

THESIS







This is to certify that the

dissertation entitled

ACQUISITION OF VISUAL SPATIAL MEMORY ORIENTATION AND REORIENTATION FLIGHTS IN THE HONEY BEE

presented by

ELIZABETH A. CAPALDI

has been accepted towards fulfillment of the requirements for

Ph.D. degree in Zoology

Major professor

Date 1 November 1996

MSU is an Affirmative Action/Equal Opportunity Institution

0-12771

#### PLACE IN RETURN BOX to remove this checkout from your record. TO AVOID FINES return on or before date due.

DATE DUE	DATE DUE

.

MSU Is An Affirmative Action/Equal Opportunity Institution ctoircidatedus.pm3-p.1

- -----

## ACQUISITION OF VISUAL SPATIAL MEMORY: ORIENTATION AND REORIENTATION FLIGHTS IN THE HONEY BEE

By

Elizabeth Anne Capaldi

## A DISSERTATION

Submitted to Michigan State University in partial fulfillment of the requirements for the degree of

## DOCTOR OF PHILOSOPHY

Department of Zoology

1996

I.

#### ABSTRACT

# ACQUISITION OF VISUAL-SPATIAL MEMORY: ORIENTATION AND REORIENTATION FLIGHTS IN THE HONEY BEE

By

#### Elizabeth Anne Capaldi

Honey bees have long served as a model organism for investigating insect navigation. Bees primarily use learned visual features of the environment to guide their movement between the nest and foraging sites. Although much is known about the spatial information encoded in memory by experienced bees, the development of large-scale spatial memory in naive bees is not clearly understood. Past studies suggest that learning occurs during orientation flights taken prior to the start of foraging. I studied what bees learn during their initial experience in a new landscape by examining the homing of bees displaced after a single orientation flight lasting only 5-10 minutes. Homing ability was assessed using vanishing bearings and homing speed. At release sites with a view of the landmarks immediately surrounding the hive, "first flight" bees, tested after their very first orientation flight, had faster homing rates than "reorienting foragers" which had previous experience in a different site prior to their orientation flight in the test landscape. First flight bees also had faster homing rates from these sites than did "resident" bees with full experience in the test landscape. At distant sites, resident bees returned to the hive more rapidly than reorienting or first flight bees, but in some cases, the reorienting bees were as successful as resident bees.

Vanishing bearings showed that displaced bees headed directly toward home from release sites up to 200-500 m away. Because systematic or random search strategies would have resulted in vanishing bearings uncorrelated with the direction of home, I concluded that homeward-oriented bees must have obtained landmark information during

the orientation flight. Thus, the orientation flight must allow bees to learn landscape features that they can see at sites up to 500 m from the nest.

Reorienting bees could fly homeward from a site that offered no direct view landmarks near the nest. This suggests that during their single orientation flight, they had formed a simple route map connecting these visually isolated parts of the terrain. First flight bees were randomly oriented at this release site. Either their orientation flights had covered a less extensive area or their lack of previous flight experience prevented them from learning the route connecting this site to the nest.

A separate series of experiments revealed that visual complexity of landscape features had no influence on the duration of orientation flights. Reorienting bees, however, completed longer orientation flights than first-flight bees. This may explain the inability of first-flight bees to form route maps during the orientation flight.

These experiments show that bees learn about landmarks very rapidly despite their brief exposure to the terrain during the orientation flight, and have provided the first insights into what information is learned by bees with different degrees of experience.

Copyright by Elizabeth Anne Capaldi 1996 .

#### ACKNOWLEDGMENTS

I would like to acknowledge my graduate committee, Tom Getty, Jim Miller, and Don Straney, and my Major Professor, Fred Dyer, for their guidance and support during my graduate studies. I thank them for their encouragement and instruction.

I gratefully acknowledge financial assistance from Michigan State University for practical contributions to my scientific development. The Department of Zoology provided travel grants and fellowships to support research and my attendance at meetings. The Ecology & Evolutionary Biology Program, under the direction of Don Hall, provided travel grants and funding for research supplies. Archbold Biological Station gave me logistical support in exchange for research assistance during spring semester 1994. The College of Natural Science provided me with fellowships for travel abroad and for the completion of my research during 1996, as did The Graduate School under the leadership of Assistant Dean Karen Klomparens. In 1995, I was awarded the Barnett Rosenberg Fellowship by the University for which I am honored and grateful. This research was funded primarily by a grant from the National Science Foundation to Fred Dyer, and an NSF Doctoral Dissertation Improvement Grant to Fred and me.

Many undergraduates working in the Dyer lab assisted with the execution of this research, and I believe their contribution merits mention here: Chelsea Kostrub, Carla Fisco, and Jennifer Anderson helped with the pilot studies in 1992 and 1993. Ryan Fletcher, Shannon Mandia, Catina Sevidal, Monica Spangus, Susan Spaulding, Carrie Thill, and Eric Weaver all had a hand in my projects in 1994 and 1995. Throughout the

duration of my graduate work, Nancy Berry and Sharon Hall were particularly helpful to the development of my research as well as my social life. Many thanks to everyone for all their hard work.

I would like to acknowledge and thank MSU's Statistical Consulting Service for advice on my linear analyses, and Alan Tessier for suggesting the use of survival analysis. Gordon Fox (University of California at San Diego) consulted with me about the use of survival analysis and SAS programming that was crucial to my research. I thank him for his attention to my work. Carmen Salsbury and Ned Walker also served as important references for the statistical analyses. Karl Geiger discussed the circular statistics with me.

Thanks to Richard Greiner and Lanny Johnson for permission to conduct the Becker experiments on their land. Walter Pett provided essential logistical support, construction skills, friendship and fresh vegetables. Tom Burton, Don Hall, Dick Hill, Kay Holekamp, Rich Merritt, and Mark Scriber contributed to my personal and scientific development for which I am most appreciative. Thanks also to the Zoology Office Staff for helping me with just about everything.

My friends and colleagues, Audrey Armoudlian, Puja Batra, Jeff 'Bird' Birdsley, Erin Boydston, Jeff Dickinson, Jill Fisher, Troy Gerhardt, Alex Hernandez, Casey Huckins, Lissa Leege, Emily Lyons, Jennifer Rosinski, Carmen Salsbury, Elizabeth Smiley, and John Wallace were crucial to my success and stability during my graduate existence. The support and comraderie that they shared with me significantly influenced my intellectual and personal growth; for that, I am most grateful. 540 residents strengthened me through the tough times and were always there to share and create joy. I am also very grateful for the friendship of Eric Fahrenkrog during the writing process. Thankfully, The Aud and Roadie were nearby to lean on and close the vault. Most importantly, I would like to thank my family for providing me with endless love and encouragement. They have made an important emotional contribution to my life that has kept me grounded through the process of graduate school. I thank them for their solid support of my adventures, for listening to me, and for helping me learn from my personal mistakes. Thanks especially to my parents for reminding me to 'keep my nose to the grindstone' and to Grandpa Shea for always being there.

# TABLE OF CONTENTS

LIST OF TABLES	x
LIST OF FIGURES	X
CHAPTER ONE	1
	T
CHAPTER TWO	
THE ACOUISITION OF HONEY BEE SPATIAL MEMORY	
DURING ORIENTATION FLIGHTS	0
METHODS 24	Ř
BESIII TS 34	5
	5
CHAPTER THREE INFLUENCES OF LANDSCAPE COMPLEXITY AND FLIGHT EXPERIENCE ON THE DURATION OF THE ORIENTATION FLIGHT	1 3 5 8
CHAPTER FOUR	
FUTURE DIRECTIONS	1
APPENDIX	7
LIST OF REFERENCES	4

# LIST OF TABLES

•

Table 1.	Position of release sites in relation to the hive at Greiner
Table 2.	Orientation behavior of honey bees released at Greiner after a single orientation flight
Table 3.	Comparisons of Fully Exposed bees homing at Greiner 69
Table 4.	Comparisons of Reorienting bees homing at Greiner
Table 5.	Comparisons of First-Flight bees homing at Greiner71
Table 6.	Wilcoxon tests of equality over treatment category (by site) for bees released at Greiner
Table 7.	Within site comparisons of bees homing at Greiner73
Table 8.	Percentage of bees that returned to the release site
Table 9.	Tests of independence: Is the number of bees that returned to the release site independent of treatment?
Table 10.	Orientation behavior of honey bees released at 4H Fields after a single orientation flight
Table 11.	Comparisons of Reorienting foragers homing at 4H Fields77
Table 12.	General linear models using time as the dependent variable to examine influences on orientation flight duration

# LIST OF FIGURES

Figure 1.	Conceptual model of orientation tasks 79
Figure 2.	Percentage of displaced bees reaching the hive from release sites in a landscape in which they have made only a single orientation flight. The data are taken from Table 7 in Becker (1958)
Figure 3.	Map of the Greiner study site showing the position of the hive (H), the 6 release positions (A-F), and approximate topography, as indicated by the contour lines which show approximate elevation in meters (converted from a survey map plotting elevations in feet). The stipled areas indicatelandscape features, such as trees, woodlots, or buildings. This site is located on the east side of Hagadorn Road, just north of Bennett Road
Figure 4.	Map of the 4H Fields study site showing relative position of the hive (H), and the 4 release sites. This site is located on MSU Agricultural Experiment Station property adjacent to the Farm Service Center on College Road. There are no contour lines because this site is entirely flat
Figure 5.	Polar histogram showing the distributions of vanishing bearings for bees from a feeder at Entomology Teaching and Research Site on Collins Road and released at Site A. The polar histogram gives the mean bearing (F), shown by the white arrow. The r-value is a measure of dispersion and corresponds to the length of the mean vector. N is the sample size; individual bees were tested only once 85
Figures 6-8.	Polar histograms showing the distributions of vanishing bearings for released at Site A. Figures 6, 7, and 8 correspond to bees in the three treatment categories, Resident bees, Reorienting bees, First-Flight bees, respectively. Each polar histogram gives the mean bearing ( $\Phi$ ), shown by the white arrow. The upper and lower limits of the 95 % confidence interval of the mean are indicated by the smaller black arrows. The true homeward direction ( $\Psi$ ) is marked by the radius line. The r-value is a measure of dispersion. N is the sample size; individual bees were tested only once. A mean bearing is considered not significantly different from a predicted angle if the prediction falls within the confidence interval

Figures 9-11.	Polar histograms showing the distributions of vanishing bearings for released at Site B. Figures 9, 10, and 11 correspond to bees in the three treatment categories, Resident bees, Reorienting bees, First-Flight
	bees, respectively. Each polar histogram gives the mean bearing ( $\Phi$ ), shown by the white arrow. The upper and lower limits of the 95 % confidence interval of the mean are indicated by the smaller black
	arrows. The true homeward direction $(\Psi)$ is marked by the radius line. The r-value is a measure of dispersion. N is the sample size; individual bees were tested only once. A mean bearing is considered not significantly different from a predicted angle if the prediction falls within the confidence interval
Figure 12-14.	Polar histograms showing the distributions of vanishing bearings for released at Site C. Figures 12, 13, and 14 correspond to bees in the three treatment categories, Resident bees, Reorienting bees, First-Flight
	bees, respectively. Each polar histogram gives the mean bearing ( $\Phi$ ), shown by the white arrow. The upper and lower limits of the 95 % confidence interval of the mean are indicated by the smaller black
	arrows. The true homeward direction $(\Psi)$ is marked by the radius line. The r-value is a measure of dispersion. N is the sample size; individual bees were tested only once. A mean bearing is considered not significantly different from a predicted angle if the prediction falls within the confidence interval
Figure 15-17.	Polar histograms showing the distributions of vanishing bearings for released at Site D. Figures 15, 16, and 17 correspond to bees in the three treatment categories, Resident bees, Reorienting bees, First-Flight
	bees, respectively. Each polar histogram gives the mean bearing ( $\Phi$ ), shown by the white arrow. The upper and lower limits of the 95 % confidence interval of the mean are indicated by the smaller black
	arrows. The true homeward direction $(\Psi)$ is marked by the radius line. The r-value is a measure of dispersion. N is the sample size; individual bees were tested only once. A mean bearing is considered not significantly different from a predicted angle if the prediction falls within the confidence interval
Figure 18-20.	Polar histograms showing the distributions of vanishing bearings for released at Site E. Figures 18, 19, and 20 correspond to bees in the three treatment categories, Resident bees, Reorienting bees, First-Flight
	bees, respectively.Each polar histogram gives the mean bearing ( $\Phi$ ), shown by the white arrow. The upper and lower limits of the 95 % confidence interval of the mean are indicated by the smaller black
	arrows. The true homeward direction $(\Psi)$ is marked by the radius line. The r-value is a measure of dispersion. N is the sample size; individual bees were tested only once. A mean bearing is considered not significantly different from a predicted angle if the prediction falls within the confidence interval

Figure 21-23.	Polar histograms showing the distributions of vanishing bearings for released at Site F. Figures 21, 22, and 23 correspond to bees in the three treatment categories, Resident bees, Reorienting bees, First-Flight
	bees, respectively. Each polar histogram gives the mean bearing ( $\Phi$ ), shown by the white arrow. The upper and lower limits of the 95 % confidence interval of the mean are indicated by the smaller black
	arrows. The true homeward direction $(\Psi)$ is marked by the radius line. The r-value is a measure of dispersion. N is the sample size; individual bees were tested only once. A mean bearing is considered not significantly different from a predicted angle if the prediction falls within the confidence interval
Figure 24.	Homing by Resident Bees homing at Greiner
Figure 25.	Homing by Reorienting bees homing at Greiner
Figure 26.	Homing by First-Flight bees homing at Greiner 100
Figure 27.	Homing by bees homing from Site A at Greiner101
Figure 28.	Homing by bees homing from Site B at Greiner102
Figure 29.	Homing by bees homing from Site C at Greiner103
Figure 30.	Homing by bees homing from Site D at Greiner104
Figure 31.	Homing by bees homing from Site E at Greiner 105
Figure 32.	Homing by bees homing from Site F at Greiner 106
Figure 33.	Polar histograms showing the distributions of vanishing bearings for bees released at 4H Fields. Each histogram corresponds to their relative compass positions on the page. North is indicated by the solitary dark
	arrow. Each polar histogram gives the mean bearing ( $\Phi$ ), shown by the white arrow. The upper and lower limits of the 99 % confidence interval of the mean are indicated by the smaller black arrows. The true
	homeward direction $(\Psi)$ is marked by the radius line. The r-value is a measure of dispersion. N is the sample size; individual bees were tested only once. A mean bearing is considered not significantly different from a predicted angle if the prediction falls within the confidence interval
Figure 34.	Homing of Reorienting bees from all sites at 4H Fields 109

Figure 35.	Map of the Corral site, showing the position of the hive (H). The stipled areas indicate buildings and the black lines represent roads. This site is adjacent to the Farrall Agricultural Engineering Building on the Michigan State University main campus
Figure 36.	Map of the Collins Road site, showing the position of the hive (H) and nearby landscape features. The dark stipled areas indicate trees or woodlots and the light stipled circle is a small gravel pit. The solid and dotted lines mark roads and the open boxes mark buildings. This site is adjacent to the Entomology Teaching and Research Facility, on the Michigan State University Farms

#### CHAPTER ONE

#### Introduction

The homing abilities of animals have long fascinated scientists and casual observers of animal behavior. Legendary feats of animal orientation, such as the seasonal migration of the North American monarch butterfly to its overwintering grounds in southern Mexico (reviewed by Brower 1996), and the intercontinental migrations of many songbird and shorebird species, generate questions about how such long-distance movement is accomplished. Even the more modest abilities of rodents and other mammals to negotiate complex mazes in search of food raise deep questions about how spatial relationships are learned by the animal (reviewed by Gallistel 1990).

Beginning with early researchers in zoology and experimental psychology (Tinbergen 1951; von Frisch 1967; Tolman 1948), studies of spatial orientation have played an extremely important role in the development of ideas about the behavioral capacities of animals. This has led to the discovery of many new sensory modalities and more clearly defined the behavioral capacities of many animals (Able 1980). However, animal orientation remains underexploited as a paradigm for the study of ecologically meaningful and naturally occurring learning. Only in the last ten years have mechanistic questions regarding the relationhips between orientation and learning come into sharp focus (Gallistel 1990; Menzel 1990; Dyer 1994).

This thesis concerns the connection between learning and orientation, an important mechanistic component to understanding how animals get around in the world. I have studied how a naive bees comes to acquire the information that it later uses for navigation. Specifically, my research examines what a bee has learned about landmarks which serve as key references for many navigating animals. The purpose of this chapter is to place the study of navigation and orientation in a broader context.

Our general knowledge of navigation mechanisms originates mainly from certain well-studied animals. Of all animals, birds are probably best known for their navigational abilities, perhaps as a result of their conspicuous feats of migration. Many bird species complete dramatic feats of long-distance navigation. Consider the Arctic tern (Sterna paradisaea), for example. These birds spend their summers breeding within the Arctic Circle, but in the fall, they depart for the Antarctic where they feed in the highly productive waters in the southernmost portion of the Atlantic Ocean. The terns follow routes that take them over the richest feeding grounds; the long journeys bring the birds to the most favorable environments throughout the year (reviewed in Dingle 1996). Questions about how these and other birds find their way have taught us a great deal about animal mechanisms of navigation. Of course, homing pigeons are probably the most renowned bird for the study of navigation. These birds can fly directly to their home loft after displacements of up to several hundred kilometers into unfamiliar areas. Experienced birds can even do so when they have been anesthetized during the outbound journey, and hence deprived of information about direction and distance of displacement (Able 1980). The literature on the navigation of homing pigeons is diverse and there is considerable debate about the mechanisms the birds use for orientation. What is becoming clear is that birds use a combination of environmental references to find their way (Berthold 1991). Birds have multiple and often redundant systems for orientation, as is the case with savannah sparrows which are reported to have a system of interacting compass senses: magnetic, star, polarized light, and possibly sun compasses (Able & Able 1996). Pigeons can use compass information derived from both the magnetic field and from solar information (Wiltschko & Wiltschko 1996). What information birds use for map-like information is still a big mystery, despite many years of investigating this behavior (reviewed in Walcott 1996).

Vertebrate animals are not alone in their ability to carry out impressive navigational feats. Invertebrates, too, show seasonal and daily movements over great distances: in the Caribbean Sea around the Bahamas, spiny lobsters (*Panulirus argus*) migrate in grand

Queues from the shallow waters where they spend the summer to deeper waters in the fall (reviewed by Kanciruk & Herrnkind 1978). Another conspicuous example that I have already mentioned is the migration of the monarch butterfly (*Danaus plexippus* L. Lepidoptera: Danaidae), a species that migrates across the North American continent in the fall, spends the winter in the mountains of Mexico, and returns during the spring to southern North America. In the southern states, the migrants lay eggs and die. This next generation metamorphoses in time to fly to the northernmost part of the species range by early summer. Two or more generations are reared throughout the summer and these individuals begin the cycle again. This behavior has been documented for many years, and yet new hypotheses are still being constructed to explain the phenomenon (Brower 1996).

While these examples of animal migration are dramatic, other ways in which animals navigate in their environment are just as impressive, even though they might not seem as miraculous. Hymenopteran insects, and other animals that forage from a central nest, face the problem of returning repeatedly to single points in the environment. Insects must be able to move efficiently between the nest and rich feeding sites; this task may not appear as fascinating as the examples of migration discussed above, but with further consideration, it becomes just as interesting. Because humans regularly explore and orient within new environments, we may overlook the challenges that orienting animals face on a daily basis, especially for small insects that range widely from the nest. The potential foraging range of one hymenopteran insect, the honey bee (*Apis mellifera* L. Hymenoptera: Apidae), is  $\approx 10$  km (Visscher & Seeley 1982). Other species of bees have been reported to have foraging ranges or 15-20 km (reviewed by Wehner 1981). Considering how easy it would be to miss the nest when setting out for it from such distance, it is remarkable that insects arrive home successfully, and almost amazing that they do so in a direct flight.

Researchers studying animal navigation generally agree that navigators need to obtain both directional (compass) and positional (e.g., map) information to head directly toward a distant goal. One major aim in studying animal orientation is to understand the features of the environment that provide directional and positional information, and the sensory and integrative mechanisms by which this information is obtained and used by the brain. In the case of bees and other insects, only visual cues (landmarks and celestial orientation cues) can provide a basis for rapid and accurate navigation over the hundreds or thousands of meters that they travel from the nest. These references in turn, are useful only if the animal has had prior experience foraging in the area. For example, as we shall see, landmarks and the sun provide no intrinsic information to a navigator about its position relative to its goal. This information is available only if the animal has had the opportunity to learn how these references define the spatial relationship between its current location and its goal. Thus, much research on animal navigation has come to focus on the role played by learning.

In recent years, both behavioral ecologists and cognitive psychologists have also become intensely interested in the question of how spatial relationships that an animal has experienced are stored internally and then used later to guide behavior in the same environment (reviews by Krebs 1990; Gallistel 1990; Shettleworth 1990; Dyer 1994, 1997a, b). This question fits neatly into the agenda of the growing fields of neuroethology and cognitive science, which are concerned with the general problem of how the brains of organisms represent information about objects and events in the outside world (Yoerg & Kamil 1991; Churchland & Sejnowski 1992; Churchland 1995). Studies of how animals orient themselves in their everyday activities can contribute much to these disciplines. The difficulty of the navigational problems that animals face, and solve, in nature, considered together with the diversity of orientation mechanisms that have evolved in different species, provide a source of both questions and tests of the theories that explain them.

In the rest of this chapter, I intend to review the mechanisms designed to solve different navigational problems faced by animals. I will first briefly review the requirements for homing behavior, and then discuss orientation mechanisms used in visually guided orientation by hymenopteran insects, primarily sphecid and vespid wasps and honey bees. Then I will outline the importance of exploring the processes by which spatial representations develop in the brain of a naive insect.

#### The **Basics**

Orientation and navigation have come to be regarded as different behavioral capacities (Schöne 1984). Orientation may be defined as the way in which an organism adjusts its body axes relative to external cues. Navigation, on the other hand, is the process by which an animal uses various stimuli to determine its position relative to a goal as it moves from place to place; effective navigation requires the ability to orient while sensory information changes along with movement. Orientation, therefore, refers to the moment to moment alignment of the organism's body, while navigation is the behavioral result of such processed sensory input.

Griffin (1955) separated navigation into three different types of homing abilities: piloting, compass orientation, and 'true' navigation. Piloting refers to the ability of animals to find a goal by referring to familiar landmarks. For animals using piloting as a strategy for homing, sensory contact with familiar features of the environment needs to be maintained (Able 1980). The second strategy, compass orientation, does not require reference to familiar landmarks but is simply the ability to orient in a given compass direction when released in an unfamiliar area. Homing can only occur if the direction leads toward home, or to a familiar area. Thus, compass orientation alone is insufficient for finding a distant goal. The third type, true navigation, is more complex. It only occurs in a small number of species (i.e., homing pigeons and many migratory birds) that can orient toward a familiar place when released in a totally unfamiliar area. Animals that use this

type of behavior do not maintain any form of sensory contact with the goal or with familiar landmarks (Griffin 1955; Able 1980). Most cases of animal homing do not involve true navigation, but instead involve some integration of the first two strategies.

For the purposes of this chapter, I use the term navigation in a broader sense than Griffin did: it is any process that leads the animal toward an unseen goal. At least two types of information are required for an animal to make the adjustments that will allow it to find a specific goal in the environment. First, the animal needs to have some method of determining the correct direction that will lead to the goal; to do this requires knowledge of its own position relative to the goal. Next, the animal independently requires the ability to discriminate different directions from one another. Each type of information is not useful without the other. For example, if you know your position ('I am south of my goal') then you need a method to discriminate north (the homeward direction) from other directions. The ability to determine direction ('I know which way is north') is only useful if the relationship between your present position and your goal is known. In other words, animals, like humans, need to be equipped with the equivalent of a map and a compass for navigation.

Wind direction, acoustic signals, and water currents are all sources of nonvisual directional information that can be used by animals (Able 1980). For most animals, however, the dominant sources of directional information in the environment come from the magnetic field (reviewed by Wiltschko & Wiltschko 1996) and the sun (reviewed by Dyer & Dickinson 1996). Foraging bees detect and use the azimuth of the sun for flight orientation and to direct their nestmates to foraging sites through their dance language (reviewed in von Frisch 1967). Insects can also use sun-linked patterns of polarized light in the blue sky as a part of their celestial compass (von Frisch 1967; Wehner 1989; Rossel & Wehner 1986).

An important source of positional information for both vertebrates (Etienne et al. 1996; McNaughton et al. 1996) and invertebrates (reviews by Dyer 1994; Wehner & Wehner 1990; Wehner et al. 1996) is path integration, or "dead reckoning". Path integration relies on a compass and a method of measuring distance and allows a foraging insect to use information experienced on the outward path of a foraging trip to determine its position relative to the hive. Deserts ants, *Cataglyphis bicolor* (Hymenoptera: Formicidae), scavenge for food and use this form of vector navigation to walk the direct route (ant-line, so to speak) toward the nest after food is found (Wehner et al. 1983). Animals using this strategy for navigation continuously integrate all angles turned with the distances covered during the outward course; at any moment during the trip the insect can select the direct route between its current location and home. This system depends upon a directional reference provided by the insect's celestial compass (Dyer & Dickinson 1994; Wehner & Müller 1993) and distance as measured by optic flow cues (reviewed by Wehner et al. 1996; Ronacher & Wehner 1995; Esch & Burns 1995; Esch & Burns 1996; Srinivasan et al. 1996).

When artificially displaced from the nest, ants and bees are denied use of their path integration ability and thus must rely on other mechanisms for homing. Insects in this situation can rely on memorized landmarks to determine their position and guide their way home. Landmarks do not play a role in orientation by path integration (Wehner 1982), and hence can be viewed as an independent source of positional information.

The ability to learn about the location of landmarks in space is crucial to an animal's ability to acquire positional information in the environment. For example, there would be no way for an insect to be preprogrammed to use the sequence of landscape features encountered along the route connecting the place where it happens to have found food and the place where its colony happens to have nested. If such landmarks could be learned, however, they provide an extremely reliable reference for navigating along this route. Landmarks such as hills and patches of vegetation are stable over the life of an insect.

Furthermore, unlike celestial cues, they are unaffected by weather. The rest of this chapter will concern the nature of the learning processes by which insects memorize spatial information provided by landmarks, and use this memorized information to guide behavior.

#### Some Perspective on Landmark Learning

Spatial memory and the nature of how it is formed has long been a focus of study in the field of animal cognition (reviews by Roitblat 1987; Gallistel 1990; Dyer 1997a). One of the major theoretical constructs employed by a cognitive perspective is the "internal representation," which is hypothesized to be an internal model of events or relationships experienced by the animal. A cognitive perspective is thus concerned to examine how such internal representations are formed and then used to control behavior (Roitblat 1987). In studying animal spatial memory from a cognitive perspective, we seek to understand what spatial relationships are represented internally, and how they are formed and used.

Psychologists generally separate studies of learning and memory into three phases: acquisition, retention, and retrieval. The first phase occurs when the animal is exposed to the stimuli to be learned. The acquired information is then stored for a period of time known as the retention interval. The third phase, retrieval, happens when the information previously acquired is reactivated and then used. In studies of learning per se, the main emphasis is placed on the first phase, which is studied via manipulations of the conditions of acquisition. It is important to note, however, that the conditions of acquisition are only of interest to the extent that they are relevant to retention and retrieval in the animal (Domjan 1993). Acquisition, then, sheds light on the contents of memory that are eventually formed. In order to study acquisition, one can ask the animal to tell us, through its behavior, what information it has acquired; we inquire as to how the animal uses what it has learned, and by doing so, we also learn about the contents of memory. The distinction

between the contents and acquisition of memory is a subtle, yet an important one to keep in mind as I review the evidence that insects use learned information about landmarks in orientation.

#### Small Scale Contents -- Visual Panoramas

Early insect naturalists wondered how bees and wasps can find their way back home from their foraging flights (Fabre 1879; Buttel-Reepens 1990; Rau 1924, 1930). Some scientists believed that an unknown sensory function had to be responsible for the homing of insects (Becker 1958). We now have ample evidence that many insects use visual landmark memories for position determination and homing behavior. Most data have been collected for honey bees whose hives have been moved over a variety of distances, but hundreds of species of insects have been shown to memorize visual panoramas and use those memories for orientation (see Table 8 in Wehner 1981).

Romanes (1885) was one of the first to suggest that honey bees had a figurative map of landmarks that would allow them to successfully orient in the environment. In 1932, Tinbergen reported that the bee-wolf, *Philanthus triangulum*, a digger wasp, was "able to orient by means of visual landmarks once, through a yet unknown method of 'distant orientation,' they have found the nest surroundings." These were the famous experiments in which Tinbergen placed a ring of pine cones around their burrow entrances in the early morning and then watched the wasp depart from the nest. While the wasp foraged, Tinbergen removed the 'cone-circle' stimulus and set up the stimulus surrounding a sham nest a few centimeters away. That the wasp upon its return searched for its burrow within the ring of cones provided evidence that the wasps use landmarks to acquire positional information.

By studying the digger wasp *Ammophila*, Baerends (1941) looked for evidence that insects could link successive sets of landmark cues together to form a route through the panorama; as the wasp moved closer to the nest, then fewer and fewer nearby landmarks would be useful. His experiments involved the transplantation of seedling pine trees in the vicinity of his focal animals. He suggested that both nearby and distant visual cues (those extending above the horizon line) would be useful to a homing wasp searching for its burrow.

Since these early studies, considerable evidence has accumulated for the role of landmarks in homing behavior (reviewed by Wehner 1981; Dyer 1994). In studies examining orientation over the small-scale, three-dimensional landmarks proved to be more effective cues for orientation than flat objects. Patterned landmarks, made of contrasting colors, and located close to the burrow were preferred by wasps; according to Tinbergen, the insects show a distinct selectiveness on what they learn, and presumable use, for homing behavior (Kruyt & Tinbergen 1938; van Iersal & van dem Assem 1964).

These studies have examined the ways in which insects use landmarks near the target to pinpoint the goal; it may be a different task for insects to orient using landmarks for travel to a goal that cannot be seen from the starting location. Baerends (1941) suggested that the wasps could build routes with which to connect the nest with feeding places. In 1953, von Frisch and Lindauer conducted a set of experiments with honey bees trained to a food dish in order to examine the relative importance of landmarks and the sun on position finding behavior. These experiments were designed to establish a competition of sorts between information provided by the celestial compass and that provided by landmarks. One experiment, for example, involved training bees from a colony to a food dish that was located 180 m to the South. A prominent visual line of trees ran along the path between the hive and the food. After bees flew this route for one day, their colony was relocated to a woodlot with an east-west edge. Three feeding stations were set up 180m from the hive and the colony was opened. Of 35 bees marked at the original feeder

(to the south), 16 bees were captured at the station to the west (along the trees), 1 was caught at the east station, while only 5 were captured at the south feeder. These results indicate that large scale landscape features can be learned and used for orientation, and that noncelestial visual information can dominate over celestial directional cues in certain contexts. Other experiments of this variety demonstrated that continuous landmarks, such as treelines, roads, or shorelines can be used for orientation, but that single landmarks, such as trees, were not used in the determination of position (von Frisch & Lindauer 1954; reviewed in von Frisch 1967).

#### Small Scale Contents - How?

After the determination of *what* types of landmarks are used in homing, the next question is *how* the landmarks encoded in memory are used (Wehner 1981). Here I review this topic in the context of studies of insects using familiar landmarks near the goal to guide their final approach. One major hypothesis is that an insect will try to match its memorized view of the surrounding visual panorama with the images that it currently experiences; by moving the body until the positions of these images match, the insect will discover the goal. Studying the behavior of male hover flies led Collett and Land (1975) to suppose that each fly has a representation of the spatial position of its "home" in terms of the visual image on its retina while it hovers. They studied flies that had established a hovering site in front of a visual marker and then watched the behavioral response of the insects when the marker was moved. The experiments showed that the fly's visual home is defined as a two dimensional picture with the size, form and relative position of landmarks imaged on its retina (Collett & Land 1975). This model would allow the fly to find its way home from a place it had never visited before by a strategy designed to decrease the mismatch between the current and memorized retinal image.

An alternative hypothesis to explain how landmarks are used was presented by Anderson (1977) in a study of how honey bees use landmarks to pinpoint the location of food. He suggested that bees do not measure the exact position, number or size of individual markers, but instead use the overall configuration of the landmarks as a guide for oriented behavior. Anderson's experimental protocol used bees trained to feeding place inside a ring of cylindrical landmarks; he reported that the bees behave as if they use the concept of 'surroundness' to return to the correct position when they were tested searching for food inside a semi-circle. He advanced the idea of van Beusekom (1948) that the bees have concepts, or 'Gestalt' ideas, about the positions of goals relative to landmarks.

More evidence has accumulated, however, for the retinal image matching hypothesis described above than for Anderson's idea. Tom Collett and his collegues at Sussex University, in particular, have greatly advanced the study of nearby landmark learning in bees. They presented a model to describe how bees locate the goal after being trained to forage at a place specified by landmarks. Cartwright and Collett (1982, 1983) suggested that bees retain in memory a representation analogous to a two dimensional snapshot of the landmarks and use this memory to guide their orientation. Experiments that presented bees with landmarks of different size during training and testing indicated that they do not learn the distance between the food and the landmarks, but that they do use how large the landmark appears when seen from the target; the bees use the retinal position of the landmark array as seen from the food source as a template, and then move themselves until their current view matches what they retain in memory. Additional evidence comes from experiments with a constellation of landmarks. When trained to an array of three landmarks and tested with the landmarks in a different spatial arrangement, the bee searches at the point where the inter-landmark angles are the same as those seen from the food source (Cartwright & Collett 1982, 1983). The apparent size and bearings of landmarks, then, are used to record positional information and that only minimal perceptual processing is required for it to be encoded in memory (Cartwright & Collett 1983). Cartwright and Collett proposed a simple computational model to explain how bees might use the discrepancy between their current and memorized retinal image to set course toward a goal.

One problem with this model, however, is that it seemed to require that the insects be able to rotate the snapshot in their heads. Insects can approach the goal from different directions, and thus the image of the landmarks would not always fall on the retina in the same way (Cartwright & Collett 1983). And yet, Cartwright and Collett's model worked well only if the snapshot were aligned in constant compass orientation. To keep the snapshot aligned, the bee would have to encode it independent of its body axis. This would resemble the human ability to mentally rotate visual images. More recent work, however, has suggested that bees actually adopt a constant body orientation during the final approach to a goal. The snapshot encoded in retinal coordinates would be maintained in a constant orientation without mental rotation regardless of a bee's direction of travel.

Cartwright and Collett (1987) went on to examine how this image matching model might work for guidance over a wider spatial area. They proposed that from each position within the terrain, the bee takes a new snapshot for the album and links it to the distance and direction to the hive. In this way, insects could construct vectors that connect the snapshot stored in memory to the direction of the nest. Cartwright and Collett (1987) introduce the concept of a distance-filtered snapshot that they suggest might help bees orient toward a goal over a wide spatial scale; these filtered snapshots would exclude nearby landmarks when the bee is at positions far from the goal. Using these two snapshot memories, the bee can orient toward the goal where there is similarity between the currently viewed retinal image and the one storied in memory (Cartwright & Collett 1987). These authors hypothesize that the bee develops, through experience, a vector map that would allow it access to a home vector for orientation; with this model, the bee could employ image matching throughout the trip toward the goal. Questions remain, however, about this model and about the presence of maps in the brains of insects.

#### Large-Scale Contents: Routes or Maps?

For the distances nesting insects travel, it will often be necessary for the animal to use landmarks beyond those that it can see at any one location in the environment. In recent years, considerable effort has focused on how large-scale featrues of the terrain are learned and used for navigation. One major question has been whether insects have maps of their environment that correspond to the cognitve maps that humans can construct and use (Byrne 1982). Such a map would require a neural representation of space that preserves the true geometrical relationships between points that may have been experienced separately. This question of whether insects have such maps is a controversial one, but the basics bear on the discussion of the contents and use of spatial memory.

Gould (1986) tested the possibility that bees have such large-scale maps by training them to a feeder, allowing them to forage there for a few days, and then capturing them on their way out of the hive and displacing them to a location that he assumed was out of sight of the training site. The bees flew immediately toward the training site upon departure from the release site, which Gould used as evidence for a landmark map in the bees' brains. He believed that the bees had integrated their experience on different routes, and then calculated the short cut path between the two points even though they had never directly experienced it. This would require encoding different routes in a common coordinate system, then using that coordinate system to compute the spatial relationships between points on different routes.

An alternative to the hypothesis that insects form such integrated maps involves an extension of the mechanism used to navigate relative to landmarks visible surrounding a goal. In this model, the insect orients in a way that continuously ensures the best match between current view of landmarks and the view experienced on previous trips. Bees could then use snapshot images arranged sequentially in memory to reconstruct the route toward home.

One way of stating the difference between these two models is to refer to the question of how insects use landmarks that are not currently in view. In Gould's model (1986), the representation encodes all seen and unseen landmarks in an integrated map, and allows the insect to refer to unseen landmarks in computing flight paths. In the route-map model, the bee would only base its flight path relative to landmarks currently seen. By necessity, this would allow bees to travel only along routes on which it would see familiar landmarks and would not allow it is compute novel flight paths.

Replications of Gould's map experiment and other tests of the cognitive map hypothesis have failed to produce evidence that bees have integrated large scale maps of familiar terrains, but have demonstrated that bees can use visual information to orient using route-maps (Menzel et al. 1990; Wehner et al. 1990; Wehner & Menzel 1990; Dyer 1991).

### Acquisition

How do insects acquire visual memories, over any spatial scale? Buttel-Reepen (1900) believed that the orientation abilities of bees had to be based on the visual and olfactory impressions that they gained from orientation flights (from Becker 1958). Because of their rather stereotypical form with respect to their temporal and spatial parameters, these flights have long been recognized in a wide variety of central-place foraging insects (review in Wehner 1981). Tinbergen (1935) proposed that the bee-wolf learned the complex configurations of visual landmarks during specialized flights that he called 'locality studies' that are equivalent to these flights. When young insects have been displaced from their nests, they are unlikely to find their way homeward if they have not completed orientation flights (Rau 1924; Becker 1958). Only recently has this behavior been studied in any depth.

When departing from the nest for the first time, bees and wasps conduct this specialized flight by turning and facing the hive and in this position begin to hover. In a typical flight, the bee or wasp will back away from the goal in a series of arcs that center approximately on the goal (Zeil et al. 1996). Often the insect then begins to fly in wider circles before leaving the local area. Orientation flights at the nest always take place before the insect begins foraging. They also occur if the visual panorama around the nest has been changed or if the nest itself is moved to a new location; in this context, they are called *re*orientation flights. The distinction between orientation and reorientation flights depends on the context of the flight. When new forgers begin orienting, it is common to describe their flights as 'first' or 'second' and so on. The second orientation flight is not considered to be a reorientation flight: rather, it is just another attempt at learning in that particular landscape. Reorientation, in contrast, implies that there is some novelty in learning through orientation flights.

Orientation flights also occur after the discovery of a rich food source, or when the insect has difficulty returning to a otherwise familiar location (Rau 1930; Opfinger 1931). In these cases, the term orientation flight generally refers only to the first phase of the flight, where the insect hovers and pivots around the landmark array. There is ample evidence that bees or wasps learn the visual characteristics of their target, whether that be a nest or a profitable food source, during these orientation flights (Wehner 1981; Lehrer 1991) but for a long time it was not clear over what the spatial scale landmark learning occurs.

Resurgent interest in orientation flight behaviors has started to fill the gaps in our knowledge about the acquisition of spatial memory by orienting insects. Lehrer (1991) described orientation flights in the context of foraging as turn-back-and-look (TBL) behavior. She investigated the function of the behavior by presenting bees with 4 different training conditions: in 3 groups, the bees could view a landmark of a particular shape and color during their (i) arrival at the food (ii) their departure from the food (iii) arrival and

departure. In the 4th group, the bees saw one landmark on arrival and another on departure. Her results suggest that learning occurs after feeding (but during the TBL) but also demonstrate that the timing of learning is dependent upon which cues are available to the bees (Lehrer 1993). She concluded that landmark learning is not based on a hard wired predisposition to learn particular visual features at specific times, rather it has a flexible nature (Lehrer 1993). There has been a flury of recent publications investigating the structure and function of orientation flights as they relate to the learning of small-scale landmarks (Collett 1992; Collett & Lehrer 1993; Zeil 1993 a, b; Collett 1995; Lehrer 1996; Zeil et al. 1996).

In spite of the progress made in understanding orientation flights and learning landmarks near the goal, not much is known about the role of orientation flights in learning large-scale landscape features, i.e. those that lie beyond the current visual horizon of a bee at the nest. It is known, however, that bees and wasps that have not completed orientation flights at the nest cannot return home when displaced into the landscape (Rau 1930; Becker 1958). During an orientation flight, bees often disappear from the local area around the hive for short periods of time; what these bees are seeing, or learning, and how far they fly from the nearby area is not known.

In the first attempt to tackle these problems, Becker (1958) studied homing by bees after a single orientation flight in a new terrain. She discovered that bees that had completed orientation flights could home from displacement points located 600 m away from the hive, from where the bees could surely not see the hive or landmarks in its immediate vicinity. This implies that insects learn aspects of the terrain during their orientation flights that they later use to guide their homeward flight (Becker 1958). Becker also found that more bees returned to the hive from release positions located close to the hive than those displaced to distant sites (1958). Finally, she also found a difference in the homing ability between bees with previous foraging experience and bees that had only one flight outside the hive. Because previously experienced bees had higher homing success from displacement points far from the nest, Becker deduced that they learned landmarks over a larger scale than first-flight bees.

Other descriptive experiments by Vollbehr (1975) suggest that the orientation flight may bias what is learned. He reported that orientation flights were directed toward the sun and that after completing an orientation flight, bees had better homeward orientation from the direction of the azimuth of the sun (Vollbehr 1975). The role played by the sun in shaping the orientation flight or influencing homing is as of yet unknown.

An attractive model for large-scale orientation behavior is the landmark matching model proposed by Cartwright and Collett (1987); for orientation to small landmarks immediately surrounding a goal, the insect matches a memorized landmark image to a currently viewed image. In the context of large-scale orientation and the task is to orient toward an unseen goal, there may be a multitude of possible snapshots between the current location and the target. Perhaps the snapshot model is extented and amplified in space, which would allow the bee use images acquired during the orientation flight to guide its flight.

Becker employed a rather crude assay of homing ability--the fraction of displaced bees that reached home successfully - making it hard to determine the scale over which landmarks are learned during the orientation flight. Specifically, her results do not decide between two very different interpretations of an ability to find home when home is over the horizon. One possibility is that the bees have learned the relationship between the landmarks at the release and those near the nest. The alternative is that bees learn only the landmarks in the near vicinity of the nest, and find their way home from distant release sites through systematic search. Both hypotheses predict decreasing homing success from more distant release sites.

### Specific Aims of My Research

There are many unanswered but important questions about the development of large scale landmark memory by honey bees. What does the orientation flight provide the bees with that they may use to guide their flight homeward? Does a bee performing an orientation flight learn about landscape scale features located over the horizon (from the point of view of a bee at the hive) or just landmarks near the nest? What accounts for the differences between naive and experienced bees? My research explores some of these questions by using Becker's work as a model. Her study needs replication in order to identify whether the distance effect in homing honey bees is due to distance per se or to the distinction between learning landmarks around the hive versus landmarks over the horizon. Becker's research examined many different aspects of bee homing without clear hypotheses or large sample sizes to support her findings. I have improved upon her methods by incorporating measurement of the bees' initial flight direction through vanishing bearings, by using improved measures of homing success and homing speed, by increasing sample sizes, and by exerting more control over visual stimuli that are available at the the release site. I have also duplicated her comparison of the behavior of bees with previous flight experience and bees with no prior flight experience, with the specific aim of investigating what accounts for the differences in the homing ability of these two groups of bees. In addition, I examine the effect of landscape features on the duration of orientation flights; this experiment builds into our model of the development of spatial memory and increases the general trends suggested in Becker's paper.

Studies of orientation flights over a large spatial scale will balance our knowledge of the acquisition of visual spatial memory with our knowledge of the contents of memory This complements and extends research done recently (Dyer 1991, 1993; Dyer et al. 1993; Robinson & Dyer 1993; Dyer & Dickinson 1994; see reviews in Collett 1996; Lehrer 1996; Wehner et al. 1996; Zeil et al. 1996; Dyer 1997a, b) and elsewhere that has characterized the contents and use of spatial memory in highly experienced bees.

### CHAPTER TWO

#### The Acquisition of Honey Bee Spatial Memory during Orientation Flights

The honey bees exhibit impressive navigational abilities over a range of spatial scales. As a central place forager, the adult bee makes repeated trips from the nest in the course of retrieving food for the colony, visiting feeding places up to 10 km from home. It is easy to overlook what orientation entails, especially for small animals that range widely from their nests, because we humans readily solve orientation problems in our day to day movements. Over the distances that bees travel, only visual information, such as landmarks and celestial orientation cues, can provide a basis for rapid and accurate orientation, and these are useful only if the animal can learn to recognize the relationships between specific visual features and specific locations in the environment. That bees can successfully navigate given these requirements is all the more impressive given their brief life span and the small size of their brain.

Navigational abilities are not exhibited by the bee until relatively late in life. After emergence from the pupal stage, adult worker honey bees typically live for 30 - 45 days. As workers age, they progress through a series of jobs within the colony. This division of labor on the basis of worker age is called "age polyethism" (reviewed by Robinson 1992). Newly emerged adult bees attend to tasks in the nest's center, such as cleaning cells, tending brood, and attending the queen. Slightly older workers shape the comb. At approximately 21 days of age, workerss begin to shift their attention to tasks at the nest's periphery; they begin to receive and store food, guard the colony from intruders, and finally, they begin to forage. Foraging continues until the end of the bee's life. This form of specialization structures the colony to function more efficiently than if it were a simple collection of individuals (Seeley 1985).
As a forager, the worker relies heavily on an ability to learn visual information (Wehner 1981). Bees must be able to learn flight directions relative to celestial cues and to landmarks to orient their dances. Orientation based on the celestial compass requires learning information about the celestial rotation relative to landmarks as well. Spatial patterns must be learned on multiple spatial scales; pattern learning and color learning are often used to recognize rewarding flowers.

The tasks faced by animals in learning landmarks vary drastically depending upon the scale over which they move (Collett 1996 a, b; Dyer 1997 a,b). The separation of tasks as 'large scale' and 'small scale' is potentially arbitrary but also useful to categorize the range of honey bee orientation behaviors. Operationally, 'small scale' includes distances over which the goal is directly detected or when landmarks marking the position of the goal are continuously visible. 'Large scale' refers to the task when the goal and nearby landmarks are not directly visible from the starting point. Small, or local, scale orientation behaviors require learning flower shape (Gould 1985), pattern orientation (Zhang & Srinivasan 1994; Giger & Srinivasan 1995), or the landmarks that fix the location of a target such as a nest entrance. Since Tinbergen's classic studies of how digger wasps (*Philanthus triangulum*) find the location of their nest entrance (Tinbergen & Kruyt 1939), many examples of orientation at this local scale have been conducted (reviewed in Wehner 1981). Experimental examinations of spatial memory tasks in insects and other animals, such as rodents or food storing birds, usually involve learning at this scale (Collett et al. 1986; Krebs 1990; Shettleworth 1990; Etienne et al. 1996).

Orientation on a larger spatial scale is more difficult to study and manipulate, but considerable progress has been made in understanding the components of the process. For efficient long-distance navigation, foraging insects like desert ants or honey bees integrate three different mechanisms: a celestial compass, path integration, and landmark learning. When an experienced individual departs from the colony in search of food, the return trip is guided by a system of path integration, or dead reckoning (Wehner et al. 1996). This

process allows the insect to use information collected about the distances and direction traveled on the outward path in order to determine its position relative to the hive. The insect continuously integrates all angles turned with the distances covered during the outward course; at any moment during the trip the insect can select the direct route between its current location and home. This system depends upon a directional reference provided by the insect's celestial compass (Wehner & Müller 1993; Dyer & Dickinson 1994; Dickinson 1994) and a measure of distance provided by optic flow cues (reviewed by Esch & Burns 1995; Esch & Burns 1996; Ronacher & Wehner 1995; Srinivasan et al. 1996; Wehner et al. 1996).

Abundant evidence suggests, however, that insects can orient homeward in the absense of information from their path integration system, and thus must have an alternative source of navigational information. For example, insects that fly homeward after artificial displacement from the nest cannot rely upon path integration, because they have no source of information about the directions and distances taken during the outward trip (Dyer 1994); the insect has not had the opporunity to track its own position using the celestial compass and optic flow cues. The principal references used for homing under these circumstances are landmarks, which provide navigational information only if the insect has learned the spatial relationship between the landmarks and the goal.

This chapter deals with the role of orientation flight behavior in learning landmarks. Many studies of this behavior have attempted to examine the contents of memory using insects that are already experienced foragers; it is also necessary to examine the acquisition of the spatial memory as a bee gains experience in the world outside the hive. The study of acquisition will provide insights into the speed and timing of learning and help us understand the contents of the representations that guide homing behavior. To date, the studies examining the acquisition of memory have examined small-scale landmark learning associated with pinpointing a food source. There is a large gap in our knowledge of the acquisition of larger-scale landmark information which needs to be investigated in order to understand the problem that faces the insect nervous system.

In Fig. 1, I present a conceptual model to describe the different problems faced by insects using landmarks for homing over different spatial scales. Recall that the basic distinction is dependent on the insects' location; does the task require orientation to visual cues directly associated with a goal (such as a food source, or the nest) or does it necessitate orientation across large distances over which the cues associated with the goal may not be visible from the starting location? Orientation at close range (or small scale) may involve the recognition of learned landmarks that are directly associated with the goal or visual characteristics of the goal itself, for example, the shape of flower, or a circle of pine cones around a nest entrance on the ground. This behavior requires that the insect match previously learned visual information with visual images that are currently experienced. For instance, if a honey bee forager finds itself displaced to either of the two points marked with a solid circle on Fig. 1, it will use aspects of the hive itself as to guide their return home. The goal, or landmarks immediately adjacent to the goal, functions as a beacon for the homing insect.

If bees are displaced to a location where they can no longer see the goal itself but they can perceive landmarks closely associated with the goal, homing can also be relatively efficient. For example, bees can learn to recognize particular tree lines (or other landscape features) that can continuously guide the approach to the unseen nest (von Frisch & Lindauer 1954). This case applies to locations indicated by the open circles in Fig. 1. The insect would learn a particular visual feature in association with the goal and orientation toward the learned feature would bring the bee quickly into the range at which the target itself could be seen.

Finally, for orientation over long distances to a familiar but unseen goal, animals must be able to use references visible at both the starting point and along its route, such as landmarks and celestial information. Honey bees' ability to choose the homeward course from a place that does not have a current view of the nest itself or of associated landmarks requires the linkage of previously learned visual associations that are strung together in a particular order (Baerends 1941; Dyer 1991) The visual images that are seen at separate times during the flight must be used, or recognized in a context dependent manner. The insect must be able to link the content of their visual images to one another to accomplish homing from distant locations. In Fig. 1, if bees can choose the homeward course upon displacement to the location of the squares, then they have developed a route memory connecting their current location with their destination. Through the recognition of familiar landmarks used to set the initial course would have to be different from those used later in the homing path.

Recent studies of how naive insects learn about landmarks have focused on the orientation flight, a conspicuous motor pattern that many insects do while departing from a place to which they will later return. Orientation flights have been observed in a variety of hymenopteran species (Wolfe 1926; Rau 1924; Frison 1930; Tinbergen & Kruyt 1939; Baerends 1941; Gaul 1951; Chmurzynski 1964; van Iersel & van den Assem 1964; von Frisch 1967; Wcislo 1992; Zeil 1993 a,b; Collett 1995). von Buttel-Reepen (1900) believed that orientation flights provided bees with visual and olfactory impressions that would allow for homing (in Becker 1958). Orientation flights at the nest entrance begin when a departing bee turns and faces the hive instead of directly departing. While facing the hive, the bee will hover back and forth, apparently looking at the hive entrance. Next, the bee shifts her body in arcs until she is circling in front of the hive, increasing the size of these movements until she is circling in the immediate vicinity of the hive. The spiraling flight takes the bee away for 4-6 minutes, after which the bee will return to the hive. She may do one or more additional orientation flights before beginning to forage. Opfinger

(1931) and later, van Iersel and van den Assem (1964), demonstrated the importance of orientation flights in the learning of small scale landmarks around a goal and numerous studies have subsequently explored the orientation flight on this small scale.

Becker (1958) was the first to establish a role for orientation flights in learning the larger scale landscape features that bees need to use in homing. She caught bees when they returned from their first flight, marked them, and then displaced them to various sites in the landscape. From a range of distances within 700 m of the nest, many bees returned home, whereas bees displaced without orientation flight experience did not. Thus, the bees that did orientation flights must have learned something about the landscape that allowed them to find home. The homing success was poorer from greater distances, which presumably reflects limits to the spatial scale that the orientation flight covers (Fig. 2). Becker (1958) also found that the ability to return home after a single orientation flight generally improves with increasing age. Intriguingly, bees with previous flight experience were more successful at homing from all distances. This suggests that something about their experience had allowed them to acquire more information during the orientation flight. Landscape features such as forests or hills did not hinder homing after an orientation flight (Becker 1958).

Becker's work, although highly intriguing, employed a relatively crude assay of landmark learning and left a number of questions unresolved. Becker's experiments did not establish whether the orientation flight enables homing by exposing bees to the visual features of the hive or the immediate surround, or whether it allows the bees to learn landmarks located far from the hive. Homing from greater distances could be based on random or systematic search strategies that would lead the bees to a place from where they could recognize the nest. Second, Becker's studies (1958) did not attempt to explain the distance effect in homing; it is unclear whether the differential homing success is a result of the bees being out of view of the nest environment or some other factor. In addition, the difference in homing ability between bees with different levels of visual experience is not

adequately explained. The difference may be the result of the distances covered by the experienced and naive bees on the orientation flight, or result from experienced bees being able to learn to form route maps during the orientation flight while naive bees cannot. These are some of the questions that motivated this study. However, the importance of compiling basic descriptions of performance by bees with limited experience in the environment needs to be recognized at the outset. It is my intention that this research should contribute to our understanding of the nature of representation of spatial information in honey bees and serve as a first step toward answering some of these questions.

As did Becker (1958), I observed the homing behavior of honey bees displaced from the nest after a single orientation flight. This protocol tests landmark learning on the landscape scale; homing is only possible if the bees could recognize familiar landmarks. Path integration is not possible because the insects were not given the opportunity to integrate their position during the outward path. Hive odor was a possible cue for homing, but the distances of the release sites made it highly unlikely. Furthermore, Becker (1958) found that bees deprived of an opportunity to do an orientation flight could not find their way home; some bees displaced to the area immediately in front of the hive were able to crawl home, but only after long periods of time. Presumably, these bees were only using odor to orient.

My assay of homing by displaced bees builds on Becker's experiments through the use of specific methodological improvements. This allows me to address questions that Becker could not. First, like Becker I chose release sites covering a range of directions and distances, but I also based my choices on the model in Fig. 1; this allowed me specifically to examine whether bees can learn only landmarks in the vicinity of the nest, or can learn the relationship between visually isolated sets of landmarks. Second, Becker released groups of bees at the same time and did not track individual bees' homing performance. I released bees singly so that I could measure homing based on individual

performance. By releasing bees one by one, I was able to measure the bees' initial departure direction upon release at each site; the determination of a bee's vanishing bearings allowed me to determine the initial orientation of bees upon release. Third, I employed an improved assay of homing success using the flight times of individual bees flying between the release site and the hive. With these data, I could make statistical comparisons between the homing success of bees in each category of visual experience. Finally, I used bees with full flight experience in the test site as a comparison by which to judge the performance of partially experienced bees.

For the main experimental series, I used 6 release sites, all far enough from the nest to deprive the bees of a view of the nest, and otherwise differing in distance, direction, and whether it offers a view of the landmarks around the nest. Three main predictions can be deduced from the model illustrated by Fig. 1. If only features of the hive itself are learned during the orientation flight, then I expected random orientation from all release positions. If the orientation flight functions for learning features of the local panorama around the hive, then I expected homeward orientation from sites with a view of such landmarks immediately surrounding the hive, and random orientation from sites without a direct line of sight of the nest environs. Finally, if route memories are formed during the orientation flight, then directed orientation from all sites would be expected, regardless of the sites' location in reference to the hive; I anticipated, however, that there would be a ceiling effect related to release site distance.

In addition, I partially replicated the experimental procedures in a different landscape in order to further document the function of the orientation flight. In this second location, I used 4 release points that differed in their compass direction from the hive, but were equidistant from it.

## **METHODS**

#### <u>Bees</u>

The colonies of bees used in the study were part of a university apiary maintained by the Dyer research group. A mix of European subspecies, predominantly the Italianderived <u>Apis mellifera linquistica</u>, characterized the bee population in the area.

# Study Sites

The primary experiments were conducted in an abandoned pasture adjacent to farm land at Michigan State University during the months of May through September of 1993, 1994, and 1995. The landscape consisted of gently rolling terrain with a mix of old fields, planted fields, and woodland (Fig. 3). This site is referred to as "Greiner," after the owner of the property. Release locations A through F were established to cover a range of factors that might affect orientation ability; most sites differ in the distance and the direction from the hive. All sites also differ in the panoramic scene visible from the release point.

In an attempt to isolate the influence of direction on orientation ability after the orientation flight, I used a second site, "4-H Fields," with bees in the two manipulated treatments. This site is located in the center of quadrat of 3 alfalfa fields and one wheat field. The wheat field occupied the northwestern quadrat of the larger area. Reorienting foragers were treated as they were in the Greiner site. The four release sites were located 100 m from the hive in the four cardinal compass directions (Fig. 4).

#### Treatment Categories

Building on Becker's (1958) studies of the orientation flight, I tested the homing ability of bees with various degress of experience in a given terrain. I studied bees in three categories of experience. The details of handling the bees prior to their use in an experiment varied with treatment which I will discuss more fully in a later section.

"Reorienting foragers" were worker bees that had foraging experience in a different landscape prior to the experiment. These bees came from small, queenright colonies that contained between 4,000-12,000 bees and were maintained in a small forest clearing 8 kilometers east of the study landscape. At this location, these bees were free to forage in the surrounding environment as members of healthy colonies. When introduced to the testing site, they performed an orientation flight on their first departure from the nest.

In contrast, "First-Flight bees" had never been outside the nest prior to the experiment, and hence were visually naive. Visually naive colonies were created using worker bees that emerged from their pupal cells in an incubator and were then installed into an observation hive. Two Langstroth frames of comb containing capped brood, pollen, and nectar served as the foundation for each colony. Except for a mated queen, the only adult bees in these hives were bees reared in the incubator. Each 'naive' colony was kept inside the lab and fed 30 % sucrose solution when not employed in an experiment. The study site was the only landscape that these naive workers had the opportunity to explore, and their only flight experience in this terrain occurred during the orientation flight for the experiment. These bees were tested starting 5-7 days after they emerged as adults, and were sufficiently mature to begin flying.

Finally, "Resident bees" were bees from a freely flying colony that was located in the test location. A two-story commercial colony was placed in the testing landscape (Site H in Fig. 3) and allowed to forage normally for at least two weeks. Foragers, therefore,

would have ample opportunity to learn the landscape features in the vicinity of the hive, and any functional differences in orientation between these Resident bees and bees in the two other treatment categories would be detectable. Bees in this third category of visual experience are unlikely to have performed orientation flights prior to foraging. I tested their homing ability after capturing them on their return from a foraging flight.

## General Protocols

On the night before an experiment with Reorienting foragers, the research assistants and I closed one of the hives after injecting smoke into the entrance, and then placed it in the back of a truck for the drive back to the lab. The next morning, we moved the hive to the testing landscape. Some aspect of this experience induces the foragers bees to perform reorientation flights on departure from the nest on the subsequent morning. Preliminary trials with this procedure indicated that at least 95% of the bees that fly out of these colonies during experimental manipulations have at least some previous flight experience, based on trials in which all bees with experience in the training site were identified with a self-marking system (Boylan-Pett 1991). For trials with First-Flight bees, we carried the colony from the lab to the field site on the morning of the experiment. Workers in these hives were sufficiently motivated to orient without the addition of smoke.

Bees in these two groups (Reorienting and First-Flight bees) were allowed to complete one orientation flight in the novel landscape by moving a closed bee hive into the pasture and placing it at the location marked with an H in Fig. 3 for experiments at Greiner and in Fig. 4 for experiments at 4H Fields. After a 20 minute settling period, I opened the colony and allowed 60-100 bees to depart. I then closed the colony and removed any bees that remained on the colony entrance. Bees that departed typically conducted orientation flights at the hive entrance. When they returned, I captured each bee singly in a 20-ml glass vial and recorded the duration of its time away from the nest during the orientation flight.

The method of capturing the bees differed by necessity for experiments with Resident bees In this group, I captured foragers as they landed on a screen that had been placed loosely on the entrance of the hive. This screen slowed the bees' entrance to the hive, and allowed us to capture them in vials. Bees carrying pollen, or bees that were obviously engorged with nectar, were preferentially chosen in order to ensure that the bees we selected were foragers and not simply bees returning from an orientation flight. The screen was removed after 40 bees were captured. After these bees were collected, they were handled like the bees in the other treatment conditions. I replaced the screen over the colony entrance when the bees were released to facilitate their subsequent identification and capture.

The vials were placed in an ice filled cooler. After chilling (2-5 minutes), each bee was labeled individually with a plastic numbered tag, placed in a clean vial, and fed with an unscented, 25% sucrose solution. This chilling procedure was performed in order to facilitate the identification of the bees; all treatment groups received the identical treatment. There is some evidence that localized cooling of small parts of the bee brain affects the formation of long term memories (reviewed in Menzel 1990), but there is little evidence that cooling the whole bee affects memory (Menzel et al. 1974) or learning in this context. For a given trial, we displaced one group of 40 bees to one of 6 different sites in the landscape and released them one by one. Observations of their homing ability were recorded by two or three human observers (see below).

I conducted a short control experiment to determine if unknown features of the Greiner landscape might have biased the orientation of bees in ways independent to the orientation flight itself. In particular, I wanted to know if there was any tendency for bees that had not completed an orientation flight but were motivated for homing to head toward the location where the test hive was normally placed. For this control, I trained bees from a colony at the Entomology Teaching and Research Site (Entomology Site) to find a feeder located approximately 3 m south of the colony. It is unlikely that any bees trained in this

way had any experience foraging at Greiner due to its distant location and the ample availability of flowers around this site. I marked the bees with plastic number tags while the bees drank sugar water from the feeder. After at least three days of foraging experience, the bees were captured from the feeder in vials just as they were about to head home. I released these bees singly at Greiner site A, which is west south west of the normal location of the test hive at Greiner. I expected that the bees would not be oriented toward the location of the test hive, and that they would not actually reach the hive in the test position. If anything, bees might be expected to fly north, the direction of the homeward flight from the feeder at the Entomology Site, or to the west, the actual direction of the Entomology Site from Greiner. If bees headed toward the location of the test hive at Greiner, then I would conclude that bias existed that tended to draw bees toward that location. This result would indicate that the orientation flight does not provide information that was not already available to bees flying in the landscape for the first time.

# Data Collection and Analysis

Except where noted, we tried to test bees only on warm and sunny days to remove any confounding influences of weather. Only one hive and one release site was used for one experiment on each test day. Each bee's vanishing bearing, or the compass bearing at the moment when the bee vanishes from human sight, was measured with a compass (Dyer 1991). Bees usually flew in a few wide circles just after release, then chose a flight direction leading away from the release site. Most bees could be followed by sight for more than 40 m from the release site. Bees lost from view within 10 m, or those still moving on a circular flight path when they vanished, were not measured. Some bees needed additional food in order to fly; we fed these lethargic bees sugar solution from a pipet and allowed them to rest for at least 3 minutes prior to their release.

In addition to the vanishing bearing, we measured the bees' travel time between the release site and their arrival at the hive using synchronized stopwatches. The hive was monitored for 45 minutes after the last bee was released on that day.

We conducted multiple trials in each treatment category until we had compiled at least 40 vanishing bearings per site for each treatment. This required many more than 40 releases per site because departing bees were often lost from view prior to obtaining a reliable vanishing bearing. There was no reason to believe that this resulted in a biased sample; the use of this measurement has become common in studies of insect orientation (Gould 1986; Dyer 1991, 1993).

The vanishing bearings were plotted on polar histograms to illustrate the directions flown by the bees. Statistical analyses of the circular distributions follow Batschelet (1981). The mean angle of a distribution of vanishing bearings is the 'center of mass' of the data on a polar histogram. The mean vector length, r, is a measure of the concentration of the data points, or the degree of clustering (Batschelet 1981). For perfectly aligned data, r = 1 while for randomly distributed data, r = 0. The Rayleigh test was used to determine if the circular distributions were nonrandomly distributed. The mean angle is considered significantly different from a predicted angle if the prediction lies outside the 95% confidence interval of the mean, as determined from Fig. 5.2.1 in Batschelet (1981). The angle of deviation is influenced by the length of the mean vector, r, and the sample size (Batschelet 1981, pg 86).

The data on homing speed of the bees were treated with survival analysis. This statistical method employs nonparametric procedures to examine data for which there is defined a point event, conventionally called "failure", that occurs after a given length of time (Cox & Oakes 1984, Muenchow 1986). In this case, the homing time, or the time elapsed from a bee's release until its arrival at the hive, is equivalent to its "failure time,"

even though by failure to stay in the field it is actually succeeding in its goal to reach home. The analysis examines the distribution of homing times from a particular treatment group or release site, and can compare different distributions statistically.

One common feature of survival data is the presence of censored data due to the termination of the experiment or to the disappearance of experimental units. (SAS User's Guide 1988). Most event time studies have data with three common elements; they are right censored, they are collected through repeated measurements over time, and they are non-normally distributed (Fox 1993). The procedures for dealing with this type of analysis originated in human demographics and in industrial reliability testing. Using this approach, the interval time of each uncensored individual in the experiment is used without the bias that results from applying parametric methods with rigid distribution requirements.

An observer stationed at the hive during the releases captured marked individuals and noted the time of their return. From synchronized stopwatches at the release point and at the hive, I could determine the flight duration to the nearest minute. Bees that did not return within 45 minutes were not included in the analysis because I only observed the hive for 45 minutes after the release of the last bee. This procedure placed an upper limit on the homing time, which resulted in a right-censored distribution of homing times. Using the SAS procedure LIFETEST in a computer program written by Gordon Fox (1993), I completed nonparametric estimates of the survival distributions and computed rank tests for association of the response variable, homing time, with the variables of treatment and release site within a single factor. Since this is a univariate procedure, I ran the LIFETEST program using treatment and release location as separate factors; that is, the data were grouped by treatment (or category of visual experience) and subjected to the analysis, and then regrouped by release site and run through the program again. By examining the data from the perspective of both factors, I can construct a picture of the influence of these factors on the response variable, return flight time. These methods allow for a description of the cumulative frequency of bees homing across strata and a comparison of the observed

with the expected number of returning bees in a given time interval. Wilcoxon tests tested the homogeneity of the survival curves based on each factor. These tests of equality ask the question, 'do the distributions differ between the three treatments?' The Wilcoxon rank statistics and covariance matrices were used to conduct pairwise comparisons among the treatments and among the release sites (Fox 1993, 1996).

Other linear statistical methods follow Zar (1996).

# RESULTS

## Duration of the Orientation Flight

For the experiments at Greiner, I used a general linear models procedure to test for the effects of the two treatments on the duration of the orientation flight. The distributions of the orientation flight times are not normally distributed, however, I tested the model using 2243 data points from individual bees, so parametric analyses are appropriate (Zar 1996). There was a highly significant effect of treatment (F=13.99, df = 1, p<0.002) on the duration of the orientation flights of bees in each treatment group. Bonferroni T tests revealed that the Reorienting bees had a higher mean return time (N = 1243, X = 9.6 min) than the First-Flight bees (N= 1001, X = 8.4 min). Therefore, Reorienting bees take a longer orientation flights than First-Flight bees, which may bear on differences in homing behavior between these 2 groups. This analysis could not be completed on the bees at 4H Fields because I studied only one treatment group

# Homing at Greiner

# Control Experiment for Orientation Bias at Greiner

The vanishing bearing for bees displaced to Greiner Site A from the Ent feeder are plotted in Fig. 5. As illustrated by the polar histogram, the bees are randomly distributed (Rayleigh test, z=0.9633, n=57, n.s.). Additionally, out of 91 bees released across three days, only 1 arrived near a hive that we placed in the experimental position. It is unlikely, then, that bees in the experiments would find the hive using some feature intrinsic to the landscape but not related to the orientation flight.

# Vanishing Bearings

Table 1 lists the distances and compass headings of the six release positions used in these experiments. Sites A, B, and D correspond to the open circles on Fig. 1. The remaining sites, C, F, and E, correspond to the open squares on Fig. 1. At all release locations, Resident bees were nonrandomly oriented and headed in the predicted homeward direction (Fig. 6, 9, 12, 15, 18, & 21). Table 2 summarizes the conclusions from the vanishing bearing data which suggest that randomly selected foragers were well acquainted with the landmarks around the release sites.

Sites A and B were both located in the same part of the landscape as the hive and offer a view of landmarks directly associated with the hive (Figs. 1 & 3). The sites were located in different compass directions from the hive, however. Both Reorienting foragers and First-Flight bees were nonrandomly oriented and headed in the homeward direction upon release at both Site A (Figs. 7 & 8) and Site B (Figs. 10 & 11). Site D was similar to Sites A and B in that it also offers a view of the landscape around the hive, although it is located quite distant from the hive landscape (Table 1). Again, Reorienting foragers and First-Flight bees were significantly oriented in the homeward direction from this site. It is

not likely that bees could see the hive from this location, but it is probable that bees could access other visual orientation cues (landscape scale features such as woodlots or treelines) that allowed them to successfully choose the homeward direction.

Site C was visually separated from the visual panorama around the hive by a woodlot and a small hill but it is located relatively close to the hive (165 m). At this site, Reorienting foragers were nonrandomly oriented and headed in the predicted direction (Fig. 13), while First-Flight bees vanish in random directions (Fig. 14). That bees with previous flight experience are clustered toward home suggests that they had learned something about its location relative to the nest during the orientation flight, while naive bees did not.

Both Reorienting foragers and First-Flight bees were well oriented upon their departure from Site D (Figs. 16 & 17). From Site D, which was located 500 m west of the hive, human observers could see the hive in the Greiner landscape, but it was unlikely that bees could see the hive itself. It is possible, however, that the bees could see the large treelines and other landmarks located near the hive to guide the bees' flight to the nest. Other possible explanations for the clustered vanishing bearings here could be wind coming predominantly from the west, or the position of this site on the high end of a hill. Additionally, if bees oriented their departures toward the azimuth of the sun (which was in the east during these experiments) (von Frisch 1967; Vollbehr 1975), then it may not be possible to attribute homeward orientation of either group to landmarks.

Site E was both visually and spatially separated from the hive and the home landscape (Fig. 3) and presents a homing task that is similar to that indicated by the open squares in Fig. 1. While the mean vanishing bearing indicates nonrandomness, Reorienting bees were not headed in the homeward direction (Fig. 19). The vanishing bearings of First-Flight bees appeared clustered, but within very wide confidence intervals (Fig. 20). Bees in both categories of visual experience generally departed in an easterly

direction, and do not appear to be oriented homeward. In addition, at this site, bees often circled high into the air before flying in a given direction, which may be an indication of their uncertainty (Wolf 1926; Menzel et al. 1990; Schöne et al. 1995)

Like Sites C and E, Site F also does not offer a direct view of the hive, or of landmarks associated with it and thus presents a task equivalent to that indicated by the open squares in Fig. 1. This site is located only 70 m behind the hive (Fig. 3), but it offers a much different visual scene than other sites; it is surrounded by a series of woodlots and a few dead trees. Reorienting foragers were oriented and headed homeward at this site (Fig. 22), but First-Flight bees, while significantly oriented, did not consistently choose the homeward direction (Fig. 23). Like the bees in these treatment categories at Site E, the bees tended to depart toward the east. Upon release from the vials, the bees often circled extensively and flew high overhead before disappearing.

#### Homing Speed

I used survival analyses to examine the flight times between release point and the hive. The cumulative frequency of bees homing (=failure) time and its standard error were calculated by the SAS procedure LIFETEST, which uses the chi square approximation for Wilcoxon tests, and plotted against time.

The homing curves of Resident bees to the Greiner site are shown in Fig. 24. Generally speaking, the homing curves for bees released from all sites are similar in shape; there is rapid initial accumulation over the first 10 minutes, followed by gradual slowing of the rate of arrival at the hive. However, separation of the curves implies that the bees at each site may differ from one another. The Wilcoxon test supports the implication that the treatment groups differ ( $X^2 = 21.7$ , df = 5, P>0.0006). This statistic means that homing from the 6 sites differs, but does not reveal which sites are differ from one another. I used the covariance matrix for the Wilcoxon statistic that is generated by the SAS procedure to calculate Z statistics for each pairwise comparison using a Bonferroni adjustment to stabilize the experiment-wise error rate, as suggested by Fox (1993). Table 3 contains the results of these multiple comparisons; in only three cases do these tests indicate statistical differences between two curves. Each of these significantly different comparisons involves Site D, a distant release point, and other sites close to the hive. There is considerable overlap on the cumulative homing frequency of bees from each site during the final time interval, which supports the notion that there are not many differences between the homing abilities of Resident bees across sites. The differences that do exist imply that homing is faster from closer sites.

Fig. 25 illustrates the cumulative homing frequency of Reorienting foragers at Greiner. The Wilcoxon test of homogeneity of the homing curves supports the inference that the release sites differ ( $X^2 = 75.63$ , df = 5, P>0.0001). Bees released at site F, the closest release location, have the highest percentage of return. The two most distance sites, D and E, have curves with the slowest rate of return and after 29 minutes, begin to diverge. The curve for Site A resembles the curve for Site B until the 15 minute mark; after this time, the accumulation rate of bees at the hive resembles bees released from Site C. The multiple comparison testing reveals statistical support for the patterns illustrated by Fig. 25; plots of homing frequency that resemble one another (for example, Sites D and E) are not statistically different from one another. Curves that appear visually distinct from one another are statistically different. The majority of the pairwise comparisons indicate statistical differences between the curves (Table 4). Homing is generally faster from the nearer sites.

The homing frequencies of bees released after their first orientation flights (First-Flight bees) are presented in Fig. 26. In this figure, there is a dramatic difference between the shapes of the curves for bees released at the most distant sites with those at the closer sites. Sites D and E have a similar form to one another, but are separated from Sites A, B, or C. Site F, which does not share a similar visual surround with the other sites, is closest release location; the cumulative homing curve at this site is intermediate to the other curves. The populations of First-Flight bees differ ( $X^2 = 269.26$ , df = 5, P>0.0001) as given by the Wilcoxon test of homogeneity across the release sites. Pairwise multiple comparisons between the release locations indicate statistical differences between most sites (Table 5), as expected based on the curves in Fig. 26. Only three sites, A & B, B & C, and C & F, are not different from one another. Once again, there is a distance effect.

In Figs. 27 through 32, I have plotted the cumulative homing frequencies of bees in the three treatment categories by release site. Table 6 summarizes the results of the Wilcoxon tests of homogeneity of the three curves at each release location. Site F, which was nearest the hive, is the only homogeneous site; at all other release points, there are significant differences between bees in each treatment (Table 6). Multiple comparisons based on Wilcoxon rank statistics and a Bonferroni adjustment to keep the experiment-wise error rate to  $\alpha = 0.05$  were conducted to determine which of the treatments differed from one another. The results of these tests are presented in Table 7. At both Site A and Site B, First-Flight bees accumulate more rapidly at the hive than bees in the two other treatment categories. Resident bees did not differ from Reorienting bees, however. The similarities between bees at these two sites is illustrated by Figs. 27 and 28. Fig. 29 describes the homing of bees released at Site C. Multiple comparison testing demonstrated that Resident bees are not statistically different than First-Flight bees, a result unexpected based on patterns of vanishing bearings at this location.

At Site D, Resident bees had a faster rate of homing than First-Flight bees, while the other two comparisons indicated no significant differences in homing. Figure 30 illustrates that Reorienting bees at this site appear intermediate to Resident and First-Flight bees, however the three treatment groups have very similar shapes. Site E differs from Site D in that the three treatments have different rates of arrival at the hive. (Figs. 30 and 31). Many of the Resident bees return from this site quite rapidly; the curve for this treatment reaches a fairly high plateau after about 15 minutes. For the other treatments, the rate of rise is much different. Resident bees have significantly different homing behavior compared to both Reorienting foragers and First-Flight bees as revealed by the multiple comparison tests. Bees in the latter two categories are not significantly different from one another (Table 7). Figure 32 is a plot of the three treatment categories at Site F; at this site, there are no differences between the cumulative homing curves.

#### Flight Behavior Of Displaced Bees On Release

I observed the bees as they departed from the vials, and although it is difficult to quantify and describe the flight behavior, I noticed a behavior that bears mention and further discussion. The bees would circle around the release position prior to departure during a typical release. Occassionally, after the bee had disappeared from the area, we would notice a marked bee flying around the site; inevitably, this bee would interfere with our ability to observe other bees. If the bee persisted in flying around the observers for more than a few minutes, we would capture the bee and record the identity of the bee that had returned to the release position. We could then continue to release other bees without the hindrance of multiple bees flying in the vicinity. Bees that were captured were never released a second time. Only a small percentage of bees that returned in this way were not captured.

While conducting the experiments, I noticed that there was a difference in the number of bees returning to the release site; the behavior occurred most often at the distant release sites and numbers seemed to vary with treatment. Bees sometimes exposed their Nasanov gland after emerging from the vial but prior to departing from the site; it became obvious that 'scenting' bees were more likely to return to us than bees that did not scent, although I have no formal data to confirm this observation. Table 8 shows the percentage of the bees in each treatment released at each site that exhibited this behavior. Resident bees at Greiner never returned to the release position. Small percentages of bees return to the release site for the Reorienting foragers, and the highest percentages occur for the First-Flight bees at Sites D, E, and F. None of these sites offer a view of the hive or its nearby landscape features. Table 9 indicates the results of six 3 x 2 G-tests of the null hypothesis that the number of bees returning to the release site is independent of treatment. At Sites A, B, and C, the number of bees exhibiting this behavior was independent of treatment, but at Sites D, E, and F, the number of bees returning to the release site was not independent of the bees' visual experience.

These results may indicate that bees at Sites D, E, and F do not have enough information with which to home efficiently. The high percentages of First-Flight bees returning to these sites after departure may mean that they cannot choose a homeward direction. (It is interesting, though, that bees that did depart Site D showed homeward orientation in their vanishing bearings.) This behavior, therefore, may be a marker of the bees' confusion; perhaps they cannot find home and return instead to the release position in order to try again, or to center their systematic search strategy at the location of their initial confusion. Becker (1958) also reported that visually naive bees return a few times to the release position before leaving the area, although she reported this behavior from a site within a 'closed' forest (in Becker 1958 pg 8).

Note also that the bees that returned to the release site and were captured were not included in the homing data that were used in the survival analyses; I assumed that because these bees were not given the opportunity to find home that they should not be included in the calculation of the homing rate. However, this decision, based largely on my decision to maximize our ability to record vanishing bearings on departing bees, may have influenced the results of the subsequent survival analysis.

An interesting situation involving this flight behavior occurred during an experiment when the weather did not cooperate with my plans. On 3 August 1994, we initiated an experiment with a naive colony. Normally, we only conducted experiments when celestial cues were available to the bees, but on this day, the First-Flight bees completed their orientation flight under a completely overcast sky. Both the sun and blue

sky were occluded by a thick cloud cover. Between the time of the orientation flight and the release time at Site E, the sky had cleared and the sun appeared. Of the 37 bees that we released, only 3 bees returned to the hive; 30 bees returned to the release site and were captured there. Four bees remained unaccounted for at the end of the experiment. In other words, 81% of the bees released on this day returned to the release point. On no other occassion did this behavior occur with such a high frequency.

One explanation for this result might be the change in the sky conditions between the time of the orientation flight and the time of the displacement. It is possible that the novelty of the celestial conditions induced confusion in the bees; the First-Flight bees were not given the opportunity to learn anything about the sun or the sun-linked patterns of polarization in the sky during their orientation flight. The appearance of solar information may have interferred with the bees' ability to use learned landmark information to choose the homeward direction. Perhaps the importance of learning celestial cues outweighed the bees' motivation for homing. Another possibility is that the shape of the orientation flight is different under an overcast sky, as would be suggested by the results of Vollbehr (1978) who reported that orientation flights are directed toward the sun's azimuth.

# Homing at 4H Fields

I used a second experimental field in order to replicate the experiments conducted at the Greiner site and to begin a systematic examination of the factors that might affect orientation in the field. The 4H Field location allowed me to vary the compass directions of the release positions within the same visual surround and allowed me to use equidistant release points.

## Vanishing Bearings

Figure 4 is a schematic diagram that illustrates the locations of the release sites in this landscape. The four release sites were located 100 m from the hive in each of the 4 cardinal compass directions. The northwestern corner of the field had been planted with wheat but it had been cut prior to these experiments. The rest of the field was planted with alfalfa. All bees studied at 4H Fields were Reorienting Foragers. Figure 33 shows 4 polar histograms of the directions flown by bees released from each site. Each circular distribution indicates nonrandom orientation behavior with high significance (Fig. 33). At the north, east, and west release sites, the bees chose the homeward direction. Bees departing from the southern release site are not significantly oriented towards the nest, but there is an obvious cluster of points in the homeward direction, indicating that most bees chose the correct route towards home (Table 10).

# Homing Speed

The homing speeds of bees at 4H Fields were analysed with survival analyses. Figure 34 plots the failure time, or the cumulative frequency of homing curves, for bees released at each of the four 100 m sites. The Wilcoxon test of equality between the sites indicated that the bees' behavior differs at each release point ( $X^2$ = 28.12, df = 3, p>0.001). I conducted multiple comparison tests to determine pairwise differences between the 4 sites; the results of these tests are provided in Table 11. Three comparisons, North & South, East & West, and South & West, indicated significant differences between the homing frequency of bees released at each site, while the other three comparisons showed no differences. It is difficult to assess why the speed and rate of homing differed between the 4 equidistant release sites. One possibility is that distant visual cues impacted the bees' capacity to orient. Another possibility is that contrast cues (such as color or motion of the ground cover) from the field itself affected the bees' homing ability. Another explanation that the shape of the orientation flight does not allow for landmark learning in

all directions; it is conceivable that the orientation flight is biased in space toward one direction or another, although it is difficult to determine which environmental factors would be most influential.

## DISCUSSION

The orientation flight is a dramatic turning point in the life of a young bee. Having worked for 2-3 weeks in the confines of the hive, the bee performs a specific behavior that allows her to begin to learn features of the landscape that she will use during the rest of her life as a forager. My research confirms that extremely rapid learning occurs during the orientation flight and the acquired information can be used immediately for homing.

My research has replicated the preliminary findings of Becker (1958) and provided new insights into the acquisition of spatial information that occurs during the orientation flight. My results indicate that the ability to find home when displaced into the landscape after a single orientation flight is partially dependent on the distance of displacement and on previous visual experience. This distance effect implies, not surprisingly, that the quality of learned information is highest for locations near the nest.

The vanishing bearings show that the selection of the homeward direction is not based upon a random or systematic search strategy, but rather that bees can select the home direction upon initial departure from a site experienced during a single flight in the landscape. From none of the release sites should bees have been able to see the hive directly, so homeward orientation implies that bees learn more than just the appearance of the hive during the orientation flight. Homing was better from sites that offered a view of the landmarks in the vicinity of the nest than from sites visually isolated from the nest environs, even when the distance was roughly the same. For example, bees in the same category homed more directly from Sites A and B than they did from Sites C and F, even though the former sites were not that much closer to the nest. In addition, there was better homing from Site D than Site E, even though they were both located quite distant to the hive.

Another important contribution of my research is the documentation of an experience effect on orientation. The vanishing bearing data from Sites C and F indicate that Reorienting foragers can construct a simple route map based on a single flight in the landscape, but newly flying bees (First-Flight bees) cannot. In the normal context of behavioral development, bees may conduct more than one orientation flight prior to the initiation of foraging in order to fully develop their knowledge of the landscape (von Frisch 1967, Wehner 1981). However, from some release sites, with only one departure from the hive, bees could orient as well as bees that have had previous flight experience from some locations. The vanishing bearing data from Site A, B, and D (Figs. 8, 11, & 17) are particularly striking. These three sites correspond to locations that have a direct view of landmarks in the immediate vicinity of the nest, like the open circles on Fig. 1. That naive bees appear as well oriented as bees in the other two treatment categories is quite interesting given their brief flight experience overall, and their limited exposure to this terrain.

From other locations, however, the orientation of the First-Flight bees upon release is generally worse than that of the other groups. The vanishing bearings are no longer as clearly clustered. At Site C for example, the distribution of vanishing bearings shows no clear peak; First-Flight bees depart in random directions (Fig. 14). This constrasts dramatically with the well oriented Reorienting bees' bearings from this site (Fig. 13).

First-Flight bees departing from Site F are not randomly distributed and they are not oriented homeward, either (Fig. 23) These bees do not consistently choose the homeward direction, while bees in the other two treatment categories do fly toward home (Figs. 21 and 22).

How can this difference be explained? One possibility is that the First-Flight bees do not travel to Site C or to Site F during their orientation flight. The structure of the flight may differ between bees with different amounts of visual experience. If they haven't visited the site during the orientation flight, then they have no information with which to guide their homeward path. It is also feasible that the First-Flight bees' orientation flight takes them to the area, but they don't acquire enough visual information there. Perhaps it takes the naive bees longer or repeated flights into the landscape to retain landmark images that do not share a view of the hive's nearby visual features. It does appear, however, that homing from Site C and Site F by First-Flight bees is a more difficult task than for the bees in the other treatment categories.

The vanishing bearings for Reorienting and First-Flight bees at Site E (Figs. 19 and 20) and for First-Flight bees at Site F (Fig. 23) are significantly oriented based on the results of the Rayleigh test, although the bees are not consistently headed in the homeward direction. In all three of these cases, the distribution of bearings is mainly spread out in the easterly direction. The bees fly away toward the east, toward the area of the sky where the sun is located when these experiments were conducted. Vollbehr (1975) has demonstrated that the first orientation flights completed by bees are aimed at the sun's azimuth and that they probably approach the hive after the orientation flight from the direction of the sun. It is possible that the sun affects the behavior of the bees when they cannot orient using noncelestial visual landmarks. The vanishing bearings collected at Site D across all treatments are also headed in the easterly direction, which corresponds to the direction homeward from this site. That there is a bias at Sites E and F may mean that the patterns at Site D are also the result of an azimuth effect. This discrepancy raises questions about the validity of our conclusions based on the bees from Site D. Replicating these experiments at a time of day when the sun was in the western sky, or using release locations with different compass directions relative to the hive, would help elucidate the affect of the sun on homing after an orientation flight.

Given that the vanishing bearing data for bees at Sites A and B in the three treatment appear equally well oriented, the results of the survival analyses are somewhat surprising. At Sites A (Fig. 27) and B (Fig. 28), the First-Flight bees accumulate at the hive more rapidly than bees in the other treatments. Their rate of return is faster than Reorienting bees or Resident bees, and this difference is highly significant (Table 7). How can I reconcile these data with the fact that the bees' vanishing bearings appear to indicate that bees in each treatment have the same orientation ability?

In addition, at Site C, where the vanishing bearings seem to indicate that the First-Flight bees are disoriented (Fig. 14), the corresponding plot of cumulative homing frequency (Fig. 29) indicates that the First-Flight bees are not different than the Resident bees. Or consider the data from Site F: from the homing rate analysis, it appears that there are no differences between the treatments (Fig. 32), but from the vanishing bearings (Figs. 21-23), First-Flight bees are not headed in the homeward direction, and although they are statistically nonrandom, appear to be distributed toward the east.

The discrepancies between the data sets may exist because vanishing bearings only indicate the bees' initial choice of the homeward direction; the bearings may not accurately reflect the flight behavior of the bees after their disappearance from the area. It is conceivable that the bees change their flight direction after I have measured their bearing. I had no way of knowing what they do (or what they see and use to guide their flight) until they arrive at the hive. Any distractions, including the choice of an incorrect landmark, may steer them off course. Alternatively, bees that are nonrandomly oriented (like the case of First-Flight bees at Site C) may encounter a familiar scene immediately after disappearing from my view and quickly discover the route towards the nest.

I made every attempt to remove possible effects of observer bias in recording the vanishing bearings. These studies were conducted over a 4 year period, however, and a number of undergraduate field assistants participated in the collection of the observations.

Due to the nature of the experimental procedure, it was not possible to conduct blind experiments. I am confident that the data represent a conservative measure of the bees' homing ability due to the strict criteria that I established for measuring the flight direction.

In this study, I found that the duration of the orientation flight is longer for Reorienting bees than for First-Flight bees. What might account for this difference? The possible explanations range from physiological to cognitive. Perhaps the First-Flight bees are in some way physically limited in the amount of time that they can spend flying on their first trip from the hive; because they are foraging precociously, there might be a constraint on their ability to fly for longer periods of time. Alternatively, perhaps they did not have enough food to complete their orientation flight. I designed my experiments to minimize weather or wind effects on the orienting bees, but there might have been environmental factors differentially affecting the treatment groups despite my attempts to dampen such effects. First-Flight bees and Reorienting bees are confronted with cognitively different tasks on their orientation flights: First-Flight bees must learn and acquire landmark information, while Reorienting bees are challenged with 're-learning' visual features in their new landscape. In addition, First-Flight bees have never had the opportunity to learn the sun's course; perhaps this knowledge is necessary for efficient orientation or homing. Re-learning might require longer orientation flights because of constraints in the way that 'old' and 'new' memories are processed, separated, or stored. I return to this issue in Chapter Three of this dissertation.

Bees in the First-Flight treatment category were housed in a colony with an even age distribution, which differs substantially from bees in a normal colony. Usually, when bees emerge from the pupal stage as adults, they are the youngest bees in the colony and their tasks are restricted to those at the nest's center. Bees progress through an array of tasks as they age; working at the nest's periphery, including foraging, usually occurs late in the bee's life. It is highly unnatural, then, for a newly emerged adult bee to begin flying on its fifth day of life. Bees are precocious foragers in my experimental colonies. In the

absense of older workers in the colony, the young bees adjust their behavioral maturation and begin foraging (Robinson 1992). This behavioral flexibility exhibited by the workers has a selective advantage; the colony requires the floral resources that the foragers collect in order to prosper. Without pollen, the queen will not lay eggs and the colony will not grow. The presence of precocious foragers in a non-manipulated colony generally indicates that there has been some change in the age distribution in the colony, as would be the case after a swarming event. My experiments involving First-Flight bees represent an extreme example of such an event. Because I repeatedly used these colonies during daily experiments, some of the bees in the First-Flight treatment group may have reached normal chronological age by the time that they managed to leave the hive to do an orientation flight.

I have no a priori reason to believe that the nature of the learning process that occurs during an orientation flight is any different for naive bees in a colony with a normal age distribution. However, questions arise about how the acquisition process may be influenced by colony state. If there are no bees bringing resources into the colony, younger bees become motivated to initiate foraging. Does the development of spatial memory preceed with its normal ontogeny? Do the precocious foragers have any deficit in learning at a younger age? In their motivation to forage, do they sacrifice learning in order to begin collecting food? Perhaps these bees are better learners so they don't need to complete as many or as long orientation flights prior to foraging. Because answers to these questions do not yet exist, I cannot determine if the behavior of the First-Flight bees is in any way different than that which would occur naturally.

The data from 4H Fields examine homing behavior from 4 equidistant release sites and replicate the basic patterns generated at the Greiner site. Each of the release locations corresponds to the open circles in Fig. 1. The 4H data allow me to show that the vanishing bearings show no strong directional bias, at least from release sites near the nest. It is unclear why bees departing from the southern release position at 4H Fields are skewed

in their orientation homeward. It is possible that the barns and woodlots located north of the field had some influence on the initial orientation of bees, but it seems unlikely that bees at the other release sites would not have been similarly influenced. The results of the survival analysis at the 4H Fields site also raise more questions than they answer; why are there differences in the bees' rate of homing? Because all sites were located 100 m from the hive's location, I did not expect to find differences in the bees' rate of accumulation at the hive after release. Again, large landmarks on the horizon may have an influence on the homing rates, but just what, or how, distant cues might affect the bees' flight remains to be further investigated. One substantual visual cue that may have altered the bees' flight is the fact that the field was divided into 2 sections: the smallest area, located in the northwest section of the field, was a harvested wheat field while alfalfa grew in the other three-forths. It is possible that the motion of the plants (alfalfa) affected the bees flight differentially at the east and south release positions. The north and west release points were located on a thin road between the fields. I have no way to determine how the ground cover, or color, might be involved with homing, although it is generally agreed that visual features that extend up vertically from the horizon are more important for orientation than 'flat' features (van Iersel & van den Assem 1964; Wehner 1981).

The orientation flight briefly exposes honey bees to a new terrain and yet this short experience in a novel area allows bees to recognize the homeward direction after displacement from the nest. It is quite remarkable that the orientation flight provides even new foragers with enough visual information for them to find their way home. After an orientation flight, bees can orient without a direct view of the nest, and in many cases, without a view of the landmarks adjacent to the nest. This ability implies that very rapid, one-trial learning occurs during the orientation flight. It is also likely that the formation of a route map can take place at this behavioral transition.

The research reported in this chapter provides a firmer basis for the further study of learning processes in honey bees. First, my experimental approach has been proven as a reliable and robust method for studying performance after known degrees of experience with spatial relationships in a natural environment. Furthermore, I have documented a variety of specific factors that influence the performance of bees in using learned information about the terrain. These include the distance of the release site, the visual isolation of the release site from the nest, and the level of the bee's previous experience in the terrain. Preliminary evidence suggests that the presence of celestial cues is another influence either on the acquisition of spatial information about landmarks or on the use of such information. Further studies of these factors and how they interact should lead to a deeper understanding of underlying learning processes.

#### CHAPTER THREE

# Influences of Landscape Complexity and Flight Experience on the Duration of the Orientation Flight

When bees or wasps leave their nests or a newly discovered food source for the first time, they embark on an orientation flight in order to learn visual aspects of their goal and the surrounding area. Orientation flights are a readily observable behavior at the front of a beehive on warm and sunny afternoons; Buttel - Reepen (1900) described the characteristic behavior as 'playing about' given that it appears that the bees are playing as they hover and shift while looking at the hive (von Frisch 1967). The bees then expand their flights into circles until they disappear from the local area. Orientation flights allow insects to acquire information about the landmarks that are later used for relocating the nest (Wehner 1981). Orientation flight behavior must have been shaped by ecological and neural constraints of acquiring, storing, and using visual spatial memory, and as of yet, there is relatively little known about those constraints (Zeil et al. 1996).

Orientation flight paths could be influenced by the magnetic field or the visual panorama, (Collett & Baron 1994), solar compass cues (Vollbehr 1975), or by local visual cues providing directional information (Becker 1958, Zeil 1993 a, b, Collett & Lehrer 1993). It is not clear at present if the spatial pattern of orientation flights is in any way affected by the local scene around the hive (Zeil et al. 1996). Wehner (1981, pg. 483), in a review of the spatial vision in arthropods, reports that there is "a positive correlation between the number of landmark cues available around the nesting site and the duration of the orientation flights being shortest in bare, featureless surroundings". Unfortunately, the details of this conclusion are not provided. His short commentary, however, raises interesting questions about the nature of the learning process that occurs during the orientation flight.

A priori, it is hard to know whether to predict an increase or a decrease in the duration of the orientation flight as the visual complexity of the environment increases. On the one hand, in relatively uniform, landscapes, with visual landmarks to learn, short initial orientation flights might be sufficient because bees are not likely to learn anything new during longer flights. Perhaps it would take the bees longer time, or repeated flights, to develop the skills necessary to orient from long distances, but the immediate orientation flight could proceed unimpeded by the requirement of learning the relationships between dominant features. On the other hand, it is easy to present an argument that the duration of the orientation should be longer in a uniform terrain. If bees have fewer landmarks with which to guide their return, then they may need more time to gather information sufficient for homing.

This chapter represents a preliminary attempt to investigate the role of landscape 'complexity' on the duration of the orientation flight. In this project, I observed the duration of orientation flights completed by honey bees in two treatment categories, First-Flight and Reorienting (see Chapter 2). Colonies of bees were displaced to two locations that had different visual surrounds. One site had relatively simple structural features, and the other had large numbers of landmarks in the vicinity. The experiments described in Chapter 2 also address this question, but the landscapes in which the releases took place (Greiner and 4H) do not clearly fit into these categories. I designed this experiment as a simple method of describing possible constraints on honey bee orientation flights. I wanted to know if the duration of the orientation flight, and presumably the spatial learning process, was dependent upon visual experience or upon the number of landmark cues surrounding the nest.

## **METHODS**

# **Bees**

The colonies of bees used in the study were part of an university apiary maintained by the Dyer research group. A mix of European subspecies, predominantly the Italianderived <u>Apis mellifera linguistica</u>, characterized the bee population in the area.

# Study Sites

One release location was located on the main campus of Michigan State University, next to the old barn adjacent to the Farrall Agricultural Engineering Building (Fig. 35). This site, situated between Science Road, Farm Lane, and Wilson Road, is referred to as the "Corral" site because it is surrounded by fence. This site was chosen because of the many nearby buildings and objects within the fenced area. Tractors and other agricultural equipment are stored within the corral. The surrounding buildings range from 1 - 5 stories. I have designated this location as a visually complex environment; it was characterized by the large amounts of structure in the nearby landscape.

The other release location is located on the Michigan State University Farms immediately adjacent to a large corn field (Fig. 36). This site is located near the Entomology Teaching and Research facility on Collins Road. In comparison to the Corral, this site is visually simple; there are some large scale landscape features including a single tree in the middle of the corn field, a gravel mound, and distant woodlots, but is devoid of distinctive landmarks that extend above the horizon. The site is 'open' compared to the cluttered Corral site, in that it is relatively devoid of conspicuous visual features. Admittedly, my evaluation of landscape structure may differ from the perception of an orienting insect. I have no way of testing if my assessment of complexity mirrors that which an insect would experience. Furthermore, in this study, I did not replicate the 'simple' and 'complex' sites; therefore, the results provide only a preliminary examination of the influences of landscape complexity and flight experience on the duration of the orientation flight.

## Treatment Categories

Building on my other studies of the orientation flight (see chapter 2), I measured the duration of orientation flights completed by bees at the two locations, the Corral and Collins Road. I studied bees in two categories of experience. The details of handling the bees prior to their use in an experiment varied with treatment.

"Reorienting foragers" were worker bees that had foraging experience in a different landscape prior to the experiment. These experienced bees came from small, queenright colonies that contained between 4,000-12,000 bees and were maintained in a small forest clearing 8 miles west of the study landscape. At this location, these bees were free to forage in the surrounding environment as members of healthy colonies. These colonies were also used in the experiments described in Chapter 2.

In contrast, "First-Flight bees" were not allowed free access to a 'home' location prior to the experiment. Visually naive colonies were created using worker bees that emerged from their pupal cells in an incubator and were then installed into an observation hive. Two Langstroth frames of comb containing capped brood, pollen, and nectar served as the foundation for each colony. Except for a mated queen, the only adult bees in these hives were bees reared in the incubator. Each 'naive' colony was kept inside the lab and fed 30 % sucrose solution when not employed in an experiment. The study site was the only landscape that these naive workers had the opportunity to explore; the only flight
experience that the First-Flight bees had occured during the orientation flight for the experiment. These bees were tested starting 5-7 days after they emerged as adults, and were sufficiently mature to begin flying. The colonies used for the study described in Chapter 2 of this dissertation were also used for these experiments.

### General Protocol

To learn how the complexity of the visual scene around a hive affects the duration of the orientation flight, I measured the duration of the orientation flights that bees completed in each of the two locations. On the night before an experiment with Reorienting foragers, the research assistants and I closed one of the hives after injecting smoke into the entrance, and then placed it in the back of a truck for the drive back to the lab. The next morning, we moved the hive to the testing landscape. Some aspect of this experience induces the foragers bees to perform reorientation flights on departure from the nest on the subsequent morning. For trials with First-Flight bees, we carried the colony from the lab to the field site on the morning of the experiment. Workers in these hives were sufficiently motivated to orient without the addition of smoke. On some occassions, the hive used in the experiments described in Chapter 2 was used on the same day. That a hive was used twice on one day should not have affected any results; bees that were released were never allowed back into the hive, even after their return to it.

Bees were allowed to complete one orientation flight in the landscape by moving a closed bee hive onto a stand in the landscape (see Figs. 35 and 36). After a 20 minute settling period, I opened the colony and allowed a small group of bees to depart. I only allowed small numbers (20-80) of bees to depart for any particular release because my goal was to accurately measure the duration of the flights; when large numbers of bees were released, it became more difficult to measure the time accurately. I made an attempt to standardize the number of bees leaving the hive, but it was difficult to control the rate of their departure. I then closed the colony and removed any bees that remained on the

colony entrance. Bees that departed typically conducted orientation flights at the hive entrance. Using a stop watch, I measured the duration of the orientation flights; I measured the interval between the moment the bees began to leave the hive to the moment that they reappeared. I used the same initial departure time for all bees leaving the hive, but measured the length of time that individual bees were away from the hive. This method of assessing the round trip orientation flight time eliminated difficulties associated with marking and tracking individually marked bees from the hives.

## **RESULTS & DISCUSSION**

The duration of the orientation flight was not affected by the site at which the orientation flight occurred (General Linear Model: F = 2.14, df = 1, P > 0.14; Table 12) but it was significantly affected by the treatment category of the bees (GLM: F = 4.06, df = 1, P > 0.04; Table 12). There was no evidence for a site by treatment interaction (GLM: F = 2.05, df = 1, P > 0.15; Table 12) in the model.

Bonferroni T tests on the means of orientation flight duration indicated a significant difference between the the two types of bees: the First-Flight bees had significantly shorter orientation flights (X = 10.33, N = 249) than Reorienting foragers (X = 12.13, N = 406). This result matches the pattern generated by the experiments in Chapter 2, although in these trials, there is a longer mean duration of flights for bees in each treatment. There was no measured effect of landscape structure on the length of orientation flights but there was an effect of previous visual experience.

What does this result tell us about the orientation flight? I will consider both factors: why there was no measured effect of landscape structure and why was there an effect of previous visual experience? When considering the first question, it is possible that my designation of complexity is not correct; in two different 'simple' and 'complex' landscapes, a difference dependent on landscape might be present. One could argue that

my 'simple' habitat, the Collins Road site, is actually characterized by large scale landscape features that are more similar to the Corral site than I envisioned. It is also possible that the orientation flight does not change in length, but changes in shape in order to accomodate landscape features. My experiments have no way to quantify a change in flight pattern within the landscape. It is possible that the flights in areas with many landmark cues are more focused on particular landmarks: that difference would not be identifiable in my paradigm.

Now I turn to the question of why Reorienting foragers seem to take longer to update their visual spatial memory than bees that are acquiring information for the first time. One possibility is that "re-learning" is a cognitively more difficult task for bees. Past studies of reorientation following colony fission by Robinson & Dyer reported that bees retain visual spatial information about their parent colony after conducting reorientation flights at the new nest location (1993). Perhaps the formation of spatial memory in this case would require more time to be acquired and built into a representation of 'new' memory. First-Flight bees may be taking flights that are restricted in their range (see Chapter 2) which may explain their shorter lengths. This does not necessarily mean that the First-Flight bees learn fewer landmarks, however.

Another possibility is that Reorienting bees, having presumably already developed a knowledge of the sun's course (Dyer & Dickinson 1994, 1996), are less likely than Firstflight bees to get lost on a long flight away from the nest. Thus, they can afford to prolong the orientation flight. Finally, it is possible that First-flight bees have not yet developed physiological capacity to undertake flights as long as the more experienced Reorienting bees.

A further interesting question which is not addressed by my study is whether the navigation abilities of bees change depending on the landscape structure. Bees may have an easier time navigating in an area marked by many distinctive landmarks because certain

features could serve as optical guideposts. The learning requirements in this scenario are quite sophisticated and, right now, we have no method of investigating the development of this form of memory. On the other hand, bees may navigate with less chance for error in a uniform landscape. For example, bees foraging from a nest in an isolated tree in a large field have little opportunity to make a mistake, or mismatch what it has retained in memory, as it makes its way homeward.

In conclusion, my results do not support Wehner's (1981) statement that the duration of the orientation flight is positively correlated with the number of landmark cues available around the nesting site. This experiment is the first explicit test of his rather anecdotal conclusion about the relationship between landscape structure and the orientation flight. Of course, further research with complete replication of the habitat types is required before the influences of landscape and experience on orientation flights can be determined. It is worth mentioning that the orientation flight times that I measured where longer than those measured by Wehner (1981); presumably, this difference is related to differences. between our experimental protocols. From what can be determined from his review, Wehner used bees that were just reaching foraging age within normal colonies; these bees may have been performing initial, short orientation flights. Many of the bees in my experiments, whether First-Flight bees or Reorienting foragers, were highly motivated. Despite the differences in our methods, his assertation about the importance of landscape structure have to be qualified in light of my results.

#### CHAPTER FOUR

# **Future Directions**

Orientation flights are a discrete and readily observable behavior which have been clearly implicated in learning (Baerends 1941; Tinbergen & Kruyt 1939; review in Zeil et al. 1996). An orientation flight is an overt indication that a learning event has occurred, which makes learning in the natural context relatively easy to study. Additionally, reliable assays have been developed for studying what is learned during the orientation flight (Lehrer 1991, 1993, 1996). In recent years, scientific interest in the orientation flight behaviors exhibited by social insects has surged and, as a result, much more is known about the active acquisition of visual information than ever before (Collett 1996; Lehrer 1996; Zeil et al. 1996). Previous research, most of which has focused on the function of orientation flights relative to small scale landmark learning has helped us better understand how the behavior is structured relative to dominant landmarks. Studies of wasps and bees orientation, especially those that require the insect to learn a specific constellation of visual landmarks, are somewhat unnatural, however, in that the insect sees a conspicuous array of landmarks; when foraging in nature, bees and wasps may not be able to rely upon such dominant cues. Nevertheless, this research has demonstrated that the learning associated with a bee's foraging task is not simple and it paves the way for future mechanistic studies of the sensory and neural mechanisms mediating the acquisition of visual information.

This dissertation goes beyond previous work in examining what insects learn about large-scale features of the terrain during their orientation flights. My experiments have established that single orientation flights allow honey bees to acquire sufficient information about the landscape to orient homeward when displaced from the nest up to 500 m from the nest. The acquisition occurs rapidly during the short flights. Successful homing may be dependent on the location of the release position and is influenced by the honey bees' past visual experience.

This research has clarified our knowledge about the acquisition of visual spatial memory thanks to improved methods, but has also left questions that need to be addressed. The purpose of this chapter is to point out important areas of future research. I have organized my suggestions around 5 main topic areas.

# Information Acquired During the Orientation Flight

First, I believe it is important to replicate the experiments using additional sites similar to Sites C and F; these 2 release positions were located close to the hive, but did not share a common visual surround with the hive itself. An experiment carefully designed to tease apart the possible influences of distance, direction, and visual scene on learning during the orientation flight would help us better understand the nature of the neural representation used during insect homing. My research has made partial progress toward this goal. By isolating each factor and employing the 'Becker' technique, the relative importance of distance, direction and visual panorama on homing would be accessible.

Second, the possibility that the sun's azimuth influences the behavior of the bees upon departure after displacement is also worthy of further examination. A structured analysis of the relationship between vanishing bearings and homing speed might also reveal information about the cues that bees use to guide their homing behavior.

# Structure of the Orientation Flight

A major gap in our knowledge concerns the actual flight path of bees during orientation and reorientation flights. In my experiments, I have used homing as an indirect measure of the spatial range of the orientation flight. If we could track the bees through the duration of their time away from the nest, we could directly might be able to reconstruct how bees acquire the spatial 'snaphots' of the landscape. Detailed analyses of orientation flights around newly discovered food sources have revealed structural elements of the flight (Collett 1992; Zeil 1993a, b; Zeil et al. 1996); by understanding the geometry of the flight, the ways that landmark learning is used to guide behavior is more clearly understood (Collett 1995). Knowing the details of the large-scale flight path will tell us the sequence of images to which bees are exposed during the flight, hence what visual cues they have the possibility of learning. The shape of the flight may provide insights into role of the orientation flight in facilitating (or constraining) the storage of large scale memories.

If bees fly in circles that widen with time (as it appears when they are in the general vicinity of the hive), then they will encounter landmarks in a particular sequence that differs from the sequence of images that would be experienced if the bees fly in a more radial pattern. The order that bees view landscape features may be important to understanding how they later use those cues for orientation. Bees are thought to retain images of the landscape in the form of mental snapshots that they use in sequence to guide their flight between points in the landscape (Cartwright & Collett 1987) but how that 'album' of images is acquired or stored in memory is unknown. Can insects place the images in the correct order, even if those images are not acquired in order? Recently, scientists have applied harmonic radar techniques to measure the trajectories of individual flying insects over distances of hundreds of meters (Riley et al. 1996). This new technology may provide the tool we need to measure individual orientation flights within a given landscape, and better understand the development of large-scale spatial memory.

## Beacons vs. Panoramas

Additional manipulations in the tradition of the 'Becker experiments' might also reveal what specific visual information is learned during the orientation flight. Of particular interest is whether bees learn beacons or panoramic views during the orientation flight. I suggest the following experiment. Allow bees to complete orientation flights in a novel landscape and when they return, displace them to another novel landscape and observe their behavior. Can newly oriented or reoriented bees become misled by landscape features? Consider a hypothetical example. If the colony was placed near a large tree in an large pasture with few visual features on the horizon, bees would presumably learn the location of the tree and be able to orient homeward by using it as a beacon. Could bees be

tricked into heading for another tree if they were displaced to a different pasture? Tests of beacon orientation by bees trained to a food dish from a hive have generally failed to find this effect when the hive was displaced (von Frisch & Linduer 1954, reviewed in von Frisch 1967). However, it is possible that homing bees might be misled by landmark cues; outbound bees with previous training are heading for a specific feeding place and thus, displaced landmark beacons compete with compass cues. Bees displaced from the nest after an orientation flight would have no compass information, and so might show beacon orientation. This type of manipulation would provide information about what features were used for homing. In a similar scenario, if bees completed orientation flights at a hive adjacent to a north-south treeline, what would they do when displaced to a site with a prominent east-west treeline? This type of experiment may inform us of the ways in which landmark cues and celestial compass information interact and are learned as references for homing. In essence, these experiments together test the same sorts of questions that von Frisch and Lindauer (1954) examined, but they did so in context of conflict between compass and landmark cues used for finding the food by highly experienced bees.

# Learning and the Brain

On the broadest level, my research bears on the question of how spatial information is represented in the nervous system. Observing observation flights allows me to catch the bee in the act of learning. It should be possible to manipulate the learning opportunities during the orientation flight to create an experimental paradigm for studying learning and for exploring the subsequent internal representation of the learned information.

With honey bees, it may be possible to study the neural correlates of memory, because adult behavioral development is accompanied by structural plasticity in the brain itself. Recent research as demonstrated that the corpora pedunculata in the insect protocerebrum, which are also known as the mushroom bodies, change in volume at the time that bees make the behavioral transition to foraging (Withers et al. 1993; Durst et al. 1994). Mushroom bodies are present in the brains of all insects but are relatively larger in the social Hymenoptera than in other insects (Chapman 1982; Strausfeld et al. 1995). These structures are highly organized brain areas that are currently the object of intense investigation by researchers interested in the molecular basis of learning (Davis & Han 1996). The mushroom bodies receive major projections from the antennal and optic lobes; there is ample neuroanatomical evidence that these structures are a site of convergence for visual, olfactory, and mechanosensory pathways (Homberg 1984; Mobbs 1985; Schürmann 1985). It is likely that the mushroom bodies play a role in cognitive tasks such as orientation in space and time (Menzel et al. 1994).

Withers et al. (1995) have determined that the neuropil of the mushroom bodies expands when the bees start to forage. These researchers have documented that the increase in volume is independent of the worker's chronological age but related to their 'behavioral age;' the change in volume accompanies the transition to foraging, but because that shift is influenced by a bee's likelihood of precocious foraging, age itself may not by an appropriate correlate. Because foraging must be preceeded by orientation, it is likely that orientation flights are the marker of neural reorganization. The honey bee, therefore, is a model system in which to study visual learning, as well as olfactory learning and the relationship between brain space and task performance. A focus on the mushroom bodies and behavioral development by honey bees would allow for the exploration of the neural mechanisms of learning in an animal that has complex behavior generated by a simple nervous system (Fahrbach & Robinson 1995).

In addition to the general linkage between the mushroom bodies and learning, behavioral development in honey bees is mediated by the endocrine system. The division of labor within a honey bee colony -- young bees work in the hive and older bees forage -is affected by the levels of juvenile hormone; high levels of juvenile hormone are associated with foraging, while low levels are present in newly emerged bees (Robinson 1992; Huang et al. 1994). Juvenile hormone is a sesquiterpenoid product of the corpora allata glands of insects that may influence the neuroanatomical plasticity in honey bee

mushroom bodies (Withers et al. 1995). The investigation of the development of orientation in bees with manipulated levels of JH (and possibly with lesions of the corpora allata or the corpora pendunculata) will teach us more about the interactive effects of hormones, neural structure, and cognitive tasks such as learning.

### Development of Orientation Flight Behavior

A further important area of future research would be to document the natural development of the orientation flight itself. We know that bees use these flights to learn the appearance and something about the location of their nests, and also use them as an opportunity to defecate outside the hive. Despite a long tradition of behavioral research on honey bees, almost nothing is known about the factors that trigger orientation flights or about the number of orientation flights that a single bee normally takes before she becomes a forager. A set of baseline studies are required for a complete picture of the behavior. It would be useful to have precise information about the age at first orientation flight, the number of flights taken per day, what time the orientation flights begin each day, the duration of the flights and age at first foraging trip. There is only brief references to the activites performed by orienting bees immediately prior to taking an orientation flight; Vollbehr (1975) simply describes the general state of agitation within the colony and attributes the trigger to the return of forager bees who create a sense of alarm among the bees preparing to fly. I believe that more structured analysis of the activities of the preforagers should be conducted. Only by understanding the natural behavior can the effects of colony manipulations truly be appreciated. My dissertation research is evidence that the orientation flight is amenable to studying learning in a natural context and is sure to tell us more about the nature of spatial representation in the nervous system.

APPENDIX

Release Site	Distance	Homeward Direction
Site A	100 m	<b>79°</b>
Site B	100 m	14°
Site C	165 m	335°
Site D	500 m	<b>94</b> °
Site E	375 m	8°
Site F	70 m	151°

Table 1. Position of release sites in relation to the hive at Greiner

Resident bees Reorienting bees   Nonrandom? Homeward? Nonrandom? Homeward?   Site A Yes Yes   Site B Yes Yes Yes   Site C Yes Yes Yes   Site D Yes Yes Yes   Site E Yes Yes No   Site F Yes Yes Yes   Site F Yes Yes Yes	Table 2 flight	. Orientation	behavior of	honey b	ees releas	ed at	Greiner	after a	single	orientation
Site AYesYesYesYesSite BYesYesYesYesSite CYesYesYesYesSite EYesYesYesYesSite FYesYesYesYesSite FYesYesYesYes		<b>Reside</b> Nonrandom?	nt bees Homeward?	Non	Reorientin random?	g bees Home	ward?	First Nonrand	t-flight lom? F	bees Homeward?
Site BYesYesYesYesSite CYesYesYesYesYesSite DYesYesYesYesYesSite FYesYesYesYesYes	Site A	Yes	Yes		Yes	Ye	S	Yes		Yes
Site CYesYesYesYesSite DYesYesYesYesYesSite FYesYesYesYesYes	Site B	Yes	Yes		Yes	Ye	S	Yes		Yes
Site D Yes Yes Yes Yes Yes No Site F Yes Yes Yes Yes Yes Yes	Site C	Yes	Yes		Yes	Ye	S	No		NA
Site E Yes Yes Yes No Site F Yes Yes Yes Yes	Site D	Yes	Yes		Yes	Ye	S	Yes		Yes
Site F Yes Yes Yes Yes	Site E	Yes	Yes		Yes	No		Yes		Yes?
	Site F	Yes	Yes		Yes	Ye	S	Yes	i	No

Comparison	Z score	Probability {same sample}	Conclusion	
A & B	z= 1.478	p= 0.069	same	
A & C	z= 2.048	p= 0.02	same	
A & D	z= 1.998	p= 0.023	same	
A & E	z= 0.783	p= 0.217	same	
A & F	z= 1.97	p= 0.024	same	
B & C	z= 0.378	<b>p= 0.353</b>	same	
B & D	z= 3.037	p= 0.001 ***	different	
B & E	z= 1.903	p= 0.028	same	
B & F	z= 0.356	<b>p= 0.361</b>	same	
C & D	z= 3.671	p=0.0001 ***	different	
C & E	z= 2.41	p= 0.01	same	
<b>C &amp; F</b>	z= 0.012	p= 0.50	same	
D & E	z= 0.944	<b>p= 0.173</b>	same	
D & F	z= 3.552	p= 0.0002 ***	different	
E & F	<b>z=</b> 2.331	p= 0.01	same	

Table 3. Comparisons of Resident bees homing at Greiner

The comparisons are based on Wilcoxon rank statistics and involve a Bonferroni adjustment to keep the experiment-wise error rate to  $\alpha = 0.05$ . The z-scores listed correspond to their single comparison probabilities. For multiple comparisons, the corrected cutoff point for significance depends on an **adjusted z > 2.932**. Significance of multiple comparison tests is indicated by \*\*\*. These statistics correspond to Figure 24.

Comparison	Z score	Probability {same sample}	Conclusion
A & B	z= 2.323	p= 0.01	same
A & C	z= 1.404	p= 0.08	same
A & D	z= 3.262	p= 0.0006 ***	different
A & E	z= 3.193	p= 0.0007 ***	different
A & F	z= 3.579	p= 0.0002 ***	different
B & C	z= 3.353	p= 0.0004 ***	different
B & D	z= 5.156	p= 1.264E-7 ***	different
B & E	z= 5.078	p= 1.912E-7 ***	different
B & F	z= 0.891	p= 0.192	same
C & D	z= 1.574	p= 0.058	same
C & E	z= 1.523	p= 0.064	same
<b>C &amp; F</b>	z= 4.595	p= 2.168E-6 ***	different
D & E	z= 0.042	p= 0.483	same
D & F	z= 6.684	p= 1.161E-11 ***	different
E & F	z= 6.589	p=2.212E-11 ***	different

Table 4. Comparisons of Reorienting bees homing at Greiner

The comparisons are based on Wilcoxon rank statistics and involve a Bonferroni adjustment to keep the experiment-wise error rate to  $\alpha = 0.05$ . The z-scores listed correspond to their single comparison probabilities. For multiple comparisons, the corrected cutoff point for significance depends on an **adjusted** z > 2.932. Significance of multiple comparison tests is indicated by \*\*\*. These statistics correspond to Figure 25.

Comparison	Z-score	Probability {same sample}	Conclusion
A & B	z= 2.459	p= 0.0069	same
A & C	z= 4.501	p= 3.381E-6 ***	different
A & D	z= 13.204	p= 0.000001 ***	different
A & E	z= 12.0	p= 0.000001 ***	different
A & F	z= 6.985	p= 1.429E-12 ***	different
B & C	z= 1.768	p= 0.039	same
B & D	z= 9.961	p= 0.000001 ***	different
<b>B &amp; E</b>	z= 8.342	p= 0.000001 ***	different
B & F	z= 4.057	p= 0.00003 ***	different
C & D	z= 8.612	p= 0.000001 ***	different
<b>C &amp; E</b>	z= 6.667	p= 1.304E-11 ***	different
<b>C &amp; F</b>	z= 2.386	p= 0.009	same
D & E	z= 3.125	p= 0.0009 ***	different
<b>D</b> & F	z= 6.332	p= 1.21E-10 ***	different
E & F	z= 4.016	p= 0.00003 ***	different

Table 5. Comparisons of First-Flight bees homing at Greiner

The comparisons are based on Wilcoxon rank statistics and involve a Bonferroni adjustment to keep the experiment-wise error rate to  $\alpha = 0.05$ . The z-scores listed correspond to their single comparison probabilities. For multiple comparisons, the corrected cutoff point for significance depends on an **adjusted** z > 2.932. Significance of multiple comparison tests is indicated by \*\*\*. These statistics correspond to Figure 26.

Site	Wilcoxon	DF	Probability	Significance
Α	81.02	2	p>0.001	***
B	23.11	2	p>0.001	***
С	44.68	2	p>0.001	***
D	7.56	2	p>0.02	**
E	29.49	2	p>0.0001	***
F	2.53	2	p>0.28	n.s.

Table 6. Wilcoxon tests of equality over treatment category (by site) for bees released at Greiner

The comparisons are based on Wilcoxon rank statistics using the standard errors of the SAS LIFETEST estimates. Each site was treated separately to compare between treatments. This test compares observed and expected numbers of bees returning in each interval between treatments, but does not indicate which treatments differ. Significance reveals heterogeneity between treatment groups. These statistics correspond to Figures 27 through 32.

Table 7.	Within	site	comparisons	of bees	homing at	Greiner		
Site	Comp	arison			Z score	Probability {same sam	ıple}	Conclusion
Site A	Resid	ent &	Reorienting		z= 2.254	p= 0.012		same
Site A	Resid	ent &	First-Flight		z= 7.293	p= 1.51E-1	13 ***	different
Site A	Reorie	enting	& First-Flight		<b>z=</b> 8.151	<b>p=</b> 2.22E-1	16 ***	different
Site B	Resid	ent &	Reorienting		z= 1.841	p= 0.032		same
Site B	Resid	ent &	First-Flight		z= 3.169	p= 0.0008	* * *	different
Site B	Reorie	enting	& First-Flight		z= 4.63	p= 1.83E-6	* *	different
Site C	Resid	ent &	Reorienting		z= 5.407	p= 3.20E-8	**	different
Site C	Resid	ent &	First-Flight		z= 1.657	p= 0.048		same
Site C	Reorie	enting	& First-Flight		<b>z=</b> 6.511	<b>p=</b> 3.73E-1	]] ***	different

Table	7. И	<i>Athin</i>	site	comparisons	of bees	homing at	Greiner	(con t )	
Site		Comp	arisor			Z score		robability	Conclusion
Site D		Resid	ent &	Reorienting		z= 1.412	d	= 0.078	same
Site D		Resid	ent &	First-Flight		<b>z=</b> 2.673	đ	= 0.003 ***	different
Site D		Reorie	enting	g & First-Fligh	ţ	<b>z</b> = 1.142	đ	= 0.127	same
Site E		Resid	ent &	Reorienting		<b>z</b> = 3.536	d	= 0.0002 ***	different
Site E		Resid	ent &	First-Flight		z= 5.339	đ	= 4.67E-8 ***	different
Site E		Reorie	enting	g & First-Fligh	¥	<b>z</b> = 1.229	d	= 0.109	same
Site F		Resid	ent &	Reorienting		<b>z</b> = 0.367	d.	= 0.356	same
Site F		Resid	ent &	First-Flight		z= 0.059	đ	= 0.059	same
Site F		Reorie	enting	g & First-Fligh	ţ	<b>z</b> = 0.141	đ	= 0.141	same
The com rate to C	npariso ( = 0.0 <sup>0</sup> ities. F	ns are t 5 for thi or mult	ased o ree trea tiple co	n Wilcoxon rank itment categories, imparisons, the co	statistics al analyzed l prrected cut	nd involve a Bor by release site. 7 toff point for sig	lferroni adju The z-scores nificance de	istment to keep the exp isted correspond to the istends on an <b>adjusted</b>	eriment-wise error neir single comparison $ z > 2.932$ .

Significance of multiple comparison tests is indicated by \*\*\*. These statistics correspond to Figures 27-32.

Table 8.	Percentage of be	es that returned to t	he release site
Site	Resident	Reorienting	First Flight
Site A	0	0.63	0
Site B	0	0.40	3.5
Site C	0	2.97	0.71
Site D	0	4.68	12
Site E	0	4.05	18.26
Site F	0	0.57	19.08

Table	9.	Tes	sts (	of	indepe	ende	nce:	is	the	num	ber	of	bees	that
returne	ed	to	the	r	elease	site	inde	epe	nder	nt of	` tre	atn	ient?	

Site	DF	G adjusted	Р
Site A	2	0.023	not significant
Site B	2	5.99	not significant
Site C	2	2.86	not significant
Site D	2	16.01	p<<0.001
Site E	2	30.69	p<<0.001
Site F	2	38.23	p<<0.001

(**α=0.05**)

Table 10. Orientation behavior of honey bees released at 4HFields after a single orientation flight

<b>Release</b> Location	Nonrandom?	Homeward?
North	Yes	Yes
East	Yes	Yes
South	Yes	No
West	Yes	Yes

Comparison	Z score	Probability {same sample}	Conclusion
North & East	z= 2.130	p= 0.017	same
North & South	z= 3.173	p= 0.00075 ***	different
North & West	z= 0.950	p= 0.171	same
East & South	z= 0.965	p= 0.167	same
East & West	z= 3.491	p= 0.0002 ***	different
South & West	z= 4.774	p= 9.042E-7 ***	different

Table 11. Comparisons of Reorienting foragers homing at 4HFields

The comparisons are based on Wilcoxon rank statistics and involve a Bonferroni adjustment to keep the experiment-wise error rate to  $\alpha = 0.05$ . The z-scores listed correspond to their single comparison probabilities. For multiple comparisons, the corrected cutoff point for significance depends on an **adjusted z > 2.635**. Significance of multiple comparison tests is indicated by \*\*\*. These statistics correspond to Figure 34.

Source	DF	Sums Squares	F value	Р
Model	3	788.12	3.23	p = 0.0222
Error	651	53023.71		
Total	654	53811.83		
Source	DF	Type III SS	F value	Р
Site	1	174.14	2.14	p = 0.1442
Treatment	1	330.51	4.06	p = 0.0444
Site*Treatment	1	167.21	2.05	p = 0.1524

•

Table 12. General linear models using time as the dependent variable to examine influences on orientation flight duration



Figure 1. Conceptual model of orientation tasks.

Figure 2. Percentage of displaced bees reaching the hive from release points in a landscape in which they have made only a single orientation flight. The data are taken from Table 7 in Becker (1958).



Figure 3. Map of the Greiner study site showing the position of the hive (H), the 6 release positions (A-F), and approximate topography, as indicated by the contour lines. The lines show approximate elevation in meters (converted from a survey map plotting elevations in feet). The stipled areas indicate landscape features, such as trees, woodlots, or buildings. This site is located on the east side of Hagadorn Road, just north of Bennett Road.



Figure 3.

Figure 4. Map of the 4H Fields study site showing relative position of the hive (H), and the 4 release sites. This site is located on MSU Agricultural Experiment Station property adjacent to the Farm Service Center on College Road. There are no contour lines because this site is entirely flat.



Figure 5. Polar histogram showing the distributions of vanishing bearings for bees taken from a feeder at the Entomology Teaching and Research Site on Collins Road and released at Greiner Site A. The polar histogram gives the mean bearing ( $\Phi$ ), shown by the white arrow. The r-value is a measure of dispersion and corresponds to the length of the mean vector. N is the sample size; individual bees were tested only once. The solid line marks the true direction of the Entomology Site from Greiner. The dashed line indicates the direction from the feeder at Entomology Site to the hive. The dotted line shows the direction from Site A to the hive at Greiner.







Figures 6-8. Polar histograms showing the distributions of vanishing bearings for released at Site A. Figures 6, 7, and 8 correspond to bees in the three treatment categories, Resident bees, Reorienting bees, First-Flight bees, respectively. Each polar histogram gives the mean bearing ( $\Phi$ ), shown by the white arrow. The upper and lower limits of the 95 % confidence interval of the mean are indicated by the smaller black arrows. The true homeward direction ( $\Psi$ ) is marked by the radius line. The r-value is a measure of dispersion. N is the sample size; individual bees were tested only once. A mean bearing is considered not significantly different from a predicted angle if the prediction falls within the confidence interval.

Figure 6. Resident bees at Site A.



Figure 7. Reorienting bees at Site A.



Figure 8. First-Flight Bees at Site A.



Figures 9-11. Polar histograms showing the distributions of vanishing bearings for released at Site B. Figures 9, 10, and 11 correspond to bees in the three treatment categories, Resident bees, Reorienting bees, First-Flight bees, respectively. Each polar histogram gives the mean bearing ( $\Phi$ ), shown by the white arrow. The upper and lower limits of the 95 % confidence interval of the mean are indicated by the smaller black arrows. The true homeward direction ( $\Psi$ ) is marked by the radius line. The r-value is a measure of dispersion. N is the sample size; individual bees were tested only once. A mean bearing is considered not significantly different from a predicted angle if the prediction falls within the confidence interval.

Figure 9. Resident bees at Site B.



Figure 11. First-Flight bees at Site B.



Figures 12-14. Polar histograms showing the distributions of vanishing bearings for released at Site C. Figures 12, 13, and 14 correspond to bees in the three treatment categories, Resident bees, Reorienting bees, First-Flight bees, respectively. Each polar histogram gives the mean bearing ( $\Phi$ ), shown by the white arrow. The upper and lower limits of the 95 % confidence interval of the mean are indicated by the smaller black arrows. The true homeward direction ( $\Psi$ ) is marked by the radius line. The r-value is a measure of dispersion. N is the sample size; individual bees were tested only once. A mean bearing is considered not significantly different from a predicted angle if the prediction falls within the confidence interval.

Figure 12. Fully Exposed Bees at Site C







Figure 14. First-Flight bees at Site C.


Figures 15-17. Polar histograms showing the distributions of vanishing bearings for released at Site D. Figures 15, 16, and 17 correspond to bees in the three treatment categories, Resident bees, Reorienting bees, First-Flight bees, respectively. Each polar histogram gives the mean bearing ( $\Phi$ ), shown by the white arrow. The upper and lower limits of the 95 % confidence interval of the mean are indicated by the smaller black arrows. The true homeward direction ( $\Psi$ ) is marked by the radius line. The r-value is a measure of dispersion. N is the sample size; individual bees were tested only once. A mean bearing is considered not significantly different from a predicted angle if the prediction falls within the confidence interval.

Figure 15. Fully Exposed Bees at Site D



Figure 16. Reorienting bees at Site D.



Figure 17. First-Flight bees at Site D.



Figures 18-20. Polar histograms showing the distributions of vanishing bearings for released at Site E. Figures 18, 19, and 20 correspond to bees in the three treatment categories, Resident bees, Reorienting bees, First-Flight bees, respectively. Each polar histogram gives the mean bearing ( $\Phi$ ), shown by the white arrow. The upper and lower limits of the 95 % confidence interval of the mean are indicated by the smaller black arrows. The true homeward direction ( $\Psi$ ) is marked by the radius line. The r-value is a measure of dispersion. N is the sample size; individual bees were tested only once. A mean bearing is considered not significantly different from a predicted angle if the prediction falls within the confidence interval.

Figure 18. Resident bees at Site E.







Figure 20. First-Flight bees at Site E.



Figures 21-23. Polar histograms showing the distributions of vanishing bearings for released at Site F. Figures 21, 22, and 23 correspond to bees in the three treatment categories, Resident bees, Reorienting bees, First-Flight bees, respectively. Each polar histogram gives the mean bearing ( $\Phi$ ), shown by the white arrow. The upper and lower limits of the 95 % confidence interval of the mean are indicated by the smaller black arrows. The true homeward direction ( $\Psi$ ) is marked by the radius line. The r-value is a measure of dispersion. N is the sample size; individual bees were tested only once. A mean bearing is considered not significantly different from a predicted angle if the prediction falls within the confidence interval.

Figure 21. Resident bees at Site F.



Figure 22. Reorienting bees at Site F.



Figure 23. First-Flight bees at Site F.











Figure 26. Homing of First-Flight bees at Greiner.



100











Figure 29. Homing of bees from Site C at Greiner.



Figure 30. Homing of bees from Site D at Greiner.









Fields. Each histogram corresponds to their relative compass positions on the page. North is indicated by The upper and lower limits of the 99 % confidence interval of the mean are indicated by the smaller black the solitary dark arrow. Each polar histogram gives the mean bearing ( $\Phi$ ), shown by the white arrow. arrows. The true homeward direction (\PV) is marked by the radius line. The r-value is a measure of Figure 33. Polar histograms showing the distributions of vanishing bearings for bees released at 4H

dispersion. N is the sample size; individual bees were tested only once. A mean bearing is considered not significantly different from a predicted angle if the prediction falls within the confidence interval.





Figure 34. Homing of Reorienting bees at 4-H Fields.

Figure 35. Map of the Corral site, showing the position of the hive (H). The stipled areas indicate buildings and the black lines represent roads. This site is adjacent to the Farrall Agricultural Engineering Building on the Michigan State University main campus.

Figure 35.



Figure 36. Map of the Collins Road site, showing the position of the hive (H) and nearby landscape features. The dark stipled areas indicate trees or woodlots and the light stipled circle is a small gravel pit. The solid and dotted lines mark roads and the open boxes mark buildings. This site is adjacent to the Entomology Teaching and Research Facility, on the Michigan State University Farms.

Figure 36.



LIST OF REFERENCES

•

## LIST OF REFERENCES

- Able, K.P. 1980. Mechanisms of orientation, navigation, and homing. In: <u>Animal</u> <u>Migration. Orientation. and Navigation</u>. (S.A. Gauthreaux ed.) pp 283-373. Academic Press: New York, NY.
- Able, K.P. & M.A. Able. 1996. The flexible migratory orientation system of the savannah sparrow (*Passerculus sandwichensis*). J. Exp. Biol. 199: 3-8.
- Anderson, A.M. 1977. A model for landmark learning in the honey-bee. J. Comp. Physiol. 114: 335-355.
- Baerends, G.P. 1941. Fortpflanzungsverhalten und Orientierung der Grabwespe Ammophila compestris. Tijdschr. Entomol. 84: 68-275.
- Batschelet, E. 1981. Circular Statistics in Biology. Academic Press: New York, NY.
- Becker, L. 1958. Untersuchungen über das Heimfindevermögen der Bienen. Z. vergl. Physiol.41: 1-25.
- Berthold, P. 1991. Orientation in birds: a final consideration. In <u>Orientation in Birds</u>. (P. Berthold, ed.) pp 322-327. Birkhäuser Verlag: Boston, MA.
- Beusekom, G. van. 1948. Some experiments on the optical orientation in *Philanthus* triangulum Fabr. Behaviour 1: 195-225.
- Boylan-Pett, W.L., R.A. Hoopingarner & D.C. Ramsdell. 1991. Bee Science 1(4): 199-202.
- Brower, L.P. 1996. Monarch butterfly orientation: missing pieces of a magnificent puzzle. J. Exp. Biol. 199: 93-103.
- Buttel- Reepens, H von. 1900. Sind die Bienen Reflexmaschinen? Biol. Zbl. 20: 9-193.
- Byrne, R.W. 1982. Geographical knowledge and orientation. In: <u>Normality and Pathology</u> in <u>Cognitive Functions</u>. (A.W. Ellis, ed.) pp 239-264. Academic Press: London.
- Cartwright, B.A. & T.S. Collett. 1982. How honey bees use landmarks to guide their return to a food source. Nature. 295: 560-564.
- Cartwright, B.A. & T.S. Collett. 1983. Landmark learning in bees. J. Comp. Physiol. A 151: 521-543.

- Cartwright, B.A. & T.S. Collett. 1987. Landmark maps for honeybees. Biol. Cybern. 57: 85-93.
- Chapman, R.F. 1982. <u>The Insects: Structure and Function</u>. 3rd ed. Harvard Univ. Press: Cambridge, MA.
- Chmurzynski, J.A. 1964. Studies on the stages of spatial orientation in female *Bembix* rostrata (Linne 1758) returning to their nests (Hymenoptera, Sphegidae). Acta Biol. Exper. (Warsaw) 24(2): 103-132.
- Churchland, P.S. 1995. <u>The Engine of Reason. The Seat of the Soul: A Philosophical</u> Journey into The Brain. MIT Press: Cambridge, MA.
- Churchland, P.S. & T.J. Sejnowski. 1992. <u>The Computational Brain</u>. MIT Press: Cambridge, MA.
- Collett, T.S. 1992. Landmark learning and guidance in insects. Phil. Trans. R. Soc. Lond. B 337: 295-303.
- Collett, T.S. 1995. Making learning easy: the acquisition of visual information during the orientation flights of social wasps. J. Comp. Physiol. 177: 737-747.
- Collett, T.S. 1996a. Short-range navigation: does it contribute to understanding navigation over longer distances? J. Exp. Biol. 199: 225 226.
- Collett, T.S. 1996b. Insect navigation en route to the goal: multiple strategies for the use of landmarks. J. Exp. Biol. 199: 227-235.
- Collett, T.S. & J. Baron. 1994. Biological compasses and the coordinate frame of landmark memories in honeybees. Nature 368: 137-140.
- Collett, T.S. & M.F. Land. 1975. Visual spatial memory in a hoverfly. J. Comp. Physiol. 100: 59-84.
- Collett, T.S. & M. Lehrer. 1993. Looking and learning: a spatial pattern in the orientation flight of the wasp Vespula vulgaris. Proc. R. Soc. Lond. B 252: 129-134.
- Collett, T.S., B.A. Cartwright & B.A. Smith. 1986. Landmark learning and visuo-spatial memories in gerbils. J. Comp. Physiol. A. 158: 835-851.
- Couvillon, P.A., T.G. Leito & M.E.Bitterman. 1991. Learning by honeybees (Apis mellifera) on arrival at and departure from a feeding place. J. Comp. Psychol. 105: 177-184.
- Cox, D.R. & D. Oakes. 1985. <u>Analysis of Survival Data</u>. Chapman & Hall: New York, NY.
- Davis, R. & K. Han. 1996. Mushrooming mushroom bodies. Current Biology 6(2): 146-148.
- Dickinson, J.A. 1994. Bees link local landmarks with celestial compass cues. Naturwissenschaften 81: 465-476.

Dingle, H. 1996. Migration. Oxford University Press: New York, NY.

- Domjan, M. 1993. <u>The Principles of Learning and Behavior</u>. Brooks/Cole Publishing: Pacific Grove: CA.
- Durst, C., S.Eichmüller & R. Menzel. 1994. Development and experience lead to increased volume of subcompartments of the honeybee mushroom body. Behav. Neural Biol. 62: 259-263.
- Dyer, F.C. 1991. Bees acquire route-based memories but not cognitive maps in a familiar landscape. Anim. Behav. 41: 239-246.
- Dyer, F.C. 1993. How honeybees find familiar feeding sites after changing nesting sites with a swarm. Anim. Behav. 46: 813-816.
- Dyer, F.C. 1994. Spatial cognition and navigation in insects. In: <u>Behavioral Mechanisms in</u> <u>Evolutionary Ecology</u>. (L. Real, ed.) pp. 66-98. Univ. Chicago Press: Chicago, IL.
- Dyer, F.C. 1997a (in press). The cognitive ecology of navigation. In: <u>Cognitive Ecology</u>. (R. Dukas, ed.) Univ. Chicago Press: Chicago, IL.
- Dyer, F.C. 1997b (in press). Spatial cognition: lessons from central-place foraging insects.
   In: <u>A Synthetic Approach to Studying Animal Cognition</u>. (I. Pepperberg, A. Kamil, & R. Balda, eds.) Academic Press: London.
- Dyer, F.C. & J.A. Dickinson. 1994. Development of sun compensation by honeybees: how partially experienced bees estimate the sun's course. Proc. Natl. Acad. Sci. USA 91: 4471-4474.
- Dyer, F.C. & J.A. Dickinson. 1996. Sun-compass learning in insects: Representation in a simple mind. Current Directions in Psychological Science 5(3): 67-72.
- Dyer, F.C., N.A. Berry, & A.S. Richard. 1993. Honey bee spatial memory: use of routebased memories after displacement. Anim. Behav. 45: 1028-1030.
- Esch, H.E. & J.E. Burns. 1995. Honeybees use optic flow to measure the distance of a food source. Naturwissenschaften 82: 38-40.
- Esch, H.E. & J.E. Burns. 1996. Distance estimation by foraging honeybees. J. Exp. Biol. 199: 155-162.
- Etienne, A.S., R. Maurer & V. Seguinot. 1996. Path integration in mammals and its interaction with visual landmarks. J. Exp. Biol. 199: 201-209.
- Fabre, J.H. 1879. Souvenirs Entomologiques. 1. Serie. C. Delagrave: Paris.
- Fahrbach, S.E. & G.E. Robinson. 1995. Behavioral development in the honey bee: toward the study of learning under natural conditions. Learning & Memory 2: 199-224.

- Fox, G.A. 1993. Failure-time analysis; emergence, flowering, survivorship, and other waiting times. In <u>Design and Analysis of Ecological Experiments</u>. (S.M. Scheiner & J. Gurevitch, eds.) pp. 253-289. Chapman & Hall: New York, NY.
- Fox, G.A. 1996. Errata for "Failure time analysis: emergence, flowering, survival, and other waiting times." In <u>Design and Analysis of Ecological Experiments</u>. (S.M. Scheiner & J. Gurevitch, eds.) pp. 253-289. Chapman & Hall: New York, NY.
- Frisch, K.von. 1967. <u>The Dance Language and Orientation of Bees</u>. Belknap/Harvard: Cambridge, MA.
- Frisch, K. von & M. Lindauer. 1954. Himmel und Erde in Konkurrenz bei der Orientierung der Bienen. Naturwissenschaften 41: 245-253.
- Frison, T.H. 1930. Observations on the behavior of bumblebees (*Bremus*): the orientation flight. Canadian Entomol. 62: 49-54.
- Gallistel, C.R. 1990. The Organization of Learning. MIT Press: Cambridge, MA.
- Gaul, A.T. 1951. Additions to vespine biology VII: orientation flight. Bull. Brooklyn Entomol. Soc. 156: 54-56.
- Giger, A.D. & M.V. Srinivasan. 1995. Pattern recognition in honeybees: eidetic imagery and orientation discrimination. J. Comp. Physiol. A. 176: 791-795.
- Gould, J.L. 1985. How bees remember flower shapes. Science 227: 1492-1494.
- Gould, J.L. 1986. The locale map of honeybees: do insects have cognitive maps? Science 232: 861-863.
- Griffin, D.R. 1955. Bird navigation. In: <u>Recent Studies in Avian Biology</u>. (A. Wolfson, ed.) pp 154-197. University of Illinois Press: Urbana, IL.
- Homberg, U. 1984. Processing of antennal information in extrinsic mushroom body neurons of the bee brain. J. Comp. Physiol. A. 154: 825-836.
- Huang, Z.Y., G.E. Robinson & D.W. Borst. 1994. Physiological correlates of division of labor among similarly aged honey bees. J. Comp. Physiol. A. 174: 731-739.
- Iersel, J.J.A. van & J. van den Assem. 1964. Aspects of orientation in the diggerwasp Bembix rostrata. Anim. Behav. Suppl. I: 145-162.
- Kanciruk, P. & W. Herrnkind. 1978. Mass migration of spiny lobster, *Panulirus argus* (Crustacea: Palinuridae): behavior and environmental correlates. Bull. mar. Sci. 28: 601-623.
- Krebs, J. 1990. Food-storing birds: adaptive specialization in brain and behavior? Philos. Trans. R. Soc. London B Biol. 329: 153-60.
- Lehrer, M. 1991. Bees which turn back and look. Naturwissenschaften 78: 274-276.

Lehrer, M. 1993. Why do bees turn back and look? J. Comp. Physiol. A. 172: 549-563.

- Lehrer, M. 1996. Small-scale navigation in the honeybee: active acquisition of visual information about the goal. J. Exp. Biol. 199: 253-261.
- McNaughton, B.L., C.A. Barnes, J.L. Gerrard, K. Gothard, M.W. Jung, J.J. Knierim, H. Kudrimoti, Y. Qin, W.E. Skaggs, M. Suster & K.L. Weaver. 1996. Deciphering the hippocampal polyglot: the hippocampus as a path integration system. J. Exp. Biol. 199: 173-185.
- Menzel, R. 1990. Learning, memory, and "cognition" in honey bees. In: <u>Neurobiology of</u> <u>Comparative Cognition</u>. (R.P. Olton & D.S. Olton, eds.) pp. 237-292. Lawrence Erlbaum Associates: Hillsdale, NJ.
- Menzel, R., J. Erber & T. Masuhr. 1974. Learning and memory in the honeybee. In: <u>Experimental Analysis of Insect Behaviour</u>. (L. Barton Browne, ed.) pp. 195-217. Springer-Verlag: New York, NY.
- Menzel, R., L. Chittka, S. Eichmüller, K. Geiger, D. Peitsch & P. Knoll. 1990. Dominance of celestial cues over landmarks disproves map-like orientation in honey bees. Z. Naturforsch.45: 723-726.
- Menzel, R., C. Durst, J. Erber, S. Eichmüller, M. Hammer, H. Hildebrandt, J.
  Mauelshagen, U. Müller, H. Rosenboom, J. Rybak, S. Schäfer & A. Scheidler.
  1994. The mushroom bodies in the honeybee: from molecules to behavior. In:
  Fortschritte der Zoologie: <u>Neural Basis of Behavioural Adaptations</u>. (K. Schildberger & N. Elsner, eds.) pp 81-102. Gustav Fischer Verlag: New York, NY.
- Mobbs, P.G. 1982. The brain of the honeybee Apis mellifera. I. The connections and spatial organization of the mushroom bodies. Phil. Trans. R. Soc. Lond. B 298: 309-354.
- Muenchow, G. 1986. Ecological use of failure time analysis. Ecology 67(1): 246-250.
- Opfinger, E. 1931. Über die Orientierung der Bienen an der Futterquelle. Z. vergl. Physiol. 15: 431-487.
- Rau, P. 1924. Notes on captive colonies and homing of *Bombus pennsylvanicus* de Geer. Annals Entomol. Soc. America. 17: 368-381.
- Rau, P. 1930. Behavior notes on the yellow jacket, Vespa germanica (Hymenoptera: Vespidae). Entomol. News 41: 185-190.
- Riley, J.R., A.D. Smith, D.R. Reynolds, A.S. Edwards, J.L. Osborne, I.H. Williams, N.L. Carreck & G.M. Poppy. 1996. Tracking bees with harmonic radar. Nature 379: 29-30.
- Robinson, G.E. 1992. Regulation of division of labor in insect societies. Annu. Rev. Entomol. 37: 637-656.
- Robinson, G.E. & F.C. Dyer. 1993. Plasticity of spatial memory in honeybees: reorientation following colony fission. Anim. Behav. 46: 311-320.

- Roitblat, H.L. 1987. Introduction to Comparative Cognition. W.H.Freeman: New York, NY.
- Romanes, G.J. 1885. Homing faculty of Hymenoptera. Nature 32: 630.
- Ronacher, B. & R. Wehner. 1995. Desert ants Cataglyphis fortis use self-induced optic flow to measure distances travelled. J. Comp. Physiol. A. 177: 21-27.
- Rossel, S. & R. Wehner. 1986. Polarization vision in bees. Nature 323: 128-131.
- SAS Institute, Inc. 1988. SAS Stat Users Guide, Release 6.03 edition. SAS Institute: Cary, NC.
- Schöne, H. 1984. <u>Spatial orientation: the spatial control of behavior in animals and man</u>. Princeton University Press: Princeton, NJ.
- Schöne, H., W.D. Kühme & H. Schöne. 1995. Take-off behavior and vanishing bearings of honeybees after displacement with open view or in a sight-proof box. Naturwissenschaften 82: 343-345.
- Schürmann, F.W. 1987. The architecture of the mushroom bodies and related neuropils in the insect brain. In: <u>Arthropod Brain: Its Evolution. Development. Structure. and</u> <u>Functions</u>. (A.P. Gupta, ed.) pp 231-264. Wiley & Sons: New York, NY.
- Seeley, T. 1985. <u>Honeybee ecology: a study of adaptation in social life</u>. Princeton University Press: Princeton, NJ.
- Shettleworth, S.A. 1990. Spatial memory in food storing birds. Philos. Trans. R. Soc. London B Biol. 329: 143-51.
- Srinivasan, M.V., S.W. Zhang, M. Lehrer & T.S. Collett. 1996. Honeybee navigation en route to the goal: visual flight control and odometry. J. Exp. Biol. 199: 237-244.
- Strausfeld, N.J., E.K. Buschbeck & R.S. Gomez. 1995. The arthropod mushroom body: its functional roles, evolutionary enigma and mistaken identities. In: <u>The Nervous</u> <u>Sytems of Invertebrates: An Evolutionary and Comparative Approach</u>. (O. Breidbach & W. Kutsch, eds.) pp 349-381. Birkhauser: Boston, MA.
- Tinbergen, N. 1935. Ueber die Orientierung des Bienenwolfes (*Philanthus triangulum* Fabre). II. Die Bienenjagd. Zs. vergl. Physiol. 21: 699-716.

Tinbergen, N. 1951. The study of instinct. Clarendon Press: Oxford.

Tinbergen, N. & W. Kruyt. 1939. Über die Orientierung des Bienenwolfes (*Philanthus triangulum* Fabre). III. Die Bevorzugung bestimmter Wegmarken. Zs. vergl. Physiol. 25: 56-63.

Tolman, E.C. 1948. Cognitive maps in rats and men. Psychol. Rev. 55: 189-208.

Visscher, P.K. & T.D. Seeley. 1982. Foraging strategy of honeybee colonies in a temperate deciduous forest. Ecology 63(6): 1790-1801.

- Vollbehr, J. 1975. Zur Orientierung junger Honigbienen bei ihrem ersten Orientierungsflug. Zool. Jb. allg. Zool. Physiol. 79: 33-69.
- Walcott, C. 1996. Pigeon homing: observations, experiments and confusions. J. Exp. Biol. 199: 21-27.
- Wcislo, W.T. 1992. Nest localization and recognition in a solitary bee, *Lasioglossum* (*Dialictus*) figueresi Wcislo (Hymenoptera: Halictidae), in relation to sociality. Ethology 92: 108-123.
- Wehner, R. 1981. Spatial vision in arthropods. In: <u>Handbook of Sensory Physiology</u>, Vol. <u>VII/6C</u>. (H. Atrum, ed.), pp. 287-616. Springer-Verlag: Berlin-Heidelberg-New York.
- Wehner, R. 1989. Neurobiology of polarization vision. Trends in Neurosciences 12(9): 353-359.
- Wehner, R. & R. Menzel. 1990. Do insects have cognitive maps? Annu. Rev. Neuroscience 13: 403-14.
- Wehner, R. & M. Müller. 1993. How do ants acquire their celestial ephemeris function?. Naturwissenschaften 80: 331-333.
- Wehner, R. & S. Wehner. 1986. Path integration in desert ants. Approaching a longstanding puzzle in insect navigation. Monitore zool. ital. (N.S.) 20: 309-311.
- Wehner, R., R.D. Harkness & P. Schmid-Hempel. 1983. Foraging strategies in individually searching ants *Cataglyphis bicolor* (Hymenoptera: Formicidae). In: <u>Information Processing in Animals</u>. (M. Lindauer ed.) pp 1- 79. Gustav Fischer Verlag: New York, NY.
- Wehner, R., B. Michel & P. Antonsen. 1996. Visual navigation in insects: coupling of egocentric and geocentric information. J. Exp. Biol. 199: 129-140.
- Wehner, R., S. Bleuler, C. Nievergelt & D. Shah. 1990. Bees navigate by using vectors and routes rather then maps. Naturwissenschaften 77: 479-482.
- Withers, G.S., S.E. Fahrbach & G.E. Robinson. 1993. Selective neuroanatomical plasticity and division of labour in the honeybee. Nature 364: 238-240.
- Withers, G.S., S.E. Fahrbach & G.E. Robinson. 1995. Effects of experience and juvenile hormone on the organization of the mushroom bodies of honey bees. J. Neurobiol. 26: 130-144.
- Wiltschko, W. & R. Wiltschko. 1996. Magnetic orientation in birds. J. Exp. Biol. 199: 29-38.
- Withers, G.S., S.E. Fahrbach & G.E. Robinson. 1995. Effects of experience and juvenile hormone on the organization of the mushroom bodies of honey bees. J. Neurobiol. 26(1): 130-144.

Wolfe, E. 1926. Orientation of bees. Z. Vergl. Physiol. 3: 615-691.

- Yoerg, S.I. & A.C. Kamil. 1991. Integrating cognitive ethology with cognitive psychology. In: <u>Cognitive Ethology</u>. (C. Ristau, ed.) pp. 273-289. Lawrence Erlbaum Associates: Hillsdale, NJ.
- Zar, J.H. 1996. <u>Biostatistical Analysis</u>. 3rd edition. Prentice Hall: Upper Saddle River, NJ.
- Zeil, J. 1993a. Orientation flights of solitary wasps (*Cerceris*; Sphecidae; Hymenoptera) I. Description of flight. J. Comp. Physiol. 172: 189-205.
- Zeil, J. 1993b. Orientation flights of solitary wasps (*Cerceris*; Sphecidae; Hymenoptera)
   II. Similarities between orientation and return flights and the use of motion parallax. J. Comp. Physiol. 172: 207-222.
- Zeil, J., A. Kelber & R. Voss. 1996. Structure and function of learning flights in bees and wasps. J. Exp. Biol. 199: 245-252.
- Zhang, S.W. & M.V. Srinivasan. 1994. Pattern recognition in honeybees: analysis of orientation. Phil. Trans. R. Soc. Lond. B 346: 399-406.

