with a different color. Population counts were made before each move and the night after each to determine population losses.

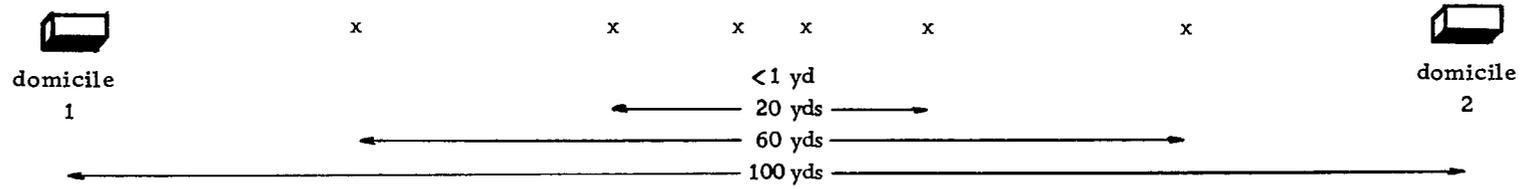


Figure 10. Progressive move experiments.

RESULTS AND DISCUSSION

Sun Orientation Studies

The characteristic pre-foraging flight behavior of females of this species presented some difficulties in obtaining the data. Except for very warm mornings when bees appear to fly directly from their domiciles to foraging areas, females usually perform several short flights around their nesting area, often resting on various objects in the vicinity, before foraging. This behavior pattern coupled with their small size and rapid flight made it extremely difficult to determine the time at which each individual left the domicile area and the direction taken of foraging flights. Therefore, the observed arrival times presented in the following experiments are difficult to evaluate in regards to flight behavior.

Experiment I. When this test was conducted on the morning of July 11, 1966, it was found that bees foraged only in alfalfa plot one (Figure 2). Of the eight bees used in this test, six were observed foraging in alfalfa plot one and the remaining two bees were not found in either plot. Bees were first observed in alfalfa plot one at the following times: bee one, 8:55 A. M.; bee two, 9:08 A. M.; bee three, 9:13 A. M.; bee four, 9:17 A. M.; bee five, 9:23 A. M.; and bee six, 9:25 A. M. Seven bees had returned to the test domicile on the

evening of July 11.

The rotation of the domicile 90° appeared to have no effect on the foraging site of the marked bees. This could indicate either that the bees are capable of sun orientation, and like honeybees learn the azimuth change, or they may have been able to locate plot one using landmarks alone. Thus a second experiment was conducted in an attempt to circumvent the latter possibility.

Experiment II. Of the twelve bees used in this test on August 17, 1966, only three were observed foraging in alfalfa plot two from 9:00 - 10:15 A. M. and none in alfalfa plot one (Figure 3). The first bee was observed in plot two at 9:00 A. M., the second at 9:05 A. M. and the third at 9:20 A. M.

Upon returning to the test area that evening, six of the marked bees were found in domicile position A (the former nesting site) and five remained at domicile position B.

Because alfalfa plots one and two were permanent plantings it is believed that some of the bees (i. e., the six that returned to domicile position A) had foraged in both plots and were therefore familiar with subtle landmarks about both plots and determined the return route to domicile position A. The other group (5 bees) appeared to have lacked previous knowledge of alfalfa plot two. Therefore a third experiment was attempted in which all plots, except plot one,

were temporary in nature.

Experiment III. This experiment was conducted on September 3, 1966. During the observation period (8:30-11:00 A. M.) six of the 13 bees were observed foraging in alfalfa plot two (Figure 4) at the following times: bee one, 9:39 A. M.; bee two, 9:50 A. M.; bee three, 10:08 A. M.; bees four, five and six at 10:33 A. M. One bee was observed near alfalfa plot three but did not forage. It landed on the observers shirt, flew away and did not return. Of the 13 marked bees, eight spent the night of September 3 at domicile position B and one at domicile position A.

Attempts to study sun orientation during the summers of 1964, 1965 and 1966 met with only limited success and therefore were terminated in 1966. Among the difficulties besetting these experiments were maintenance problems with tank trucks used to haul water to alfalfa plots, inability to control rabbit populations feeding on alfalfa plots and native hymenopterans which competed with test bees for the limited available forage. Attempts to control rabbits by shooting, poisoning and fencing and hymenopterans by insecticidal treatments and netting met with only limited success.

In spite of the minimal data it is believed that the results obtained from experiment III may indicate that a sun compass exists in M. rotundata. The data shows that all observed bees foraged only in

alfalfa plot two which was positioned in the same relative compass position to the relocated domicile (domicile position B) as alfalfa plot one was to domicile position A (Figure 4).

Since sample sizes in these experiments were small, accurate probability values could not be assigned to the data.

Color Discrimination Studies

Using procedure one in color methods (Figure 5), the following results were obtained.

The responses of bees, conditioned to various colors, during interchange with grays, black and white are recorded in Table I.

Table I. Responses of color conditioned bees when relocating their training colors. *

Color	<u>Response to conditioning color**</u>			Number of Observations	Number of Bees Tested
	Positive	Negative	Unclassified		
red	0	7	1	8	3
orange	9	0	0	9	1
yellow	18	0	0	18	4
green	10	0	0	11	3
blue	8	0	0	8	2
purple	7	0	0	7	1

* See appendix for details of these results.

** See text for explanation.

The data in Table 1 show that bees gave positive responses in most trials for all colors except red. Two types of positive responses were observed depending upon the color being tested. Bees trained to yellow returned from the field and usually without hesitation entered the straw associated with the yellow card regardless of its new position on the face of the test domicile. With all other colors tested to which bees gave positive responses they returned first to the original locations of the training color squares, hovered, then flew directly to the displaced colors and entered the straws. Behavioral responses designated as unclassified differed from positive responses in that bees hovered about the face of the domicile showing some selective preference for training colors but not entering straws associated with them on the initial sightings. Negative responses, found only in red test, were those observations in which the bee hovered before the test domicile for several seconds, without any selective preference for training colors. However, in all tests bees eventually entered straws associated with the correct training colors.

These observations suggest that yellow provided a stronger orientation stimulus for bees than all other colors tested. All other colors, except red, appeared to be intermediate in this respect.

The results obtained in red tests are interesting when considering that although bees appeared to react negatively to it when

displaced, eventual entrances were always made into straws associated with red conditioning cards. An olfactory explanation appears inconsistent with observations because bees passed over the area of the training square several times before finally entering the straw associated with the red square. A more reasonable explanation can be found in the spectrographic analysis of the red paint used (Figure 11). It can be seen from the spectrograph that the color pigment was not pure red but possessed some orange. Therefore, it may be that bees were responding to pigments other than red present but their low concentration made their detection by homing bees much more difficult (i. e., of extremely low stimulus strength).

Bees returning to the test domiciles above first approached the original positions of training colors in all but tests with yellow. It was thought that, with colors other than yellow, bees may have been comparing only the gray square at the color conditioning site with the test color formerly positioned there. If this were true, then the possible combinations of moves necessary to test all grays at each position would be astronomical. Therefore, it appeared desirable to use the domicile described in methods for testing colors against single shades of gray. (Figure 6). Two primary colors were tested using this apparatus: red, because bees appeared to react negatively to it in the above studies, and blue because it was one of the colors which appeared to be distinguished by bees although without the

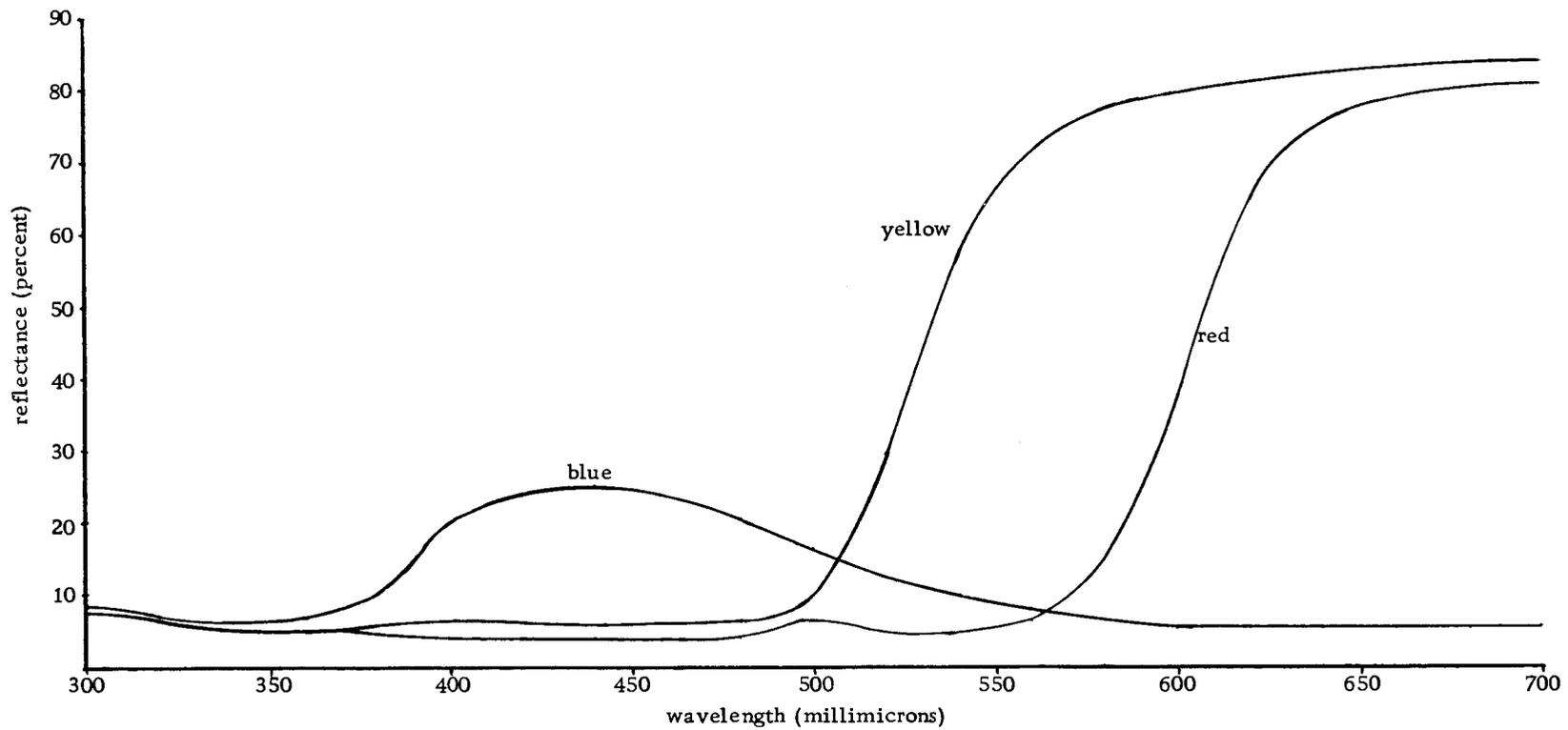


Figure 11. Spectrographic diagram of three paints used in color experiments.

strong stimulus value exhibited by yellow.

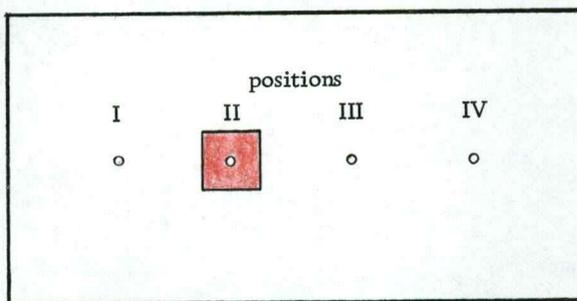
The first series of experiments using this method were conducted with red as the training color. Results of these experiments appear in Tables 2 through 10 which include hovering, alighting and entrance data for test arrangements one, two and three (Figure 12).

The first of these testing procedures, arrangement one (Figure 12), was conducted to determine if in changing the location of the conditioning red card the homing bee was able to distinguish it from three selected shades of gray and black.

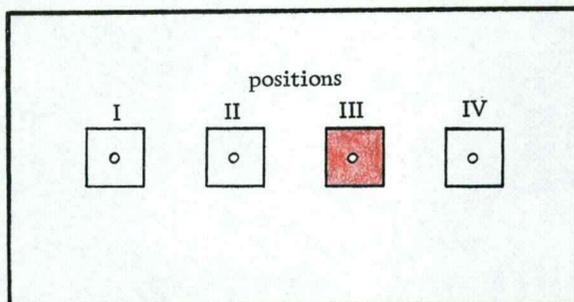
Entrance data for arrangement one (Figure 12) are presented in Table 2. These data show that the bee entered the repositioned red card significantly more times when tested with Gray A ($P < 0.002$)³, Gray B ($P < 0.002$) and Gray C ($P = 0.002$) than with black. Further, the entrance data suggest that the homing bee may be unable to distinguish black from red since in 10 of 22 trials she entered a black instead of the red card.

The second testing procedure, arrangement two (Figure 12), was designed to see if the homing bee responded differently to shades of gray and black when the red conditioning card was not present on the test board than when present as in arrangement one above.

³Probability values were assigned using tables dealing with "Significance Limits for the Fourfold Table Test" from Scientific Tables (Geigy and Basel, 1962).

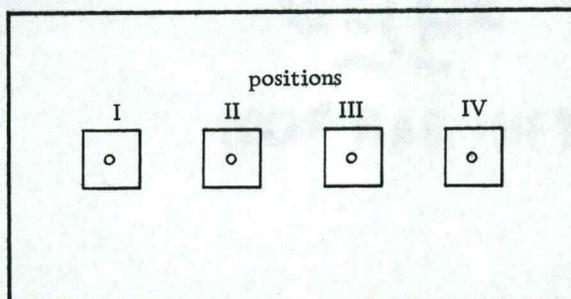


- a) Bee conditioned to colored card at position II.
- b) All other positions without cards.



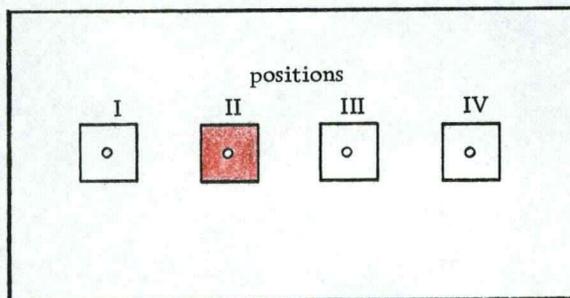
Arrangement one

- a) Colored card moved from position II to position III.
- b) Nesting straw removed and a new one put in its place.
- c) Gray or black card (same shade as those at positions I and IV) placed at position II.



Arrangement two

- a) Colored card removed from test board.
- b) Gray or black card (same shade as those at positions I, II, IV) placed at position III.



Arrangement three

- a) Color card placed at position II.
- b) Original nesting straw returned to position II.

Figure 12. Outline of color experiment manipulations.

Table 2. Entrance data for red color studies (Arrangement one).

	Position I	Position II	Position III	Position IV	Totals
Gray A	0	0	22	0	22
Gray B	0	0	22	0	22
Gray C	0	2	20	0	22
Black	1	9	12	0	22
Totals	1	11	76	0	88

Table 3. Entrance data for red color studies (Arrangement two).

	Position I	Position II	Position III	Position IV	Totals
Gray A	7	11	3	1	22
Gray B	0	14	7	1	22
Gray C	4	13	3	2	22
Black	4	6	11	1	22
Totals	15	44	24	5	88

Table 4. Entrance data for red color studies (Arrangement three).

	Position I	Position II	Position III	Position IV	Totals
Gray A	0	22	0	0	22
Gray B	0	22	0	0	22
Gray C	0	22	0	0	22
Black	3	18	1	0	22
Totals	3	84	1	0	88

Table 5. Hovering data for red color studies (Arrangement one).

	Position I	Position II	Position III	Position IV	Totals	Mean hoverings per entry
Gray A	11	30	23	1	65	2.95
Gray B	7	20	19	2	48	2.18
Gray C	8	34	24	2	68	3.09
Black	12	32	15	5	64	2.91
Totals	38	116	81	10	245	

Table 6. Hovering data for red color studies (Arrangement two).

	Position I	Position II	Position III	Position IV	Totals	Mean hoverings per entry
Gray A	106	228	156	29	519	26.59
Gray B	49	138	82	7	276	12.55
Gray C	15	73	64	6	158	7.18
Black	12	41	35	5	93	4.23
Totals	182	480	337	47	1046	

Table 7. Hovering data for red studies (Arrangement three).

	Position I	Position II	Position III	Position IV	Totals	Mean hoverings per entry
Gray A	6	16	2	0	24	1.09
Gray B	4	17	3	0	24	1.09
Gray C	4	15	5	0	24	1.09
Black	3	16	7	1	27	1.23
Totals	17	64	17	1	99	

Table 8. Analysis of variance on transformed hovering data from red color studies. (Transformation: $Y = \log_{10}(1+x)$)

	d.f.	S. O. S.	M. S.	F
Totals	263	35.6683		
Moves	2	17.7338	9.8669	211.0759***
Shades	3	1.3984	.4661	9.9719***
Shades x moves	6	2.7561	.4594	9.8267***
Residual	252	11.7799	.0467	

*** $P < 0.001$

Table 9. Alighting data for red color studies (Arrangement one).

	Position I	Position II	Position III	Position IV	Totals	Mean alightings per entry
Gray A	0	0	26	0	26	1.18
Gray B	1	0	20	0	21	0.95
Gray C	0	1	26	0	27	1.23
Black	1	4	13	1	19	0.86
Totals	2	5	85	1	93	

Table 10. Alighting data for red color studies (Arrangement two).

	Position I	Position II	Position III	Position IV	Totals	Mean alightings per entry
Gray A	2	6	5	1	14	0.64
Gray B	0	5	5	0	10	0.45
Gray C	1	11	5	0	17	0.77
Black	2	4	5	1	12	0.55
Totals	5	26	20	2	53	

The distribution of entrances for arrangement two (Table 3) were more widely spread than those recorded for arrangement one (Table 2). Comparisons of entrance results for each gray shade in arrangement one (Table 2) with those of the same shade in arrangement two (Table 3) indicate that their distributions are significantly different (Gray A ($P < 0.002$), Gray B ($P < 0.002$) and Gray C ($P = 0.002$)). However, similar computations with black indicate no significant differences which further suggests that black and red may have appeared similar to the homing bee.

A third series of trials (arrangement three, Figure 12) were conducted to reinforce conditioning to the red card located at position II and to observe any deviations in behavior which may have been attributed to preceding arrangements one and two.

The data in Table 4 indicate that the homing bee entered the red card in all gray trials and all but four when black cards were tested. Again these data suggest some confusion by the bee between black and red.

Hovering data for arrangements one, two and three are presented in Tables 5, 6, and 7 respectively. An analysis of variance on hovering data from these arrangements (Table 8) indicates that the bee's hovering responses to these arrangements were significantly different ($P < 0.001$). The analysis also suggests that the number of hoverings before the bee entered varied significantly

($P < 0.001$) between the shades of gray and black tested. The analysis further indicated a significant interaction ($P < 0.001$) between shades and arrangements suggesting that hovering means for each shade of gray and black were distributed differently in arrangements one, two and three.

Mean hoverings per trial, Tables 5, 6 and 7, are plotted in Figure 13. Little variation between means is observed among shades of gray and black within arrangements one and three. It will be remembered that these two test arrangements included the red training card. Judging from the hovering responses for arrangements two, without the red training card, its presence probably contributed by reducing confusion of the bee which is expressed through fewer hoverings before entry.

Hovering means for arrangement one are slightly larger than arrangement three. (Figure 13) It may be that moving the red training card from position II to III on the test board disoriented the homing bee sufficiently to result in more hoverings for arrangement one than three, for in the latter the red card was located at position II (position of training).

Figure 13 shows mean hoverings for arrangement two (red training card absent) to increase almost linearly from black to the lightest shade of gray tested (Gray A). A possible explanation for these results is as follows. The homing bee has a conditioned

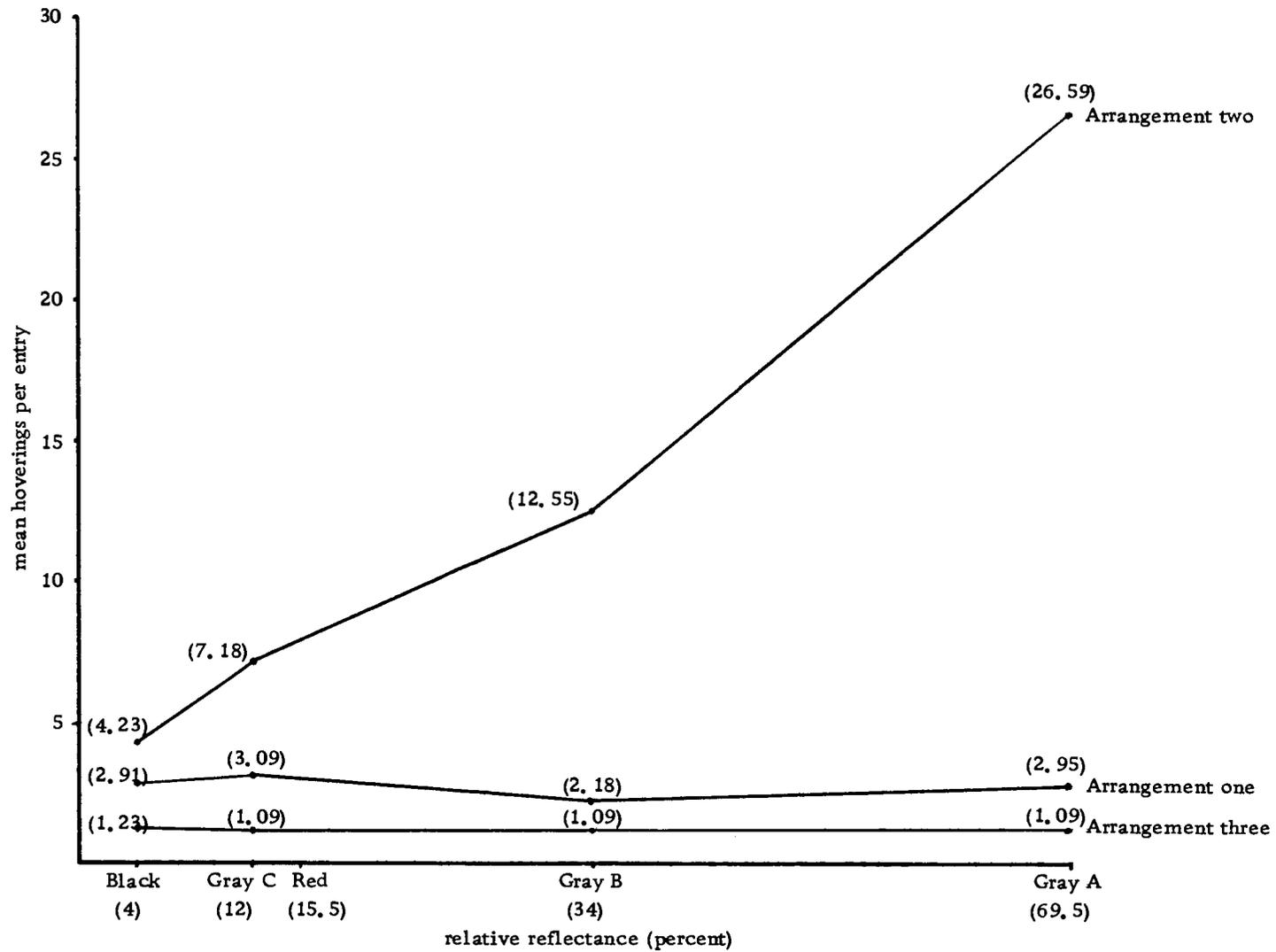


Figure 13. Mean hoverings per entry for red color studies. Exact hovering means appear in parentheses.

"mental image" of the red training card. It is not necessary for red to be sensed as the color red because the bee could perceive it only as black, if pure, or possibly as a hue of some other color if impure. As the bee approaches the test situation she surveys the area for a stimulus object (red card) which matches her "mental image of red." In arrangements one and three the match is accomplished resulting in fewer hoverings before entry. However, the conditioning stimulus object (red card) is not present in arrangement two and the bee responds by increased hoverings as the contrast between the stimulus objects (gray cards) and the "mental image of red" becomes greater. If the above explanation is correct, then the "mental image of red" and black cards used in these tests may have appeared similar to the bee as suggested by the low mean hoverings for black in all test arrangements.

Table 5 shows more hovering at positions II and III (80.41%) than at I and IV (9.59%). These results are expected since both the training position (position II) and relocated color card (position III) occupy inner positions.

The data in Table 6 show, as did those in Table 5, that more hoverings were made at position II (45.89%) than at any other position and that the majority of hoverings (78.11%) occurred at positions II and III. In comparing Table 6 with its associated entrance data (Table 3), more hovering and entrances occurred at position II than

at any other position for all shades of gray, but fewer entrances in black studies (6) than at position III (11).

These data comparisons suggest that the homing bee is able, at least to some degree, to orient itself in relation to the position of training without the red training card being present.

Alighting data for arrangement one (Table 9) appear to indicate a direct relationship with arrangement one entrances (Table 2) in that 91.40% of the alightings and 86.36% of all entrances occurred at position III. Table 9 data further show alighting means of approximately one per entrance (0.86-1.23).

Arrangement two entrances (Table 3) and alightings (Table 10) show similar distributions with about half, 50.00% of the entrances and 49.06% of the alighting, of their respective data occurring at position II. Mean alighting per entry are less than one (0.45-0.77).

Arrangement three alighting data have not been presented in tabular form because only six were recorded in all trials.

It is felt that alightings represented abortive entrance attempts which may account for the data similarities discussed above. The biological significance relating to why the bee did not enter after alighting was not determined.

The results of experiments conducted with blue as the training color appear below in Tables 11 through 19.

Table 11. Entrance data for blue color studies (Arrangement one).

	Position I	Position II	Position III	Position IV	Totals
Gray A	0	0	7	0	7
Gray B	1	0	6	0	7
Gray C	0	1	6	0	7
Black	0	0	6	0	6
Totals	1	1	25	0	27

Table 12. Entrance data for blue color studies (Arrangement two).

	Position I	Position II	Position III	Position IV	Totals
Gray A	2	4	1	0	7
Gray B	1	5	1	0	7
Gray C	2	3	2	0	7
Black	3	2	0	1	6
Totals	8	14	4	1	27

Table 13. Entrance data for blue color studies (Arrangement three).

	Position I	Position II	Position III	Position IV	Totals
Gray A	0	7	0	0	7
Gray B	0	7	0	0	7
Gray C	0	7	0	0	7
Black	0	6	0	0	6
Totals	0	27	0	0	27

Table 14. Hovering data for blue color studies (Arrangement one).

	Position I	Position II	Position III	Position IV	Totals	Mean hoverings per entry
Gray A	6	12	21	0	39	5.57
Gray B	9	14	26	2	51	7.29
Gray C	2	4	15	0	21	3.00
Black	0	7	12	0	19	3.17
Totals	17	37	74	2	130	

Table 15. Hovering data for blue color studies (Arrangement two).

	Position I	Position II	Position III	Position IV	Totals	Mean hoverings per entry
Gray A	72	112	66	8	258	36.86
Gray B	71	111	60	12	254	36.29
Gray C	20	53	38	9	120	17.14
Black	15	46	30	4	95	15.83
Totals	178	322	194	33	724	

Table 16. Hovering data for blue color studies (Arrangement three).

	Position I	Position II	Position III	Position IV	Totals	Mean hoverings per entry
Gray A	1	7	2	0	10	1.43
Gray B	2	7	1	0	10	1.43
Gray C	0	7	0	0	7	1.00
Black	0	6	0	0	6	1.00
Totals	3	27	3	0	33	

Table 17. Analysis of variance on transformed hovering data from blue color studies. (Transformation: $Y = \log_{10}(1+x)$)

	d. f.	S. O. S.	M. S.	F
Totals	80	17.4014		
Moves	2	12.6696	6.3348	116.1285***
Shades	3	0.7125	0.2375	4.3538**
Shades x moves	6	0.2568	0.0428	0.7850 ^{n. s.}
Residual	69	3.7639	0.0545	

** $P < 0.01$

*** $P < 0.001$

n. s. = $P = 0.05$

Table 18. Alighting data for blue color studies (Arrangement one).

	Position I	Position II	Position III	Position IV	Totals	Mean alightings per entry
Gray A	1	0	8	0	9	1.29
Gray B	1	1	8	0	10	1.43
Gray C	0	0	6	0	6	0.86
Black	0	0	11	0	11	1.83
Totals	2	1	33	0	36	

Table 19. Alighting data for blue color studies (Arrangement two).

	Position I	Position II	Position III	Position IV	Totals	Mean alightings per entry
Gray A	1	1	2	0	4	0.57
Gray B	3	5	0	0	8	1.14
Gray C	1	1	2	0	4	0.57
Black	0	1	3	0	4	0.67
Totals	5	8	7	0	20	

Entrance data for arrangements one and two appear in Tables 11 and 12 respectively. A comparison between entrance totals in these tables shows them to be significantly different ($P = 0.002$)⁴ with most entrances occurring at position III (position of the blue card) for arrangement one (25 or 92.59%) and position II for arrangement two (14 or 51.85%).

All entrances were made at position II, the position of training (Table 13). These data indicate strong conditioning by the bee, possibly because the stimuli associated with position and color are integrated at position II.

Hovering data for arrangements one, two and three appear in Tables 14, 15 and 16 respectively. Analysis of variance on these data (Table 17) reveals that the bee's responses to arrangements one, two and three were significantly different ($P < 0.001$). The bee also exhibited significant differences ($P = 0.01$) in the number of hoverings before entry depending upon the shade of gray or black being tested. However, interactions between shades and arrangements were not significant, suggesting that for each shade of gray and black the hovering means for arrangements one, two and three are similarly distributed.

Mean hoverings per trial, Tables 14, 15 and 16, are plotted in Figure 14. The graph shows a great many more hoverings for

⁴See footnote on page 48 for method of computation.

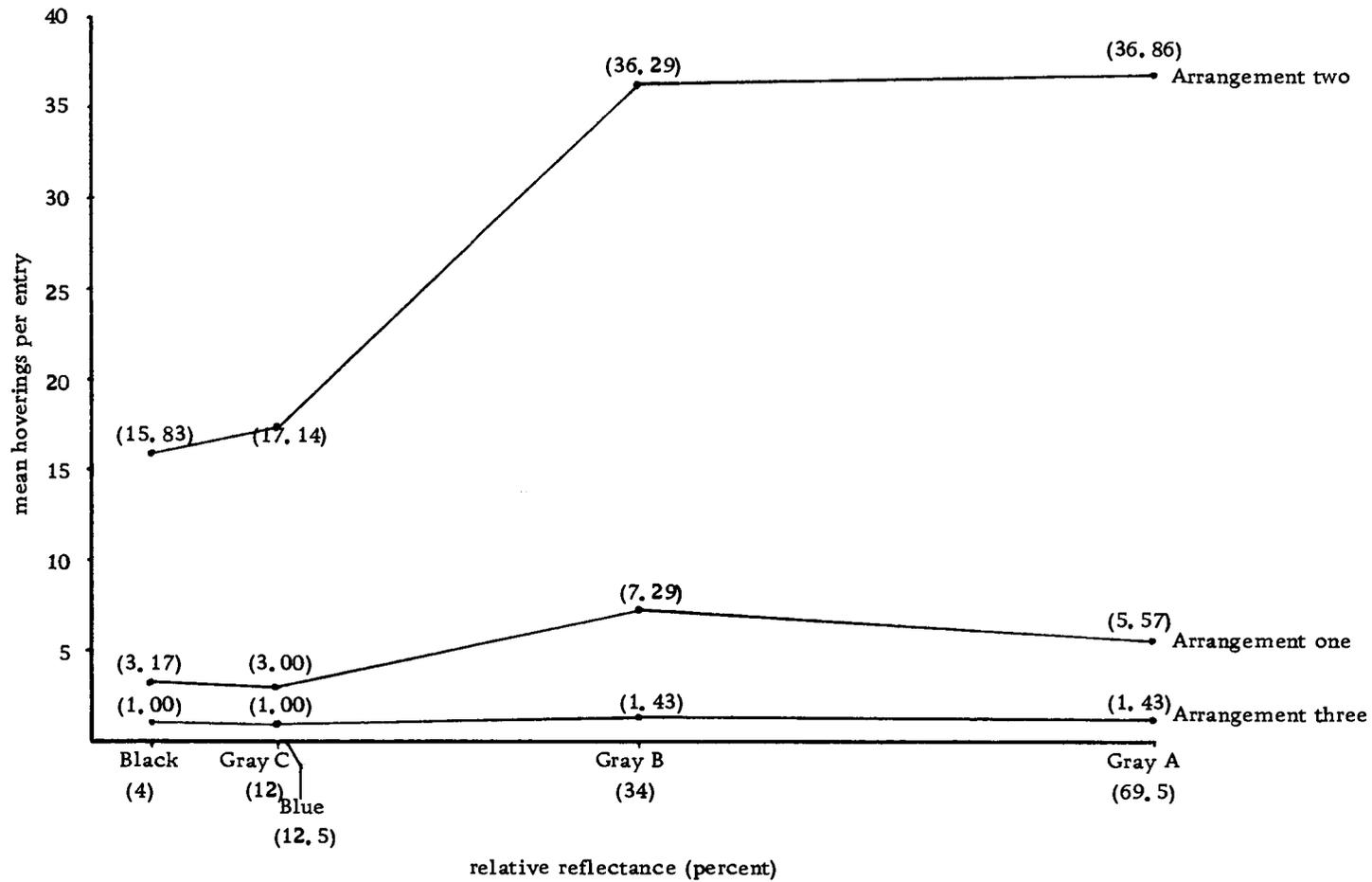


Figure 14. Mean hoverings per entry for blue color studies. Exact hovering means appear in parentheses.

arrangement two than for one or three. The absence of the blue training card in arrangement two probably explains the increased hoverings observed. The curve for arrangement two also shows almost 100 per cent fewer hoverings when black and Gray C were tested than for Grays A and B. The homing bee may be responding to this situation in a fashion hypothesized in the discussion of the red color studies. Since the blue paint used in these studies was quite dark (see Figure 11 for spectrograph) the contrast in brightness between the bees "mental image of blue," black and Gray C should be less than for Grays A and B. Therefore, the homing bee exhibits greater confusion, expressed as more hoverings, when tested with Grays A and B.

It is apparent from Table 14 that over half (56.92%) of the total hoverings were made before the blue test card (position III). These data agree with associated entrance data (Table 11) in which 92.59% of the entrances were also made at position III.

In contrast, arrangement two entrance and hovering data (Tables 12 and 15 respectively) indicate more total hoverings (44.48%) and entries (51.85%) at the position of training (position II) than at any other position.

Again, as in red studies above, arrangement three entrance (Table 13) and hovering (Table 16) data indicate little confusion by the hovering bee in locating and entering the conditioning color card at

position II.

Alighting data presented in Table 18 indicate that almost all alightings (91.67%) occurred at position III where the blue card was affixed. Mean alightings per entry show little variation (0.86-1.83) between the gray shades and black tested.

Table 19 shows the alightings to be distributed between positions I(5), II(8), and III(7). Mean alighting per entry are low, varying between 0.57 and 1.14.

If we consider alighting as abortive entrances, then the single alighting recorded for arrangement three further supports the accuracy shown by the homing bee in the hovering and entrance data presented in Tables 16 and 13 respectively.

The data for red and blue studies suggest some interesting contrasts. For example, the curve for hovering means in arrangement two of the red studies (Figure 13) differ in slope as well as magnitude from that for blue (Figure 14).

If, as suggested earlier, blue is a definite hue for M. rotundata and red is not, then a homing bee conditioned to blue may be more disoriented when the blue card is removed from the nesting site than it is when the red is removed. If this explanation is correct, then it may explain the much higher hovering means obtained for arrangement two in blue than in red experiments.

The hovering means for black in the red color studies are

closely grouped while those in blue studies show much higher means for arrangement two (15.83 per entry) than for arrangement one and three (3.17 and 1.00 per entry respectively). These data suggest that red and black appear similar to the homing bee and that she may be color blind to red. It is further suggested by the data that blue and black appear dissimilar to the homing bee and may indicate that blue is distinguished as a definite hue.

All data tables for blue and red color studies show that most hoverings, alighting and entrances occurred in association with positions II and III. Bees probably tended to orient on these positions because they were trained at position II and training color cards were located at position III in arrangement one.

Ultra Violet Perception

In order to prevent multiple nesting in the domicile used for yellow discrimination studies (Figure 5), white cotton plugs were placed in each straw to a depth of one inch except that straw to which the colored card was affixed. After a bee had nested in the domicile for several days and testing was about to commence, these plugs were removed. It was observed, with no manipulation of the domicile except cotton plug removal, that the returning bee was greatly confused and demonstrated great difficulty in finding its correct nesting straw. This behavior contrasted sharply with its usual, direct

accurate entry.

When these cotton plugs were placed under an ultra violet light, they were found to be highly reflective.

Strikingly different results were obtained with the same bee when the above procedures were repeated using black cotton plugs. After these plugs were removed the returning bee demonstrated no noticeable deviations in behavior from that observed when plugs were in place.

Bees nesting in a greenhouse cage at Oregon State University during March 1966 were observed to aggregate on the side of their cage near an ultra violet lamp, even though other intense light sources were proximal to the cage.

These observations suggest that M. rotundata may be sensitive to ultraviolet wavelengths which are similar to the results of Kühn (1927) and later workers demonstrating ultraviolet sensitivity in honeybees.

Color Stimulus Strength

Color experiments with M. rotundata provided a method of manipulating certain entrance stimuli (color cards) and determining their relative stimulus strength for bees homing on the nest entrance. Conditioned females of M. rotundata normally home accurately on the exact position of nest entrances and usually enter without hesitation.

However, when objects of very strong stimulus strength associated with the entrance are displaced but still within their visual field, such as a yellow card, bees fly directly to the displaced object without regard to the former nest entrance position.

If objects providing strong stimuli are completely removed, e. g., the removal of cotton plugs with high ultraviolet reflection, bees are confused but search predominantly in the immediate area of the nest entrance. This behavioral reaction also was evident in the color studies in which the conditioning color card was completely removed from the test board. In these situations bees were observed to hover more often at the former training position than at any other. Therefore, it appears that when major orientation aids are no longer present, subordinate ones are sometimes sufficient to allow bees to home on their correct nest position. In other words, a hierarchy of stimuli are employed in orientation with the strength of the respective stimuli determining their placement in the hierarchy.

Another category of color stimuli designated as intermediate was here defined. This group was exemplified in the color experiments by blue, purple, orange, and green. When these colors were transferred to other portions of test domiciles, bees first hovered near the correct entrance position before flying to the position of the training color card. Upon removal of these color cards from the experimental situation, bees again exhibited confusion but generally

confined hovering movements to the area of the nest entrance. The stimulus strength of these colors was apparently greater than the stimulus provided by nest entrance position, but only slightly.

Figure Discrimination Studies

Initial figure discrimination studies were conducted using a four by eight foot sheet of plywood with four, twelve inch diameter figures affixed to it (Figure 7). Observations on bee behavior in these studies suggested that the position of the nest at the time of conditioning strongly influenced the behavior of returning bees. In other words, bees nesting at either of the two outer positions, irrespective of the figures associated with them, tended to return to an outer position during testing even though their nest-associated figures had been moved to an inner position.

This "edge effect" was dominant and bees conditioned to figures at the lateral positions hovered there for long periods before extending the range of their flight patterns to include middle positions. Upon extension of their flight patterns, entrances were usually made into the correct training figure (Table 20).

It appears that "edge effect" results from greater stimulus strength or "clues" being present at that position than is provided by figural configuration. This situation may prevail because outer positions on the test board are more readily defined from a distance.

Table 20. Figure studies using a four position test board

Training figure	Figure replacing training figure	Position of conditioning	Position of testing	Reaction to training figure		Number of observations
				positive	negative	
▲ *	Y	3	4	x		7
▲	I	3	2	x		3
Y	▲	4	3	x		4
Y	●	4	3	x		2
●	○ **	1	2	x		2
●	I	1	2	x		3
I	●	2	1	x		2
Y	I	4	2		x	1
○	●	1	3		x	1
▲	I	3	1	x		1
Y	●	4	1		x	1

* blackened figures painted entirely yellow

** lined figures perimeter only painted yellow, center neutral gray

In approaching the nesting site from a distance a homing bee may orient itself in relation to these strong stimuli for position even before differences between test figures are perceived.

Bees nesting in inner positions tended to be more flexible in their homing behavior. Instead of hovering before a given middle position on the test board for great periods of time, the bee generally moved more rapidly from one position to another. It may be that in returning to central positions on the test board bees were able to scan greater positions of the test area therefore appearing better oriented than those nesting in outer positions.

To overcome the "edge effect," a second sheet of plywood was positioned adjacent to the first, providing a surface four by sixteen feet on which nine figures were placed approximately 19 inches apart (Figure 8). Experiments on figure recognition were confined to bees nesting towards the middle of the series, where the stimulus effects of the board edges were minimized.

The results of these experiments appear in Table 21. The data show that bees were capable of distinguishing between figures and in only seven of 43 trials entered figures other than those to which they were conditioned.

It was observed in the above tests that when test figures were moved one position (i. e., 19 inches), the time required for the bee to find and enter was much less than moves of two (i. e., 38 inches)

Table 21. Figure studies using a nine position test board

Training figure	Figure replacing training figure	Position of conditioning	Position of testing	Reaction to training figure		Number of observations
				positive	negative	
● *	○ **	5	6	x		3
●	○	7	6	x		2
●	○	5	7	x		1
●	○	7	5	x		1
○	●	6	5	x		1
○	●	6	7	x		2
○	■	6	5	x		1
○	Y	6	3	x		1
△	▲	7	6	x		5
△	▲	7	5		x	2
△	▲	8	6	x		1
△	■	7	5	x		1
▲	■	6	7	x		1
▲		1	4		x	1
■ ***	▲ ***	2	3	x		2
■ ***	▲ ***	3	2	x		1
■ ***	▲ ***	7	6	x		1
■ ***	★	2	4	x		1
Y	+	2	3	x		2
Y	+	3	5		x	1
Y	+	3	1	x		1
Y		6	7	x		1
Y		3	4	x		1
	Y	4	3	x		1
	Y	8	7	x		1
+	+	3	2	x		1
+	+	2	3	x		1
+	●	6	5		x	1
+	●	6	4		x	1
+	■	6	7		x	1
+	■	6	8	x		2

* blackened figures painted entirely yellow

** lined figures perimeter only painted yellow, centers neutral gray

*** six inch diameter figures (all others 12 inches in diameter)

or three positions (i. e., 57 inches). Usually the foraging bee would return directly to the original position of her nesting figure on the board and, not finding it, would hover before the new figure now occupying its place. She would examine its contours and gradually increase her searching area until the correct figure was found. Except for the initial return to the original nesting position, the search pattern behavior of the test bees was highly variable.

Trial and error searching was quite evident when figure displacements were greater than 19 inches. Indeed, bees were often unable to find their displaced figures because their seemingly random searching did not take them to the area to which this conditioned figure had been moved.

It appeared from these observations that the distances between the figures was greater than the perception range of a bee hovering a few inches in front of the board and that both the six and 12 inch figures themselves may be beyond this field of vision. As indicated above, bees were often observed hovering at the margins of the figures as if orienting on a portion of the figure rather than the figure as a whole because of their inability to include the entire figure in their visual field. Nevertheless, homing females seemed to be responding to the entire figure, either by viewing it from distances as they approached the test board or possibly through summation upon closer examination of figure perimeters.

This visual range problem prompted construction of a smaller test board, 24 x 12 inches, on which figures three inches in diameter were placed (Figure 15). This test board size, 24 x 12 inches, was selected because, in the tests above, bees appeared to have little difficulty re-orienting on figures spaced 19 inches apart.

Using test boards of this type a series of experiments was conducted to determine if bees conditioned to cross-shaped figures could distinguish between them and triangles, circles and squares.

One group of trials (arrangement one, Figure 16) was conducted to determine if changing the location of conditioning crosses on test boards would influence subsequent figure selection by homing bees. The data for these studies (Table 22) indicates that bees were able to distinguish crosses from triangles, circles and squares and in 85.19% of the trials entered the crosses which had been moved from position II to III.

Table 22. Entrance data for figure discrimination studies (Arrangement one).

	Position I	Position II	Position III	Position IV	Totals
Triangle	1	2	24	0	27
Circle	2	4	22	0	28
Square	2	1	23	0	26
Totals	5	7	69	0	81

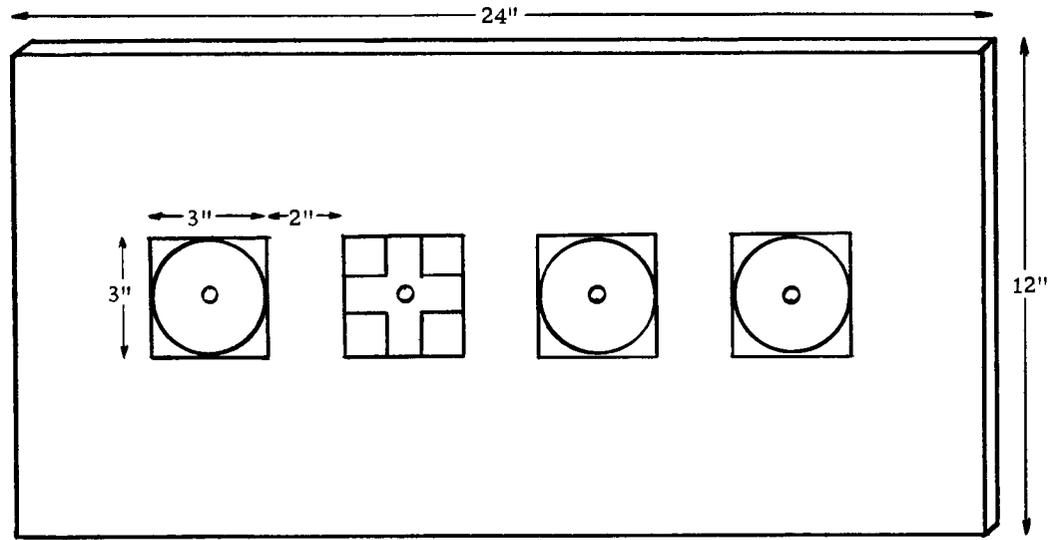
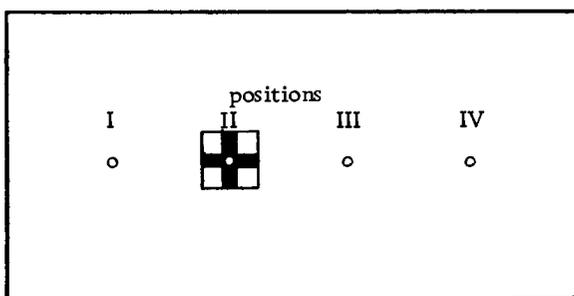
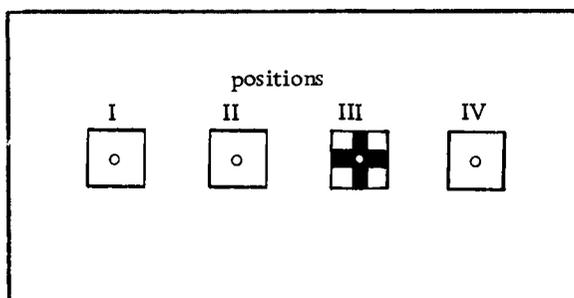


Figure 15. Domicile used for testing cross with other figures (triangles, circles and squares).

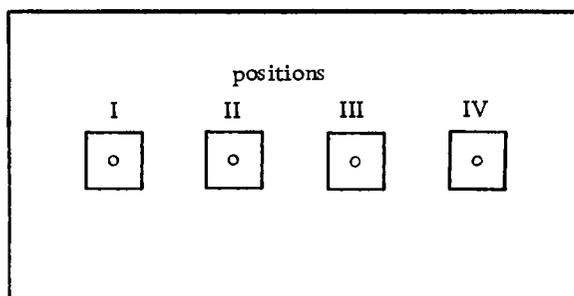


- a) Bee conditioned to cross card at position II.
- b) All other positions without cards.



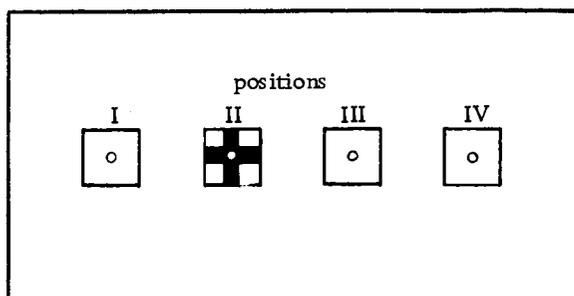
Arrangement one

- a) Cross card moved from position II to position III.
- b) Nesting straw removed and a new one put in its place.
- c) Figure card (same configuration as those at positions I and IV) placed at position II.



Arrangement two

- a) Cross card removed from test board.
- b) Figure card (same configuration as as those at positions I, II, IV) placed at position III.



Arrangement three

- a) Figure card placed at position II.
- b) Original nesting straw returned to position II.

Figure 16. Outline of figure experiment manipulations.

A second group of tests (arrangement two, Figure 16) was designed to observe the reactions of homing bees to situations in which conditioning crosses had been removed from the test board and only comparison figures (i. e., triangles, circles and squares) were present. Entrance data (Table 23) for these trials indicated that 59.26% of the total entrances were made at position II, the position at which bees were conditioned to the cross configuration.

Table 23. Entrance data for figure discrimination studies
(Arrangement two)

	Position I	Position II	Position III	Position IV	Totals
Triangle	3	19	5	0	27
Circle	6	14	6	2	28
Square	5	15	5	1	26
Totals	14	48	16	3	81

A chi-square analysis comparing total entrances for arrangement one (Table 22) vs. two (Table 23) shows their distributions to be significantly different ($P < 0.001$). It appears from the data that these differences in distribution may be due to the strong stimulus provided by the training cross when present (arrangement one) and the subsequent shift to positional orientation when it is absent.

A third series of trials (Arrangement three, Figure 16) was designed to reinforce conditioning to training crosses and to observe

any deviations in behavior which may have been attributed to preceding arrangements one and two. The entrance data obtained in these studies (Table 24) indicate strong orientation to conditioning crosses at position II (98.77%) and may represent the combined stimulus strengths of position as well as training figure indicated from data for arrangements one and two above.

Table 24. Entrance data for figure discrimination studies (Arrangement three).

	Position I	Position II	Position III	Position IV	Totals
Triangle	0	27	0	0	27
Circle	1	27	0	0	28
Square	0	26	0	0	26
Totals	1	80	0	0	81

Hovering data associated with entrances for arrangements one, two and three appear in Tables 25, 26 and 27 respectively. An analysis of variance on these data (Table 28) reveals that bee's hovering responses to these arrangements were significantly different ($P < 0.001$). The analysis also indicates no significant differences between the distributions of hoverings made by bees when testing with triangles, circles or squares. Nonsignificant interaction differences were obtained, suggesting that for each figural configuration tested against crosses, hovering means for arrangements one,

two and three were similarly distributed.

Table 25. Hovering data for figure discrimination studies
(Arrangement one).

	Position I	Position II	Position III	Position IV	Totals	Mean hoverings per entry
Triangle	15	61	96	12	184	6.81
Circle	17	48	80	11	156	5.57
Square	18	44	74	7	143	5.50
Totals	50	153	250	30	483	

Table 26. Hovering data for figure discrimination studies
(Arrangement two).

	Position I	Position II	Position III	Position IV	Totals	Mean hoverings per entry
Triangle	44	125	76	21	266	9.85
Circle	90	175	130	47	442	15.79
Square	63	150	104	32	349	13.42
Totals	197	450	310	100	1057	

Table 27. Hovering data for figure discrimination studies
(Arrangement three).

	Position I	Position II	Position III	Position IV	Totals	Mean hoverings per entry
Triangle	2	27	7	4	40	1.48
Circle	7	27	8	3	45	1.61
Square	5	24	3	1	33	1.27
Totals	14	78	18	8	118	

Table 28. Analysis of variance on transformed hovering data from
figure studies. (Transformation: $Y = \log_{10} (1+x)$)

	d.f.	S. O. S.	M. S.	F
Totals	242	39.1150		
Moves	2	13.9253	6.9626	65.3401 ***
Figures	2	0.0689	0.0343	0.3218 ^{n. s.}
Figures x moves	4	0.2547	0.0637	0.5975 ^{n. s.}
Residual	234	24.9351	0.1066	

*** $P < 0.001$

n. s. = $P > 0.05$

Mean hoverings per trial, Tables 25, 26 and 27, are plotted in Figure 17. The graph shows greater hovering means in arrangement two than for one and three. This situation probably occurred because conditioning crosses were absent in arrangement two.

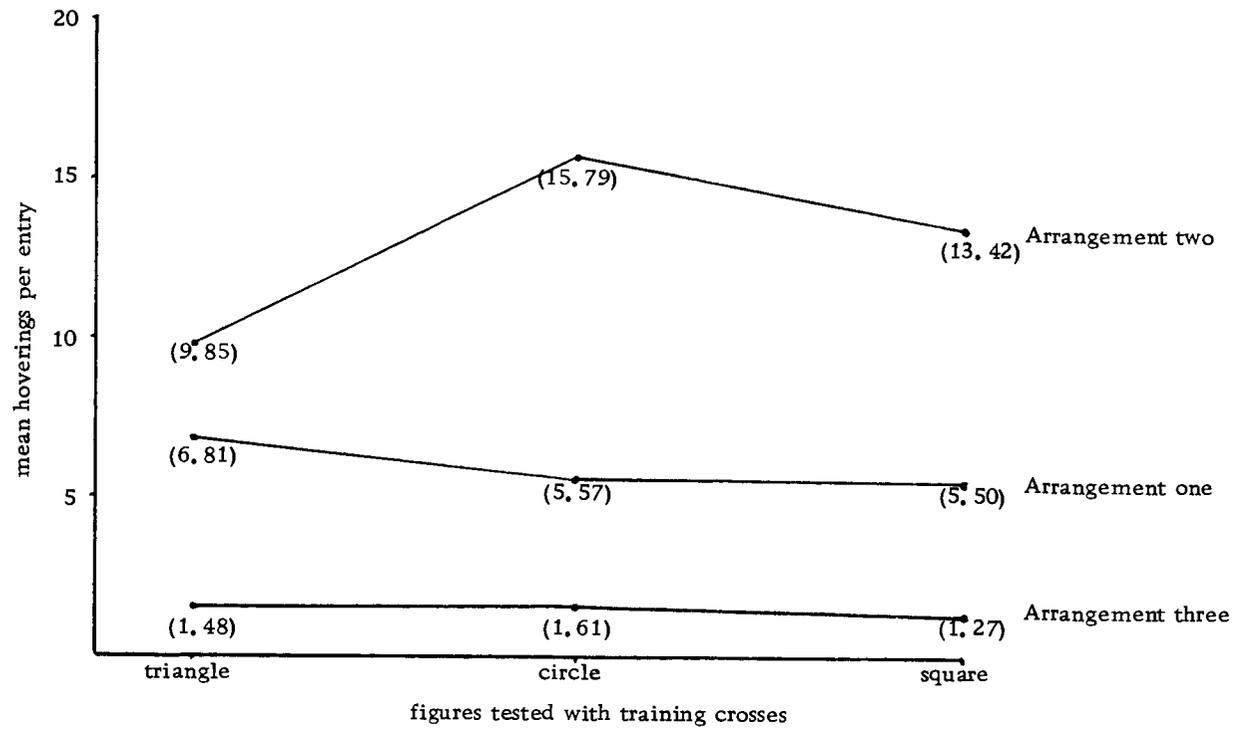


Figure 17. Mean hoverings per entry for figure discrimination studies. Exact means appear in parentheses.

Hovering means for arrangement one were higher than arrangement three because the relocation of training crosses from position II to III resulted in competition between stimuli associated with position and figural configuration. Perhaps, as suggested in previous figure studies, homing bees oriented to position at some distance from the test board and upon arrival at the position were confused by the displacement of their conditioning crosses.

Alighting data for arrangement one (Table 29) shows that 91.30% of the total alightings occurred at position III where crosses were affixed. Associated entrances (85.19%, Table 22) and hovering data totals (51.76%, Table 25) also indicate strong responses to crosses at position III. It appears from these data that, for arrangement one, bees centered their orientation activities in proximity to the conditioning cross at position III.

Table 29. Alighting data for figure discrimination studies (Arrangement one).

	Position I	Position II	Position III	Position IV	Totals	Mean alighting per entry
Triangle	0	6	40	0	46	1.70
Circle	1	1	30	0	32	1.14
Square	1	1	35	0	37	1.42
Totals	2	8	105	0	115	

Arrangement two alighting data presented in Table 30 shows 52.78% of the total alightings occurring at position II. Entrance data (Table 23) for this test arrangement show 59.26% and 42.57% of the entrances and hoverings respectively to have been made also at position II. These data show a tendency for homing bees to orient to position when the conditioning cross was not on the test board.

Table 30. Alighting data for figure discrimination studies (Arrangement two).

	Position I	Position II	Position III	Position IV	Totals	Mean alighting per entry
Triangle	1	16	4	0	21	0.78
Circle	4	11	8	3	26	0.93
Square	3	11	10	1	25	0.96
Totals	8	38	22	4	72	

Arrangement three entrance (Table 24) and hovering (Table 25) data show, as discussed separately above, strong orientation by homing bees to the conditioning position (position II) and training crosses affixed there. Only three alightings were recorded for this procedure which further attests to the accuracy of homing by the bees. It is believed that this accuracy is a result of the additive effects of complementary stimuli, correct position and correct figure.

It is apparent from the above studies that females of M. rotunda are able to distinguish between various figures associated with their nesting sites. After females had become conditioned to the landmark configurations associated with their nest entrance, short distance displacements of key stimuli were sufficient to cause abnormal homing behavior.

As would be expected, radical changes in the appearance of the immediate area of the nest entrance causes great orientation difficulties. For example, in some of the initial figure discrimination studies (Figure 15), bees were permitted to nest in straw positions two and three on the experimental boards. After they had become established, the training figure card was positioned over the nesting straw. Returning bees tended to avoid the immediate area of the nest entrance and searched instead at the other three positions on the boards, presumably because they provided characteristics similar to the original nest entrance.

After a bee had been nesting in a given straw with an associated figure for a few days, figures of different configurations could be placed on the board in the remaining positions without affecting the returning bee's behavior. This response was of interest since the closest margins of the figures on either side of the training figure were only three and one-half inches from the center of the bee's nesting straw. Apparently orientation stimulus provided by

the conditioning figure, and perhaps other landmarks, was sufficiently strong to override the effect of the additional surrounding figures.

It would appear from the above discussion that markers used by these bees immediately before entering their nesting hole are highly limited to those in the immediate area of the nest entrance and must be quite well defined.

Displacement Studies

As noted in the introduction, several attempts to relocate adults of M. rotundata in 1962 and 1963 resulted in high population losses. Of the 1111 bees moved in 1962, only 108 bees (9.7%) remained after the last introduction, and in 1963 only 210 of 1521 bees (13.8%) re-established at the new site. In both years, the bees and their domiciles were removed from under the eaves of barns and other dwellings, about each of which existed a multitude of prominent landmarks and an abundance of blooming alfalfa. In contrast, the relocation area was a vast sagebrush desert free of prominent landmarks and with relatively little alfalfa bloom. In addition, bees were housed in a shelter quite dissimilar in structure to the dwellings from which bee domiciles had been taken. This desert area was further characterized by having strong southwesterly winds which prevailed at the time of bee relocations described above.

A series of relocation experiments was designed in an attempt

to determine causes of the high population losses noted above.

The first two populations represented in Table 31 were conditioned to nesting habitats in which prominent landmarks (e. g. farm buildings) were present and the sun was visible during most of the day. Populations 3 and 4 were taken from a nesting site in a residential area shaded by a dense canopy of trees. Each of these populations was moved into similar alfalfa fields with few prominent landmarks and housed in shelters of the same type (Figure 1) at distances varying from 3.5 to 25 miles from the original nesting site.

Table 31. Relocations from dwellings to field stations.

Bee populations	# bees before move	# bees after move	% loss	Remarks	distance and direction of move
1	67	33	50.7	nesting in open area, sun and prominent landmarks	east 25 miles
2	40	17	57.5	"	east 5.5 miles
3	91	16	82.4	nesting under canopy of trees	southwest 4 miles
4	89	7	92.1	"	east 3.5 miles
5	116	19	83.6	high wind	east 4.5 miles
6	109	6	94.5	high wind	east 5.5 miles

A chi-square analysis indicates that losses in populations 1 and 2 (Table 31) were significantly lower ($P < 0.005$) than losses in populations 3 and 4. While losses in all of these populations exceeded 50 per cent, it is felt that populations 3 and 4 were more completely dependent upon landmarks prior to relocation since they had previously nested in dense shade. The magnitude of change from this habitat into an open field would seemingly be greater than that experienced by populations 1 and 2 and may possibly account for the greater losses observed. If a sun compass is operative in M. roundata, then it is possible that populations 1 and 2 would be better able to reorient in the new environment since they had full view of the sun at their former nesting site. [It has been shown that for the sun compass to be operative in the honeybee, novice bees must learn the course of the sun through visual observation (von Frisch, 1967).] However, it is difficult to believe that a sun compass explanation is probable since losses were high in both populations (50.7 and 57.5 per cent respectively). This is not meant to imply that a sun compass does not exist. It may be that orientation stimuli provided by landmarks at the original nesting site were strong and therefore used to the exclusion of the sun compass. Von Frisch and Lindauer (1954) report that prominent landmarks may be of such stimulus strength that they override the use of sun compass orientation in the honeybee.

The losses as a result of relocating populations 1 to 4 (Table 31) suggest that at least part of the difficulty experienced in 1962 and 1963 was a result of the strong conditioning of the bees to landmarks at their former nesting areas and an apparent inability to break this conditioning when moved into a new area.

Populations 5 and 6 (Table 31) were moved from the same nesting locality as populations 1 and 2 but to different alfalfa fields. Strong winds (approximately 30 m. p. h.) on the day following these moves were believed responsible for high population losses observed (83.6 and 94.5 per cent respectively). These losses were significantly greater (chi-square $P < 0.005$) than those recorded when populations from the same nesting site were moved on relatively calm days.

Since high winds also occurred during the 1962 and 1963 moves, these results indicate that wind may have been a contributing factor in population losses.

It is interesting that those populations nesting in shaded conditions (populations 3 and 4, Table 31) yielded nonsignificant chi-square values when compared with population relocations conducted during windy weather. It may be that the two situations, disruption of landmark conditioning and wind, are equatable in terms of disorientation effects when bees are relocated.

Another series of experiments was conducted to determine if

bee populations conditioned to field shelters painted different colors would experience losses similar to those in unpainted shelters when moved within their shelters into new fields. The fields to which the bees were to be moved were carefully chosen for their similarities with those accommodating the original nesting sites. It was assumed that painted boxes would be visible from greater distances and provide stronger orientation stimuli for bees than unpainted shelters when population relocations were made. The results of these population relocations are cited in Table 32. A chi-square analysis indicates that population losses were similar in painted and unpainted shelters.

Since painted shelters did not provide any greater orientation assistance in the relocations, it may be that stimulus provided by the shelter itself was sufficient to assist bee reorientation at the new site. Because original and relocation sites were chosen for the absence of prominent landmarks, the shelters were the only major landmarks present.

Population losses as a result of these relocations (Table 32, populations 1 through 5) were significantly lower ($P < 0.005$) than those recorded in populations 1 through 4 in Table 26. The relocations recorded in Table 26 were made between greatly dissimilar areas and populations were moved from the eaves of buildings into small field stations. However, populations represented in Table 32

were moved with their respective field shelters into areas quite similar to those at the original nesting site. It appears from these comparisons that bees are strongly conditioned to landmarks about their nesting sites and that high population losses result when they are moved into a greatly dissimilar area.

Table 32. Relocations from one field to another.

Bee Populations	# bees before move	# bees after move	% loss	Remarks	distance and direction of move
1	52	43	17.3	Shelter unpainted	East 4 miles
2	26	24	7.7	Shelter unpainted	East 4 miles
3	106	90	15.1	Shelter painted (turquoise)	North 10 miles
4	20	18	10.0	Shelter painted (blue)	South 4 miles
5	28	20	28.6	Shelter painted (yellow)	South 4 miles
6	73	12	83.6	Little forage near area	South-south-west 4 miles
7	86	21	75.6	"	South-south-west 8.5 miles

Populations 6 and 7 (Table 32) were moved, in their respective field shelters, from large alfalfa fields to small alfalfa plots with little available bloom. Prominent landmarks were visible on

the horizons at the original nesting places but not in the relocation areas. However, it is believed that minimal amounts of bloom in the new area was the major contributing factor in high population losses observed.

A series of experiments was designed to determine if population losses would occur as a result of relatively short moves (100 yards) within the same alfalfa field and to determine to what extent painting the shelters affected losses under these circumstances.

The results of four relocations are recorded in Table 33, using unpainted shelters. Populations 1 and 2 were moved within one field and populations 3 and 4 were relocated within a second. All relocations were conducted on different days.

Table 33. Relocations within the same field (shelters unpainted).

# bees before move	# bees after move	% loss	Remarks
37	21	43.2	100 yards east of original site
21	13	38.1	100 yards north of original site
83	63	24.1	100 yards north of original site
110	67	39.1	100 yards north of original site

Relocation data using colored shelters appear in Table 34.

Populations 1 and 2 (Table 34) were moved within a third field and populations 3 and 4 in a fourth, all on different days.

Table 34. Relocations within the same field (shelters painted yellow).

# bees before move	# bees after move	% loss	Remarks
99	70	29.3	moved 100 yards west of original position
79	70	11.4	moved 100 yards north of original position
60	52	13.3	moved 100 yards north of original position
62	51	17.7	moved 100 yards northeast of original position

Populations associated with color (yellow) marked shelters (Table 34) had significantly less loss (chi-square $P < 0.005$) than those in shelters not color marked (Table 33).

It appears that color may have assisted bees either in reorienting to or relocating their displaced shelters. Since these relocations were only 100 yards from the original nesting sites, bees may have been familiar with the entire area in which each move was made. They may have flown from their relocated domiciles, without reorienting, and returned to the original nesting site,

possibly guided by characteristics on the horizon. Not finding the shelter there, bees may have searched the area with more of them being attracted to the colored boxes because of the color stimulus provided.

An alternative explanation is possible. Because of their normally short flight range when forage is abundant, the bees may have been unfamiliar with the new area about their shelters. At the new site more bees were able to return to their relocated domiciles because of the additional stimulus provided by colored shelters.

Another series of experiments was designed to determine if two populations moved progressively closer together would retain their fidelity to painted and unpainted shelters. Three types of moves were conducted, one in which both shelters were unpainted (Table 35), two with one shelter unpainted and the other painted (Tables 36 and 37), and one with both shelters painted different colors (Table 38).

Interchange between populations nesting in unpainted shelters did not occur until the shelters were 20 yards apart (Table 35). However, unaccountable population losses occurred, especially from domicile one. The total loss from domicile one (30.6%) is significantly greater (chi-square $P < 0.005$) than that from domicile two (9.2%). No adequate explanation can be given for the

differential loss since both shelters and bee populations were believed to be comparable. Population losses and interchanges, although few, were greater than those recorded for similar progressive move studies in which one or both of the shelters were painted (Tables 36-38).

Table 35. Progressive move studies (both shelters unpainted)

Distance between domiciles (yards)	# bees Domicile 1	# bees Domicile 2	Interchange	
			# incorrect bees in Domicile 1	# incorrect bees in Domicile 2
100	72	87	0	0
60	67	86	0	0
20	56	84	1	3
< 1	50	79	5	2

Table 36. Progressive move studies (one shelter painted and one unpainted)

Distance between domiciles (yards)	# bees		Interchange
	Yellow shelter	Unpainted shelter	
100	27	55	0
60	27	88	0
20	27	94	0
< 1	22	91	0

Table 37. Progressive move studies (one shelter painted and the other unpainted).

Distance between shelters (yards)	# bees in unpainted shelter		# bees in painted shelter (turquoise)	
	# bees in correct shelter	# bees in incorrect shelter	# bees in correct shelter	# bees in incorrect shelter
100	18	0	21	0
60	18	0	21	0
20	18	0	21	0
<1	17	1	20	0
10 yards domiciles interchanged	8	5	15	9
10 yards domiciles returned to correct shelter	15	3	17	2

Data in Table 36 indicate that all marked bees remained with their respective shelters when one shelter was painted (fluorescent yellow = Sherwin William's Solar Yellow) and the other was unpainted. It can be seen from the table that the population nesting in the unpainted shelter increased in size while the other maintained a relatively constant number of individuals. The population increase was due to the influx of bees from a large adjacent nesting population. No interchange between populations was observed.

The test was repeated using one shelter painted turquoise and the other unpainted. There was no bee loss and the only interchange occurred when the domiciles were < 1 yard apart (Table 37). The evening after the shelters were adjacent to one another (< 1 yard) each was moved five yards (10 yards apart) and domiciles containing sleeping females were interchanged. This manipulation was designed to determine if the bees would remain faithful to their original domiciles or to their shelters and to determine the extent to which searching occurred.

On the night following domicile interchange it was found that, while not significant at the 5% level (chi-square), 15 of 20 bees originally nesting in the painted shelter (turquoise) and 8 of 17 in the unpainted shelter returned to the wrong domicile but to the original shelter.

The domiciles and sleeping bees were returned that evening to shelters in which they were originally positioned. On the following day most bees returned to their correct shelters now possessing their original domiciles.

The data obtained from an experiment similar to the preceding one (Table 37) but in which both shelters were painted, one yellow and the other blue is presented in Table 38. The data show that no interchange occurred between populations even when shelters were positioned adjacent to one another (< 1 yard).

Table 38. Progressive move studies (both shelters painted).

Distance between shelters (yards)	# bees in painted shelter (blue)		# bees in painted shelter (yellow)	
	# bees in correct shelter	# bees in incorrect shelter	# bees in correct shelter	# bees in incorrect shelter
100	49	0	48	0
60	49	0	48	0
20	49	0	48	0
<1	48	0	43	0
10 yards domiciles interchanged	44	3	36	0
10 yards domiciles returned to correct shelter	40	0	39	3

When domiciles were interchanged all but three bees returned to the shelters to which they were conditioned. Upon returning the domiciles to their correct shelters all but three bees returned again to their correct shelters and domiciles.

A chi-square analysis comparing the results of domicile interchanges performed when one shelter was painted and one was not (Table 37) shows significantly greater interchange ($P < 0.005$) than for similar data when both shelters were painted (Table 38). It may

be that the distinction between the two shelters was much greater when both were painted and thus provided a stronger orientation stimulus for both resident populations.

GENERAL DISCUSSION

Orientational Classification

Although there are numerous studies on the orientation of solitary wasps and social bees, little has been done with the solitary bees. Recently Chmurzynski (1964) proposed a classification of the stages of orientation based on the position of the homing insect in relation to its nest. He considered the following stages to be distinctive:

1. distant orientation - locating the general area of the nest
2. proximate orientation - recognition of the nest vicinity
3. immediate orientation - finding the nest entrance
4. recognition of the nest
5. orientation inside the nest

Recognizing that the categorization of biological phenomena is highly subjective, this proposed classification is useful in attempting to systematically characterize homing flight behavior. It appears that the behavior of M. rotundata can be categorized in a manner consistent with at least some of the stages in the above classification.

The sun orientation experiments and observations by Stephen (1967) suggest that the maximum flight range for distance orientation in M. rotundata is usually between one-quarter and one-half mile. This is only an estimate since the flight range may be influenced by

the position of the nest relative to the location of forage, age and experience of bees, abundance of forage, landmarks, and a host of other factors. While the maximum flight range may be at some distance from the nesting site, under normal field conditions, where an abundance of bloom is present, this species tends to utilize forage closest to the nesting site before extending its range. Since most populations of M. rotundata are found in association with alfalfa fields, the stage of orientation designated above as distant is usually within 400 yards of the nesting site. It is believed that landmark clues within the distant orientation region are most important and guide returning bees to within a few yards of the nesting site.

At this point, proximate orientation clues appear to become functional such as characteristics of structures housing nesting domiciles and possibly relative domicile position in or on these structures (e. g., domicile positioning under the eaves of dwellings).

The importance of distant and proximate orientation was demonstrated in displacement studies in which bees and their domiciles were relocated and placed in distinctly different shelters in surrounding areas differing from their former nesting places (Table 31). The high population losses observed in these relocations were believed due to radical changes in distant and proximate orientation clues in the two areas and the inability of the bees to break conditioning to the former clues.

The role of proximate orientation clues was also demonstrated in the progressive move studies (Tables 37 and 38). In these studies, domiciles were exchanged between two shelters positioned ten yards apart and differing only in their color. This presented a situation in which proximate clues (shelter color) were changed while distant orientation remained the same. The results suggest, especially when both shelters were painted (Table 38), that the strong stimulus provided by shelter color was sufficient to guide bees to, and cause them to remain in, incorrect domiciles.

When displacement studies were conducted by moving bee populations and their shelters from one field lacking prominent background markers to another of similar background pattern, losses were greatly reduced (Table 32). It was apparent that differences between the two locations were not sufficient to greatly disturb bee orientation. In these experiments, the shelters themselves may have acted as distant and proximate markers or if bees foraged great distances, sun orientation may have been predominantly used in distant orientation.

It is apparent from the above experiments that distant and proximate orientation clues are of paramount importance in guiding bees into the immediate areas of their nests.

The broader categories (distant and proximate) of Chmurzynski's homing classification are poorly defined for most

hymenopterans because of the difficulties in continuously observing their flight when away from their nests. However, observations made on behavior in the immediate area of nesting sites are more readily obtainable.

Malyshev (as cited by Chmurzynski, 1964) found that if nest entrances of Osmia rufa (L.) were moved as little as two centimeters these bees hovered at the former position of the entrances before going to the new entrance positions. Kathariner (as cited by Chmurzynski, 1964) demonstrated that when rotating a honeybee hive 45°, bees hovered at the former entrance location before entering at the repositioned entrance. A rotation of 90° greatly confused returning bees and resulted in large numbers hovering at the former entrance position. Observations on immediate orientation similar to those cited above have been recorded for M. rotundata. When a nesting domicile was moved as little as 12 inches under the eaves of a barn, bees hovered at the original position for long periods of time before locating their domicile. Apparently they had been led to the original domicile position by proximate clues which provided an irreversible fix on the position which they were unable to overcome.

When nesting in drinking straw domiciles, it was found that displacing a nesting straw as little as one centimeter was sufficient to cause disorientation. While it is true that these domiciles present some orientation problems due to the large number of similar nesting

straws (250 and more), these bees are surprisingly accurate in finding their own nesting tunnels.

It is evident from the above discussion that recognition of the nest entrance position is extremely well defined in M. rotundata.

It seems more appropriate for this study to integrate Chmurzynski's categories of recognition of the nest and orientation inside the nest since they are believed to be intrinsically related in M. rotundata.

Orientation inside the nest was studied in 1962-63 (Osgood, 1964). In these experiments, various portions of the nesting straw or contents were removed, exchanged or rearranged and behavioral reactions of the returning bee to the modification were recorded. Although possible olfactory stimuli were not researched it was found that tactile stimuli were important to nest acceptance. Slight disruptions in nesting cells were sufficient to cause bees to react abnormally and reject the nest.

Management Recommendations

Relocating adult bees. It is recognized that the best practice to insure maximum population increase is not to move adult populations of M. rotundata. However, if such procedures become necessary, the following recommendations should help to minimize population losses.

As evidenced from the above studies, adult bees should always be relocated "en masse" at night, that is with shelter, domiciles and respective bee populations. When moves are to be made over short distances (20-100 yards) painted shelters appeared to assist bees in orienting to the new location.

Long distance relocations (one mile or more) may become necessary, if alfalfa bloom is lost or if alfalfa is cut in rotation, to better utilize pollinators. It is important that background markers in the new area be similar to those at the original site. However, since most moves of this type are generally made from one open alfalfa field to another a minimum number of prominent landmarks usually exists. It would appear to be a good practice to position shelters in the new area so that their openings face the same compass directions in relation to the alfalfa fields as were present in the former area. If a number of shelters are to be moved into the same field it is important that their relative position to one another be maintained in distance as well as their associated color sequence. One method of accomplishing these moves would be to construct movable shelters. Being mobile these shelters and respective populations could more easily be moved as a unit.

One alfalfa seed grower in eastern Oregon has mounted his nesting domiciles on a farm wagon. This apparatus enables the farmer to remove his bee population before insecticide applications

as well as better utilize his bees for pollination if they are needed in other fields.

Moves should be conducted only when strong winds do not prevail since population losses were extremely high under these conditions.

Orientation aids. It is evident from these studies that M. rotundata can perceive certain colors as well as distinguish among various figures. Of the colors, yellow appears to provide the greatest stimulus strength but blue, orange, green and purple also were distinguished. Although red appeared to be similar to black, it may also assist as an orientation aid when used in combination with the above colors if black is not being used.

Among the figures tested, crosses were found to be readily distinguished from circles, squares and triangles. Many figures painted only at the perimeters appeared to be differentiated from those with their entire surfaces painted one color. Combinations of different colors and figures may greatly assist bees in orienting to their nesting sites, especially when populations and numbers of nesting holes are large.

The importance of these orientation aids cannot be overemphasized. Commercial nesting boards each with 2,000 holes, present special orientation problems due to their large surface area and the

uniform spacing of nesting tunnels. Bees nesting in these domiciles experience great difficulty in locating their correct nesting holes. Unfortunately, many farmers interpret this disordered behavior and its concomitant flight activity as an indication of population size when in reality he is viewing the inefficiency of his management practices.

It is common practice to construct field stations containing 50-100 nesting boards. This situation compounds the orientation problem mentioned above due to the unbroken pattern of nesting holes presented. A more desirable arrangement would be to leave spaces between these boards or perhaps alternate straw domiciles with nesting boards to assist bees in reorientation.

Small figures of various design and colors or even colors painted in irregular designs on nesting boards can assist bee orientation in small shelters containing few nesting boards. Figures of small size (e. g., 3 inches) are desirable since they appear to be more easily seen in their entirety and would more effectively define the location of a given nesting tunnel.

From an orientation standpoint, small shelters (i. e., <10 boards) would tend to minimize homing difficulties experienced by bees and promote efficiency. However, the economics of shelter construction and maintenance often dictate a reduction in the number of field stations with larger populations of bees nesting in each. Where large shelters are used, painting of nesting boards in

vertical groups should reduce lateral drift of homing bees and assist them in orienting to the general area of their nests. Perhaps figures of various sizes and configurations superimposed on the painted surfaces of the nesting boards would further assist bees in finding their correct tunnel entrances.

When many field stations are located in a single field it is desirable to paint each of them a different color. This procedure assists bees in finding their nesting domiciles when returning from great distances.

SUMMARY AND CONCLUSIONS

Sun orientation studies indicate that a sun compass may exist in Megachile rotundata. However, further studies will be necessary before definite conclusions can be made.

It was demonstrated that females of M. rotundata can distinguish various colors. Orange, yellow, green, blue and purple gave positive responses by color conditioned bees when tested against a background consisting of 22 shades of gray, black and white. In these tests bees responded negatively to red.

Yellow appeared to provide a stronger orientation stimulus than any other color tested. Upon returning from the field, bees conditioned to yellow flew directly to yellow cards regardless of their position on the test surfaces. In contrast, bees conditioned to other colors (i. e., orange, green, blue and purple) flew first to the former position of their respective color cards before reorienting and flying to their position of relocation.

These data suggest that bees are conditioned to color as well as to the position of their nests on test boards. Yellow, because of its strong stimulus effect, apparently overrode the conditioned stimuli associated with position and directed homing bees to it. However, the other colors providing weaker color stimuli than yellow were unable to compete with landmarks associated with position. Homing

bees in these situations flew first to the former position of the color card but in the absence of the conditioned color stimulus, reoriented in search of the color card.

A second series of color experiments demonstrated that M. rotundata may be color blind to red as was suggested by the color experiments above. In these experiments the homing bee was tested against three shades of gray and black. It was apparent from the data that she was able to distinguish red from the three shades of gray but had great difficulty discriminating red from black.

Bees were capable of distinguishing blue from all the shades of gray and black tested. These data further supported the results of the first series of color experiments in which blue appeared to act as an intermediate stimulus color for M. rotundata.

Preliminary observations also indicate that M. rotundata is sensitive to ultraviolet light. When white cotton plugs were removed from straws in a color testing domicile, homing test bees appeared to be greatly disoriented. These cotton plugs were found to be highly reflective in the ultraviolet wavelengths. The same manipulations, but using nonultraviolet reflecting black yarn for plugs, caused no disorientation when removed from the straws. Apparently the bee was using the pattern of ultraviolet reflections to orient to its nesting straw.

Figure studies indicated that females of M. rotundata are able

to perceive differences in form. Among those figures tested and distinguished by bees were squares, circles, crosses, triangles, stars, Y and I configurations. Initial studies were conducted by conditioning bees to certain figures and then exchanging these figures with others of different configuration on the test board. Although homing bees had varying degrees of difficulty locating the displaced figures to which they had been conditioned, they usually found and entered the correct figures.

Again, as in color experiments, bees appeared to be strongly position oriented and usually returned to the former nesting positions before increasing their searching flight range to include the new positions of their nesting figures. Figures used in these initial studies were believed too large (i. e., six and 12 inch maximum diameters) and their distances apart too great (i. e., 19 inches) to be encompassed in visual fields of homing bees when near the test board.

A second series of experiments was conducted using three inch figures positioned five inches apart, in which bees were conditioned to cross figures. It was found in subsequent tests that bees were able to distinguish crosses from circles, squares and triangles.

A number of adult population relocations were made. It was found that when moving bees from the eaves of various dwellings

into field stations high losses occurred. These losses were attributed to strong landmark conditioning at the old nesting site which bees were unable to break in order to reorient to the new nesting sites.

Losses were low when bees conditioned to nesting in field shelters were subsequently relocated with their shelters into other fields with characteristics similar to those of original sites. Population losses were low, but similar when using either painted or unpainted shelters. Apparently background markers at the original and relocation areas were similar enough so that major disorientation did not occur.

When relocations were made within the same field (i. e., 100 yards) population losses were significantly greater in those nesting in unpainted shelters than in painted ones (yellow). It appeared that bees may have been confused by the relocation of their shelters. Since the surrounding area was familiar to them they may have foraged and attempted to return to the original location of the nesting shelter. Color conditioned bees may have been assisted in returning to their shelters by the color stimulus provided.

Relocation studies were also conducted in which two shelters were moved progressively closer together in the same field. It was found that more interchange occurred when both shelters were unpainted than when one or both shelters were painted.

After completing the above progressive moves it was found, in those studies with both shelters painted (i. e., one yellow, one blue) that if they were moved ten yards apart and their domiciles exchanged, bees returned to the shelter to which they were conditioned even though it contained the wrong domiciles.

Population losses were also high when bees were moved from fields with abundant bloom into others with little forage. Strong winds also contribute to high population losses when relocating bees.

It appeared from these relocation studies that the requirements necessary to provide minimal population losses when relocating adult bees are: moving them and their respective domiciles and shelters into areas having a landmark composition similar to that of the original nesting sites; conditioning bees to color painted shelters before moves, especially for short relocations (i. e., 20-100 yards); reestablishing them in areas containing ample forage; and moving bees only when strong winds do not prevail.

General orientation was characterized for M. rotundata. Although females usually forage within 400 yards of the nesting site when bloom is abundant (e. g., alfalfa field), observations suggest that they may have a maximum flight range of between one-quarter and one-half mile. Obviously flight range is dependent on local conditions near the nesting site. When homing bees returned from

the field general landmarks appeared to guide them to the immediate area of their nests, at which time patterns of smaller landmarks served to define nest entrances more precisely. Once close to the nesting site slight variations in the position of the nest entrance were sufficient to cause deviations from normal flight behavior. Homing flight patterns were also disrupted when landmarks (e. g., color cards or figures) were displaced in relation to the nest entrance. It was evident that certain landmarks had greater stimulus value than others.

From the data obtained, bee management recommendations were made concerning methods of relocating adult populations and the use of figures and color as orientation aids.

It was suggested that short moves (e. g., between adjacent fields) could best be accomplished in progressive steps by moving domiciles and shelters 20-100 yards each day. Using this method, bees are gradually conditioned to changing surroundings, thus minimizing losses. Painted shelters were also found to reduce population losses in short moves.

For long moves (e. g., over one mile) it is important that characteristics of the new site be similar to those of the original nesting area. Bees should always be moved with their associated shelters and if a number of shelters are moved from one field to another their relative positions should be maintained. With

multiple shelter moves differential painting should assist bee re-orientation at the new site.

Orientation aids consisting of colors and figures found to be distinguished by M. rotundata are of invaluable assistance when used at nesting sites to better delineate the positions of nesting tunnels for homing bees. In most commercially produced nesting media bees are confronted with hundreds of similar nesting tunnels and experience great difficulty returning to their correct nesting tunnels. It is believed that these difficulties can be largely circumvented using appropriate figure and color schemes to assist bee orientation.

BIBLIOGRAPHY

- Baerends, G. P. 1941. Fortpflanzungsverhalten und Orientierung der Grabwespe Ammophila campestris Jur. Tijdschrift voor Entomologie 84:68-275.
- _____. 1959. Ethological studies of insect behavior. Annual Review of Entomology 4:207-234.
- Beusekom, G. van. 1948. Some experiments on the optical orientation in Philanthus triangulum Fabr. Behaviour 1:195-225.
- Bohart, G. E. 1963. How to manage the alfalfa leaf-cutting bee (Megachile rotundata Fabr.) for alfalfa pollination. Logan, 7 p. (Utah. Agricultural Experiment Station. Circular 144).
- Burkhardt, Dietrich. 1964. Colour discrimination in insects. In: Advances in Insect Physiology. Vol. 2. New York, Academic Press. p. 131-173.
- Chmurzynski, J. A. 1963. The stages in the spacial orientation of the female digger wasp Bembix rostrata (L). Animal Behavior 11:607-608.
- _____. 1964. Spatial orientation in flying Hymenoptera. Przegląd Zoologiczny 8:119-137.
- Daumer, K. 1956. Reizmetrische Untersuchung des Farbensehens der Biene. Zeitschrift für vergleichende Physiologie 38:413-478.
- Evans, H. E. 1966. The behavior patterns of solitary wasps. Annual Review of Entomology 11:123-154.
- Frisch, K. von. 1915. Der Farbensinn und Formensinn der Biene. Zoologische Jahrbücher, Abteilung für allgemeine Zoologie und Physiologie 35:1-182.
- _____. 1948. Gelöste und ungelöste Rätsel der Bienen-sprache. Die Naturwissenschaften 35:38-43.
- _____. 1949. Die polarisation des Himmelslichtes als orientierender Faktor bei den Tänzen der Bienen. Experientia 5:142-148.

- Frisch, K. von. 1950. Bees, their vision, chemical senses and language. Ithaca, New York, Cornell University. 118 p.
- _____. 1967. The dance language and orientation of bees. Cambridge, Harvard University Press. 566 p.
- Frisch, K. von. and M. Lindauer. 1954. Himmel und Erde in Konkurrenz bei der Orientierung der Bienen. *Naturwissenschaften* 41:245-253.
- Geigy, J. R., and A. G. Basel. 1962. Scientific tables. 6th ed. Ardsley, New York, Geigy Pharmaceuticals. 778 p.
- Goldsmith, Timothy H. 1961. The color vision of insects. In: A symposium on light and life, ed. by William D. McElroy and Bentley Glass. Baltimore, Johns Hopkins Press. p. 771-794.
- Hertz, Mathilde. 1929. Die Organisation des optischen Felder bei der Biene. I. *Zeitschrift für vergleichende Physiologie* 8: 693-748.
- _____. 1939. New experiments on colour vision in bees. *Journal of Experimental Biology* 16:1-8.
- Hobbs, G. A. 1967. Domestication of alfalfa leaf-cutter bees. Ottawa. 19 p. (Canada. Department of Agriculture. Publication 1313).
- Hurd, Paul D., Jr. 1967. The identity of Megachile rotundata (Fabricius) and M. argentata (Fabricius) (Hymenoptera: Apoidea). *Entomologiske Meddelelser* 35:3-10.
- Iersel, J. J. A. van. 1952. On the orientation of Bembix rostrata L. In: Transactions of the Ninth International Congress of Entomology, Amsterdam, August 17-21, 1951. Vol. 1. Amsterdam. p. 384-393.
- Iersal, J. J. A. van, and J. van den Assem. 1964. Aspects of orientation in the diggerwasp Bembix rostrata. In: Learning and associated phenomena in invertebrates; Proceedings of a Conference, Cambridge, England, July 1964. London. p. 145-162. (Animal Behaviour Supplement).
- Jacobs-Jessen, Una F. 1959. Zur Orientierung der Hummeln und einiger anderer Hymenopteren. *Zeitschrift für vergleichende*

Physiologie 41:597-641.

Jander, R. 1963. Grundleistungen der Licht-und Schwereorientierung von Insekten. Zeitschrift für vergleichende Physiologie 47:381-430.

_____. 1963. Insect orientation. Annual Review of Entomology 8:95-114.

Kornerup, A., and J. H. Wanscher. 1962. Reinhold color atlas. New York, Reinhold. 224 p.

Kühn, A. 1927. Über den Farbensinn der Bienen. Zeitschrift für vergleichende Physiologie 5:762-800.

Kunze, P. 1961. Untersuchung des Bewegungssehens fixiert fliegender Bienen. Zeitschrift für vergleichende Physiologie 44:656-684.

Lindauer, Martin. 1961. Communication among social bees. Cambridge, Harvard University Press, 1961. 143 p. (Harvard Books in Biology. No. 2).

Linsley, E. Gorton. 1958. The ecology of solitary bees. Hilgardia 27:543-599.

Lubbock, Sir J. 1882. Ants, bees and wasps, a record of observations on the habits of the social Hymenoptera. New York, D. Appleton and Company. 448 p.

Michener, C. D. 1955. Apoidea. In: A century of progress in the natural sciences, 1853-1953. San Francisco, California Academy of Sciences. p. 575-578.

Osgood, Charles E. 1964. Foraging and nesting behavior of the leafcutter bee Megachile rotundata (Fabricius). Master's thesis. Corvallis, Oregon State University. 104 numb. leaves.

Schricker, B., and W. P. Stephen. 1968. Factors influencing orientation of the wasp Steniolia scolopacea albicantia Parker to its sleeping plant. (Manuscript)

Stephen, William P. 1962. Propagation of the leaf-cutter bee for alfalfa seed production. Corvallis. 16 p. (Oregon.

Agricultural Experiment Station. Bulletin 586).

Stephen, William P. 1965. Temperature effects on the development and multiple generations in the alkali bee, Nomia melanderi Cockerell. Entomologia Experimentalis et Applicata 8:228-240.

_____. 1966. Native bees - an untapped pollinator resource. In: Second International Symposium on Pollination Southhall, Middlesex, England. 1966. p. 191-194. (Bee World. Vol. 47. Supplement)

_____. 1967. Professor, Oregon State University, Dept. of Entomology. Personal communication. Corvallis.

Stephen, W. P., G. E. Bohart and P. F. Torchio. 1968. Bees of northwestern America. (Manuscript)

Tinbergen, N. 1932. Über die Orientierung der Bienenwolfes (Philanthus triangulum). Zeitschrift für vergleichende Physiologie 16:305-334.

_____. 1935. Über die Orientierung des Bienenwolfes. II. Die Bienejagd. Zeitschrift für vergleichende Physiologie 21:699-716.

Tinbergen, N. and W. Kruyt. 1938. Über die Orientierung des Bienenwolfes. III. Die Bevorzugung bestimmter Wegmarken. Zeitschrift für vergleichende Physiologie 25:292-234.

Tinbergen, N. and R. J. van der Linde. 1938. Über die Orientierung des Bienenwolfes (Philanthus triangulum Fabr.). IV. Heimflug aus unbekanntem Gebiet. Biologisches Zentrablatt. 58:425-435.

Tsuneki, Katsuji. 1950. Experimental analysis of sensory cues working in the return to the nest of the Pompilidae (solitary Hymenoptera). Annotationes zoologicae japonenses 23:75-84.

_____. 1961. Colour vision and figure discriminating capacity of the solitary diplopterous wasp, Odynerus frauenfeldi Saussure. Memoirs of the Faculty of Liberal Arts, Fukui University, ser. II, Natural Science 11:103-160.

U. S. Department of Agriculture. Statistical Reporting Service. 1961. Seed crops by states, 1954-59. Washington, D. C. 31 p. (Statistical Bulletin 288)

_____. Statistical Reporting Service. 1966. Seed crops by states, 1959-64. Washington, D. C. 22 p. (Statistical Bulletin 385)

Weiss, Harry B. 1943. Color perception in insects. *Journal of Economic Entomology* 36:1-17.

Wolf, E. 1927. Über des Heimkehrvermögen der Bienen. II. *Zeitschrift für vergleichende Physiologie* 6:221-254.

Wolf, E. and G. Zerrahn-Wolf. 1936. Flicker and the reactions of bees to flowers. *Journal of General Physiology*. 20:511-518.

APPENDIX

Appendix 1. Key to positions on the face of the color domicile illustrated in Figure 5 for data presented in Table 1.

Gray, black or white colors used	Position on test domicile	Gray, black or white colors used	Position on test domicile
white	13	25.0	3
29.9	2	24.0	1
29.75	25	23.0	7
29.65	24	22.0	20
29.5	23	20.5	11
29.4	9	20.0	12
29.0	19	18.0	14
28.5	17	17.0	10
28.0	6	15.0	18
27.5	4	13.0	21
27.0	16	12.0	22
26.0	15	Black	5

1	2	3	4	5
6	7	8	9	10
11	12	13	14	15
16	17	18	19	20
21	22	23	24	25

Appendix 2. Detailed data for yellow color studies summarized in Table 1 (See key in Appendix 1)

Initial position of yellow card	Relocated position of yellow card	Type of Manipulation	Response
8	18	domicile rotated	positive
8	14	"	"
8	1	color card moved	"
8	25	"	"
8	18	"	"
8	14	"	"
8	12	"	"
8	25	"	"
8	21	"	"
8	5	"	"
8	3	"	"
8	23	"	"
8	11	"	"
8	19	"	"
8	21	"	"
8	5	"	"
8	25	"	"
8	1	"	"

Appendix 3. Detailed data for red color studies summarized in
Table 1 (See key in Appendix 1)

Initial position of red card	Relocated position of red card	Type of manipulation	Response
18	8	domicile rotated	negative
13	15	color card moved	"
13	23	"	"
13	3	"	"
13	11	"	"
14	3	"	"
14	7	"	unclassified
14	17	"	negative

Appendix 4. Detailed data for green color studies summarized in Table 1 (See key in Appendix 1)

Initial position of green card	Relocated position of green card	Type of manipulation	Response
18	8	domicile rotated	uncertain
18	8	"	positive
18	8	"	"
15	23	"	"
18	14	color card moved	"
18	14	"	"
18	21	"	"
18	21	"	"
18	5	"	"
18	5	"	"
25	15	"	"

Appendix 5. Detailed data for blue color studies summarized in Table 1 (See key in Appendix 1)

Initial position of blue card	Relocated position of blue card	Type of manipulation	Response
6	4	domicile rotated	positive
6	4	"	"
6	4	"	"
6	22	"	"
6	13	color card moved	"
6	13	"	"
9	7	"	"
9	17	"	"

Appendix 6. Detailed data for orange color studies summarized in Table 1 (See key in Appendix 1)

Initial position of orange card	Relocation position of orange card	Type of manipulation	Response
13	11	color card moved	positive
13	15	"	"
13	23	"	"
13	3	"	"
13	14	"	"
13	14	"	"
13	17	"	"
13	17	"	"
13	12	"	"

Appendix 7. Detailed data for purple color studies summarized in Table 1 (See key in Appendix 1)

Initial position of purple card	Relocated position of purple card	Type of manipulation	Response
19	9	domicile rotated	positive
19	17	color card moved	"
19	17	"	"
19	12	"	"
19	12	"	"
19	13	"	"
19	13	"	"