

Polarization-Based Navigation in *Drosophila*

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To Oliver Finn,
Sophia Grace,
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Abstract

Insects maintain a constant bearing across a wide range of spatial scales. Monarch butterflies and locusts traverse continents (Williams, 1957; Wehner, 1984), and foraging bees and ants travel hundreds of meters to return to their nests (Dyer, 1996; Wehner, 1984, 2003), whereas many other insects fly straight for only a few centimeters before changing direction. Despite this variation in spatial scale, the brain region thought to underlie long-distance navigation is remarkably conserved (Loesel et al., 2002; Homberg, 2008), suggesting that the use of a celestial compass is a general and perhaps ancient capability of insects. Laboratory studies of *Drosophila* have identified a local search mode in which short, straight segments are interspersed with rapid turns (Mayer et al., 1988; Bender and Dickinson, 2006). However, this flight mode is inconsistent with measured gene flow between geographically separated populations (Jones et al., 1981; Slatkin, 1985; Turelli and Hoffmann, 1991), and individual *Drosophila* can travel 10 km in a single night (Yerington, 1961; Jones et al., 1981; Coyne et al., 1982, 1987)—a feat that would be impossible without prolonged periods of straight flight. One well-known cue relevant to orientation and navigation is the pattern of polarization of skylight. To study possible mechanisms of orientation to skylight polarization, we built an arena in which we could observe individual flight responses to rotating the angle of polarized light in the laboratory. We found that flies robustly steer in response to changes in the polarization angle of light. Individual flies also stabilize a particular polarization plane when they are given closed-loop control of such a stimulus. To directly examine orientation behavior under outdoor conditions, we built two portable flight arenas in which a fly viewed the natural sky through a clear aperture. In the first we examined the ability of flies to compensate

for external rotations with or without the aid of skylight polarization. The second arena contained a liquid crystal device that could experimentally rotate the polarization angle of the skylight. In both outdoor arenas we tracked fly orientation using a digital video camera and custom computer vision system. Our findings indicate that *Drosophila* actively orient using the sky's natural polarization pattern.

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Chapter 1

Introduction

When the child of morning, rosy-fingered Dawn, appeared, I called a council and said, "My friends, we are in very great difficulties; listen therefore to me. We have no idea where the sun either sets or rises, so that we do not even know East from West. I see no way out of it; nevertheless, we must try and find one..."

—Homer, *The Odyssey*

(Translated by Samuel Butler)

An animal's movement through the environment is an aspect of its behavior that is both one of the simplest and one of the most complex to study. It is simple because visual observation alone often is sufficient to provide a wealth of detail about the organism's behavior. It is complex, because the act of observation potentially disrupts the animal or can only cover a limited part of the animal's movement range. Nonetheless, it cannot be doubted that the study of such behavior is important, given the relevance movement has in the life of animals. For a huge portion of the animal kingdom almost every evolutionarily relevant task, including capturing prey, foraging for food, finding a mate, avoiding predators, competing or cooperating with conspecifics, all involve elaborate coordination of movement (Fraenkel and Gunn, 1961). Large parts of the nervous system are responsible for directing movement. Hence understanding the operation of nervous systems will rely on understanding how motion is controlled. Insects have proven to be amenable to the study of movement because of their small size and their abundance. Moreover, the remarkable success

with which this class has differentiated to exploit almost every conceivable niche indicates that it has much to teach us about robust control of movement.

Of the many ways that animals move, spatial orientation and long-distance movement require unique types of neural control. (We consider long-distance movement to be movement across spatial scales orders of magnitude greater than the animal's body length, be it a migrating whale or a termite on its nuptial flight.) Unlike an escape response, long-distance movement requires maintenance of a course over long timescales. This type of sustained activity relies on sensory systems capable of detecting the direction and rate of travel, as well as some type of memory to keep the organism on course. Studies on various insect species have elucidated different parts of the strategies used by animals during long-range movement.

1.1 Behavioral evidence for detection of light's polarization angle

In the life of a hive-dwelling bee there are two types of long-range movement: Flights to a new hive location after swarming, and foraging trips to and from food resources. In studies of the latter, Karl von Frisch, one of the fathers of ethology, uncovered a vast array of interesting behaviors exhibited by bees (von Frisch, 1954, 1974). He discovered the "waggle-dance" through which bees communicate the direction and distance to food resources. After an individual forager discovers a patch of flowers with abundant nectar or pollen, she returns to the nest and indicates the distance and direction to the patch relative to the location of the sun in the sky with her dance. This observation reveals a surprising amount about the capacity of the bee's nervous system. It implies that the bee in some way knows which direction she flew relative to the sun. Furthermore, other bees can steer a course based on this information with enough accuracy to find the food resource. (Von Frisch studied many aspects of bee behavior, including sensory systems other than vision, but here we can only give a brief review of findings relevant to the current work.) In a series of studies, von Frisch

discovered that bees could correctly navigate and communicate directions even when the sun itself was not visible, as long as they could view a small patch of clear sky. As discussed in the next section, skylight is partially polarized from scattering in the atmosphere. When the direction of polarization of the skylight was experimentally rotated, the bees altered their dances as though the sun's location had been rotated (Figure 1.1). This indicated that bees are able to perceive the angle of polarization of light and use it to direct their flights.

1.2 The polarization of skylight

Before we go into more detail about the responses of insects to polarized light, we need to make a brief aside into the sources of polarized light in nature (for a more complete discussion of polarized light in nature from a biological perspective, see Wehner, 2001). An electromagnetic light wave is characterized by the direction of oscillation of its electric and magnetic fields. Because these are always perpendicular to one another (and to the direction of propagation), we can entirely specify the relevant axes by determining the direction of oscillation of the electric field, or e-vector. For most sources of light, these e-vectors are randomly distributed in all directions perpendicular to the direction of propagation. When all of the electric field oscillations are in one direction, however, we say that the light is linearly polarized. The direction of the e-vector oscillation is termed the polarization angle or plane of polarization. If the e-vectors are partially aligned, such that the out-of-polarization-plane oscillation is nonzero, we call the light partially linearly polarized. There is a second important type of polarization, which occurs when the e-vector of light measured at one location traces a circle (or ellipse) over time. This type of light is said to be circularly (or elliptically) polarized. Throughout this document, if light is referred to as polarized, without specifying linear or circular, our convention will be that it is linearly polarized.

Several processes are common sources of polarized light. Artificial linear and circular polarizing filters are available commercially. Linear polarizing filters, called

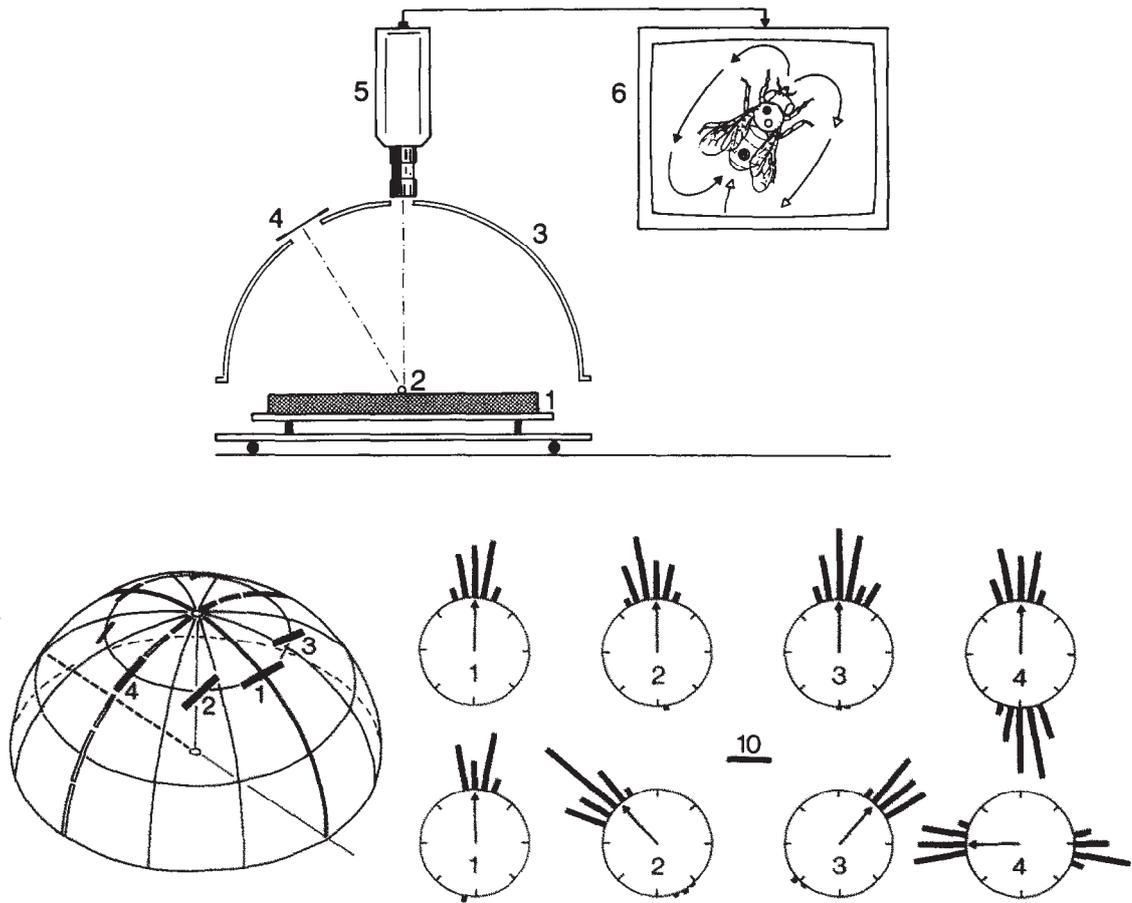


Figure 1.1: Bees alter their dance direction when the polarization angle is rotated (modified from Rossel, 1993). A schematic of the experimental arena is shown in the top panel. A camera (5) records movies (6) of bees (2) dancing below a translucent dome (3) with a single window (4) cut in it. The bottom-left panel shows the four positions of the window in the experiment (1–4) and approximate natural polarization angles seen by the bee through those window positions (bold bars). The dotted line points toward the sun's position. In the bottom-right panel, circular histograms show the bee's dance directions (scale bar is 10 dances). In the top row the bee dances in the correct (sun-ward) direction regardless of the window's position. Only when the window is 90° from the sun is the bee unable to disambiguate the 180° ambiguity of the polarization signal (far-right plot). The bottom row shows data from experiments in which the window was covered with a linearly polarizing filter. In all cases the window was in position 1. From left to right, the filter's polarization axis was aligned horizontally, 45° counterclockwise from horizontal, 45° clockwise from horizontal, and vertically. These filter orientations mimic the polarization signal that was transmitted by the window in location 1, 2, 3, and 4, respectively. The bee alters her dance directions with respect to the arena, indicating that although the window was in the same physical location in all four conditions, she interprets it as if it were in different locations. Hence, she no longer dances in the true sun-ward direction (up here) but instead in the direction implied by the polarization signal (arrow direction).

linear polarizers (or just polarizers, for short) function by blocking off-axis e-vectors. Thus, when illuminated with unpolarized light, an ideal polarizing filter would transmit only half of the total incident intensity. Circular polarizers are constructed by affixing a quarter-wave plate to the output side of a linear polarizer. This optical element retards the electric field vector in one direction by an amount equal to one quarter of the light's wavelength. When correctly aligned, this delay results in an e-vector that shifts with the oscillation of the light, tracing out a circle at any single location along the direction of propagation.

Clearly, bees did not evolve to respond to light transmitted by man-made filters. There are two common sources of partially linearly polarized light in nature. The first is reflections and refractions at the interface between substances with different indexes of refraction, such as air-water interfaces or glassy plant surfaces. Because different proportions of orthogonal e-vector directions are reflected at such interfaces, the reflections and refractions are partially polarized (see Section 2.2.4). More importantly for our present purposes is the polarization of skylight. When light from the sun scatters from charged particles in the atmosphere, the direction it scatters in depends on its angle of incidence and e-vector (Strutt, 1871). This dependence results in partial polarization of the sky seen from a location on the surface of the earth. The degree of polarization increases as the angle from the sun increases, reaching a maximum 90° from the sun, and falling off to a second minimum at the anti-sun location (the location diametrically opposite to the sun through the location of the observer). The direction of polarization at any location is perpendicular to the direction to the sun at that location. This results in a global pattern of linear polarization aligned in concentric circles around the sun (Figure 1.2). Because the polarization pattern is determined by the location of the sun, it can be used to infer the sun's location. There is one ambiguity, however—the polarization pattern is symmetrical about the great circle 90° from the sun, so the sun's location can actually only be determined to lie along one axis through the observer (the sun and anti-sun cannot be discriminated).

The ability to navigate with respect to fixed compass directions is clearly important to the lifestyle of a bee, who must return to the nest after foraging. Since the

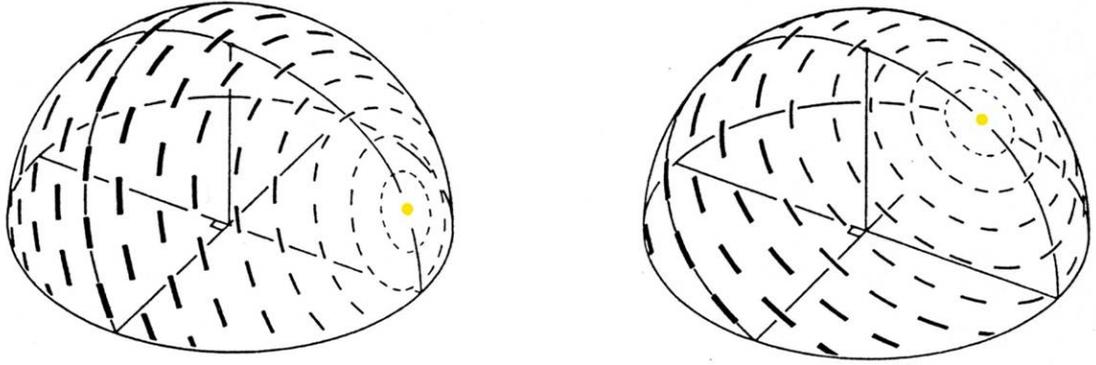


Figure 1.2: Global pattern of sky polarization (modified from Wehner, 1982). The yellow circle represents the sun, the origin indicates the location of the observer. The short segments are drawn in the direction of polarization of light from that sky location. Their length indicates the relative degree of polarization (longer segment implies more polarized). The two panels represent different times of day—on the left the sun is lower in the sky, on the right it is closer to its midday position.

pattern of polarization is determined by the location of the sun, it changes over the course of the day. In the northern hemisphere its azimuth changes by an average of 180° over a day, or 15° per hour, in the clockwise direction (in the southern hemisphere it appears to travel in the opposite direction). Bees correctly compensate for this change in the sun's position over the course of the day when navigating during different times of day (Kalmus, 1956; Dyer and Dickinson, 1994)—when tested in the afternoon after being allowed to forage at a given location only in the morning, bees can correctly account for the movement of the sun and find the foraging location. If transported to the southern hemisphere, however, northern-hemisphere bees will navigate in the wrong direction.

1.3 Insect eyes

Another important characteristic of the detection of polarization information was discovered in bees: There is evidence that a region of the bee's eye is specialized for this sensory modality. In order to describe this, we must briefly review the anatomy of insect eyes. Insects have two sets of eyes: The ocelli have arrays of photoreceptor

cells under a single lens (similar in structure to our eyes) and are sometimes called simple eyes. The second set of eyes are referred to as compound eyes because they have many lenses, each with its own small set of photoreceptor cells (Figure 1.4, panel A). Each lens and its associated photoreceptors are called an ommatidium. In flies each ommatidium contains six outer photoreceptors, labeled R1–6, that each express the same rhodopsin, *rh1*, sensitive to blue/green light, and an additional sensitizing pigment that confers sensitivity to ultraviolet (UV) light (Kirschfeld et al., 1977). There are two inner photoreceptors, one, R7, located distally to the other, R8. These can express the same or different opsins (Figure 1.3). It is thought that they exist in one of three configurations:

- Both R7 and R8 expressing a single pigment, *rh3*, sensitive to UV light (called DRA ommatidia)
- R7 expressing *rh3* while R8 expresses *rh5*, sensitive to blue light (sometimes called “pale” and labeled with a “p”)
- R7 expressing *rh4*, sensitive to UV light, and R8 expressing *rh6*, sensitive to green light. These ommatidia are termed “yellow” and labeled with a “y” (Salcedo et al., 1999).

Insect photoreceptor cells are different in morphology than vertebrate photoreceptors. In vertebrates, the opsins are located in disks arrayed orthogonally to the direction of incoming light. In the photoreceptor cells of insects, however, the opsins are held in the membrane of microvilli—tube-shaped structures whose long axis is perpendicular to the angle of incoming light (Figure 1.4, panel C). The opsins preferentially align along the long axis of the microvillus in which they are held. In addition, the opsins preferentially absorb light when their dipole moment is aligned to the e-vector of the light. Thus, the response of a microvillus is intrinsically polarization sensitive—it will respond more readily to light with polarization angle parallel to its long axis than to the polarization angles perpendicular to it. In most parts of the compound eye, the microvilli directions in a photoreceptor are twisted, such that they

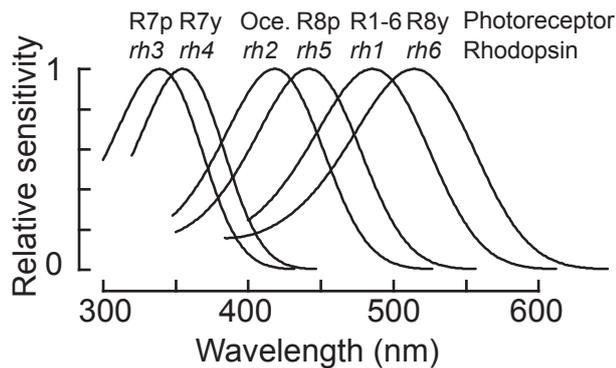


Figure 1.3: Relative sensitivities of *Drosophila* rhodopsins. These sensitivity curves were determined from electroretinograms of flies expressing a single rhodopsin gene ectopically in the R1–6 photoreceptor cells. Since the sensitivities were not measured in their normal cellular environment, it is possible that they are not fully accurate. Further, *rh1* interacts with a sensitizing pigment that bestows significant UV sensitivity to cells R1–6 (Kirschfeld et al., 1977; Feiler et al., 1988), not shown here, and it is possible that this type of mechanism exists in other cell types. (Oce. stands for Ocelli.) This figure was modified from Salcedo et al. (1999).

are oriented in different directions at different depths (see, for example, Wernet et al., 2012). Because of this, the polarization sensitivity at the level of the microvilli is eliminated when responses from many microvilli are combined at the cellular level. In cells in which there is not this twist, however, polarization sensitivity will be preserved in the cell.

In bees a polarization sensitive region of the compound eye has been identified (Labhart, 1980; Wehner and Strasser, 1985). It is located along the dorsal rim of the eye, and is often referred to as the dorsal rim area, or DRA. Blocking this region with paint abolishes polarization-dependent behavioral responses. The DRA ommatidia exhibit specializations that make them especially suited for detecting the angle of polarization of skylight. The cells receive light from the sky, one of the major sources of polarized light in nature, and they have relatively wide fields of view, enabling them to sample from large areas of the sky. In addition these cells have higher degrees of polarization sensitivity, resulting from low degrees of twisting along their lengths.

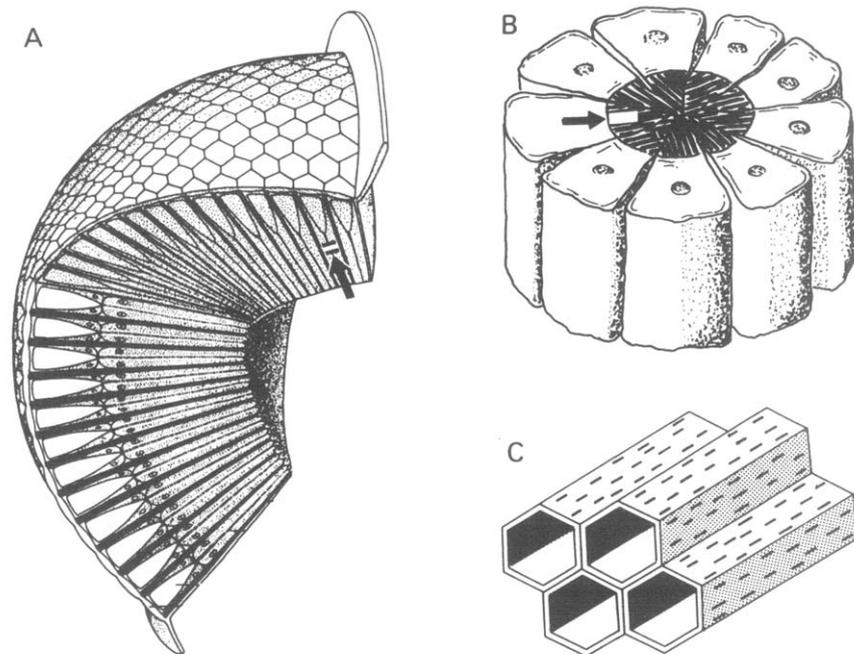


Figure 1.4: The insect eye (modified from Rossel, 1993). The left panel shows the complex eye is divided into many ommatidia. The top right panel depicts a cross section through the nine photoreceptor cells (arrow in A) in a single bee ommatidium (in flies this configuration is slightly different, with 6 outer cells and two vertically stacked inner cells). The bottom right panel shows the alignment of opsins in the microvilli of a photoreceptor (arrow in B).

1.4 Light polarization sensitivity in other species

Subsequent to the discovery of polarization sensitivity in bees, numerous other species of insect have been shown to possess the ability. Among them, perhaps the best studied is the desert ant, *Cataglyphis*. This ant, which forages across tens or hundreds of meters in the desert of northern Africa, uses the celestial polarization pattern to navigate directly back to its nest after finding food (reviewed by Wehner, 1984, 2003). Monarch butterflies and locusts, both migratory species, use celestial information to direct their flights (Williams, 1957; Mouritsen and Frost, 2002; Heinze and Reppert, 2011; Homberg et al., 2011).

All of these species have a clear need for navigational faculties, either to return to a hive or nest, or to migrate successfully. Do animals that do not migrate and are not central place foragers have any use for sun-based navigation? A look at the dung beetle *Scarabaeus zambesianus* is informative. These beetles scavenge balls of dung from fresh droppings. These sources are sites of intense competition, so once a beetle collects its ball, it tries to roll it away from the source as quickly as possible. A straight course away from the fresh droppings is therefore the ideal route, but the beetle is rolling a ball larger than its own body, and often encounters obstacles that make keeping a straight path difficult. By rotating linear polarizing filters above beetles rolling their balls in the field and in the lab, researchers have shown that these beetles rely on polarized light to hold a straight course (Dacke et al., 2003). The use of the polarization of skylight for this type of long-range movement the researchers call “orientation for ‘leaving’ rather than homing”.

Reflection on the case of the dung beetle reveals that perhaps any small motile creature could benefit from a cue that allowed it to correct for perturbations from a straight course. Keeping a straight course is not only useful when navigating to a specific goal, but helps animals reach even an unknown goal. Upon examination of a fruit fly, one of the most striking characteristics is its large red eyes. In normal flight or walking posture, the dorsal hemisphere takes up a large proportion of its field of view. Of course this is useful in detecting predators, which can come from

any direction, but it also points to the possibility that flies are extracting some useful information from the sky, such as polarization angle. In the next section we will briefly review the work on how far flies tend to travel in the wild. This will give an indication of whether they do rely on the ability to navigate a straight course in their natural environment.

1.5 Implications of population genetics studies for neurobiology

Starting with the work on *Drosophila* by Thomas Hunt Morgan elucidating the role of chromosomes in heredity, this genus quickly became an important model for evolutionary biologists. As early as the 1930s, Theodosius Dobzhansky, working with Morgan, pioneered field studies of flies aimed at determining how genes were propagated through natural populations (“gene flow”). From the earliest stages, these experiments could be divided into one of two categories: Direct measurements of the movement of individual flies through the environment, and indirect measurements of the movement of genes by sampling native populations in different areas and inferring the rate of genetic exchange between them. Although there was significant interplay between these lines of research, we will treat them separately.

Among the *Drosophilids*, two species, *pseudoobscura* and *melanogaster*, have been most often examined in the context of dispersal and gene flow. We will focus on experiments on *melanogaster*, since it has emerged as the primary model in neurobiological studies, but we will also attempt to include relevant work on other species where possible.

1.5.1 Direct measurements of dispersal: Mark and recapture studies

Some of the first experiments aimed at quantifying fly vagility were conducted by Nikolay Vladimirovich and Elena Aleksandrovana Timoféeff-Ressovsky in the late

1930s (Timoféeff-Ressovsky and Timoféeff-Ressovsky, 1941a,b,c). These researchers released *Drosophila melanogaster* and several other species that were recognizable by mutant characters in the midst of a grid of baited traps spaced 10 meters apart. The entire experimental field was 110 meters on a side or smaller in all of the releases. They reported numbers of flies entering the traps over the weeks following the release. Although they stated that flies did not escape the experimental field, these experiments were not designed to address possible long-distance flights.

Dobzhansky and Sewall Wright, beginning in the 1940s, published a series of studies in which they measured dispersal rates of *pseudoobscura* and *melanogaster* (Dobzhansky and Wright, 1943). They released laboratory-reared orange-eyed mutant flies in late afternoon in the mountains of Southern California. They monitored recapture rates along trap lines baited with fermenting banana extending in four directions either 220 meters or 300 meters away from the release point. The traps were opened starting the afternoon after the release, and recapture continued for several days. The average distance traveled in the first day after release varied from 59 to 118 meters. In all of the experiments, however, several mutant flies were captured in the farthest eight traps, indicating that the trap lines were not sufficiently long to ensure that individual flies were not flying significantly farther than the area covered in the experiments.

In the one experiment of this study in which the researchers used *melanogaster* it was not necessary to release mutants, because they report that there was no native population of this species in the study area. 3083 individual flies were released in the late afternoon at the center of a two trap lines, as before. On the next afternoon they recaptured 38 flies, and another 21 were recaptured 24 hours later. They report that the average distance traveled were about 20 times lower than that for *pseudoobscura*, but the low recapture rate makes any interpretation of the result difficult.

One final observation from these early studies deserves note: Dobzhansky and Wright reported that the distributions of recapture rates of flies over different distances one or two days after release were distinctly leptokurtic—the extreme ends of the distributions contained disproportionately high numbers of flies (individual

flies who had traveled the greatest distances) compared with a normal distribution, a situation sometimes referred to as “heavy-tailed.”

While not a direct measure of dispersal in the wild, an important piece of evidence relevant to dispersal was reported by Wigglesworth (1949). He measured the duration of flight before complete exhaustion in tethered *Drosophila melanogaster*, and found that on average a one week old fly could fly for 278 minutes, over 2.5 times longer than that measured for *Drosophila funebris* earlier (Williams et al., 1943). Given even a moderate average forward speed of 0.5 meters per second (van Breugel and Dickinson, 2012), this would imply that a well-fed adult *Drosophila melanogaster* has the capacity to fly over 8 kilometers without eating or drinking (see also Götz, 1987; Lehmann and Dickinson, 1997). This, in conjunction with intermittent observations of *Drosophila* captured at sea tens of kilometers from the nearest land (Gressitt et al., 1962; Harrell and Yoshimoto, 1964), suggested that it would be worthwhile to look for dispersal at the range of kilometers.

In a report provocatively entitled, “Are we winning the *Drosophila* fight?” published in 1961, A. P. Yerington discussed *Drosophila* from the point of view of farmers who viewed this organism as an agricultural pest. Of importance to this community was the range of flies, since this indicated the radius around a facility from which they must be eradicated. By radioactively tagging flies, releasing them, then recapturing them with baited traps, he observed individual flies traversing 10.3 kilometers in 24 hours. In another publication, Yerington and Warner (1961) described the method in more detail: Lab-reared or field-caught *Drosophila melanogaster* were starved for several hours, then fed fermenting ground figs with a radioactive isotope of phosphorus, P^{32} , in aqueous solution distributed over the surface. Flies who had ingested this food could be identified later using a Geiger counter. Many experiments were conducted, but perhaps the most impressive took place on October 13, 1959. At 5PM 40,000 marked *Drosophila melanogaster* were released 6.4 kilometers south of an isolated Calimyrna fig orchard near Clovis in the San Joaquin Valley in California. The temperature was about 26.7° C and wind under 0.5 kilometers per hour. 24 hours after release, 61 marked flies had been recaptured, and some of them had ended up

in traps over 7 kilometers upwind of the release point. Although this was the farthest distance reported, in many of the experiments marked flies flew distances on the order of kilometers in several days, even when released in the presence of abundant food.

Given the potential problems with releasing large quantities of radioactively labeled flies into the wild, an important advance was made by Wave et al. (1963) when they tested the efficacy of various fluorescent stains as markers of *Drosophila*. They reported that spraying flies with 0.5% rhodamine B reliably marked flies. In a test study near Beltsville, Maryland they observed stained *Drosophila melanogaster* traversing 4.8 kilometers. They released flies over a course of several weeks and only checked the recapture traps once a week, so no details are available for exact timing of the dispersal events.

The mark and recapture experiments on *Drosophila melanogaster* described above were not widely cited by population biologists. In 1973, Crumpacker and Williams followed up on the work of Dobzhansky by conducting a series of mark and recapture experiments on *Drosophila pseudoobscura* in Colorado (Crumpacker and Williams, 1973). They marked flies with fluorescent dust and released them in the center of a field of baited traps. The distance to the farthest trap was 702 meters along four primary directions, and 427 meters along 4 intermediate directions. In this field, considerably larger than that used by Dobzhansky and Wright (1943), they observed greater dispersal distances. After one day the mean distance of marked flies was 176 and 202 meters in two different experiments. They noted, however, that these were “probably serious underestimates of the true dispersal rates” because many flies traveled to points outside the range of traps. They estimated that after two morning activity periods more than half of the flies had gone beyond the range of the experimental field.

McKenzie (1974), conducting an experiment on *Drosophila melanogaster*, released marked flies near an Australian vineyard, and noted only “relatively restricted distances moved”. Given that the farthest trap from the release point was only 60 meters away, however, these conclusions cannot be considered reliable. Also in 1974, Dobzhansky and Powell conducted another set of mark and recapture experiments

in Mather, California (Dobzhansky and Powell, 1974) using a larger array of trap lines. By releasing wild-caught *Drosophila pseudoobscura* marked with fluorescent dust, they measured a greater rate of dispersal than that measured in 1943: After one day the marked flies had moved an average of 182.6 meters (compared to between 59 and 118 meters measured previously).

At this point it is clear that experimental design plays an important role in the magnitude of dispersal measured by experimenters. In a report containing both experimental data and a meta-analysis of previous experiments, Johnston and Heed (1975) pointed out that when attractive baited traps were placed at larger intervals, longer dispersal distances were measured. As a case study, they conducted a mark and recapture experiment on *Drosophila nigrospiracula* in Tucson, Arizona. By monitoring recapture in a series of banana-baited traps, they measured an average dispersal rate of 4.8 meters per day. This estimate is an order of magnitude less than the rate observed for populations of the same species in the absence of baited traps. Perhaps more importantly, in the wild these flies subsist on transiently available cactus rots that are separated by an average of over 121 meters. Hence, the dispersal rate measured in the presence of baited traps would not permit species survival. In a following study (Johnston and Heed, 1976) these experimenters reported extremely high dispersal rates in *Drosophila nigrospiracula*, concluding that the entire population can be treated as panmictic. Dobzhansky et al. (1979) extended the observation of the effect of traps to natural environments: They observed that *Drosophila* dispersed less in favorable habitats (e.g., dense woods) than less favorable ones.

Returning to release and recapture experiments on *Drosophila melanogaster*, in a study remarkable for sheer numbers, Guest et al. (1979) released 12.3 million¹ sterile flies marked with fluorescent dye in New Jersey over the course of several months. In the days after the releases they recaptured 1000 marked flies. Of these 956 were within 31 meters of the release point, four were 1.93 kilometers away, 14 were 3.22 kilometers distant, and 26 were 4.18 kilometers away. Unfortunately the authors do not report

¹The authors of this study did not report how they counted such a vast number of flies, but a volumetric analysis seems the most likely method.

exact times at which these individuals were recaptured. In Raleigh, North Carolina McInnis et al. (1982) marked native *Drosophila melanogaster* with fluorescent dust and released them at the intersection of 800- or 1200-meter-long trap lines. They observed average movement of 150 meters per day, although they remarked that this may have been an underestimate because of flies moving beyond the range of the traps.

In the 1980s four studies effectively made the case for long-distance dispersal in *Drosophila*. The first, (Jones et al., 1981), reported results from both allelic analysis and mark and recapture experiments. We will focus on the latter here. The researchers first released fluorescently marked wild-caught *Drosophila pseudoobscura* at the intersection of a cross of trap lines extending in approximate cardinal compass directions for 1600 meters. The experiments were conducted in a region of California's Death Valley desert with no native *Drosophila* population. They reported that the flies reached the end of the trap lines within 12 hours, and that very few flies could be found within the study area after 24 hours. In three different experiments, the mean distances moved by the flies were 405, 509, and 392 meters in 15 hours. That is, these flies exhibited dispersal rates over five times higher than those reported by Dobzhansky and Powell (1974). Because of the extraordinary dispersal rates they observed in these trap line experiments, the researchers expanded the observed range in later experiments: They released marked flies in the middle of the desert, then observed traps located several kilometers away in distant oases. They reported that a large number of flies dispersed over 2 kilometers in a single day, with some traveling over 10 kilometers in that time span². Given the angular size of the oasis from their release point, the experimenters estimate that in fact the majority of *Drosophila pseudoobscura* would travel 10 kilometers over unfavorable terrain in a single day.

These studies, while intriguing in their report of long-distance flights, leave open two questions: Are these flights only a result of the release point? That is, are the

²The investigators were careful to eliminate the possibility that they transported flies with them from the release point to the recapture areas by executing the following procedure: "...investigators walloped themselves vigorously about the head and body to remove any flies" (Coyne and Milstead, 1987).

long distances traveled an artifact of the inhospitable region in which the released flies found themselves? And second, do *Drosophila melanogaster* also exhibit this behavior? With these in mind, Coyne et al. (1982) undertook another set of mark and recapture experiments in Death Valley. In order to quickly identify species in the field, they classified flies as either “black” or “yellow,” after determining that the black flies were *Drosophila pseudoobscura* and roughly 60% of yellow flies were *Drosophila melanogaster* and 40% were *Drosophila simulans*. They found that the mean migration distances were similar in black and yellow flies, but that the recapture percentage was much lower for yellow flies. The most relevant result for the present discussion was the finding that after releasing 30,000 marked yellow flies in a desert oasis at 6PM, by 9:30AM the next morning they had recaptured 14 yellow flies in traps at an oasis 6.8 kilometers away, and 3 yellow flies in traps located in an oasis 14.6 kilometers away. Because the release occurred after the evening activity period, the researchers suggested that most of the movement took place during only a few hours of the morning activity period. Thus, *Drosophila melanogaster* exhibit long-range movements over inhospitable terrain even when released in favorable environmental conditions.

The third study in this series, (Coyne et al., 1987), addressed two points. The first was the question of how far away from an oasis can a fly detect it as an attractive location. By releasing *Drosophila melanogaster* at various distances from an oasis, they found that past 100 meters away flies were as likely to be caught traveling away from the oasis as towards it. This indicates that flies perceive this type of habitat at approximately 100 meters. The second question was whether undisturbed flies living in nature can be found far from suitable habitats (that is, was the behavior of the flies in the mark and recapture experiments changed by the experimental intervention.) In order to address this, the researchers captured wild flies in remote desert locations. They found *Drosophila melanogaster* present in areas several kilometers from the closest spring or tree, indicating that this species indeed travels through desert terrain even in the absence of experimental disruption.

The main limitation of these studies from the point of view of population biol-

ogists was that they took place in unusually inhospitable terrain, so it was difficult to generalize their findings on dispersal rates to other types of habitats. In the final report Coyne and Milstead (1987) examined fly behavior in more temperate environmental conditions—an isolated Maryland orchard. In this study they released pupal *Drosophila melanogaster* that had been bred to be heterozygotes for two linked recessive alleles. By monitoring the levels of these alleles in the surrounding area, they were able to determine that flies had spread 10 kilometers over 3 months. While this experimental procedure is not amenable to exact timing of dispersal events, it demonstrated that long-range gene flow is not unique to desert environments.

Later experiments by other researchers (e.g., Markow and Castrezana, 2000) have confirmed the observation of kilometer-range movement in 24 hours by various *Drosophila* species.

1.5.2 Indirect measures of gene flow: Population genetics studies

Most of the studies mentioned in Section 1.5.1 were designed to understand how much exchange of genetic material takes place between geographically separated populations of flies. In fact, they were often conducted as tests of hypotheses generated by sampling the genetic structures of various natural populations. The first such population genetics studies were based on the analysis of recessive lethal alleles (e.g. Dobzhansky and Wright, 1941 for *Drosophila pseudoobscura* and Paik, 1960 for *Drosophila melanogaster*.) Since a lethal allele represents a significant fitness cost, chances are that such an allele would arise once before being eliminated. Thus, if two flies both carry the allele, it is likely that they both inherited it from a common ancestor in the fairly recent past. Given estimates of mutation rate, the relative concentration of lethal alleles can be used to estimate the amount of genetic exchange between two populations. From this type of analysis many more complex methods have been developed, and it is out of the scope of this work to review them here. (Slatkin, 1985, presents a general overview of the methods for determining gene flow.) Although

there is a history of substantial debate in this field, recent consensus supports the existence of extensive gene flow among populations of *Drosophila melanogaster*.

By examining the frequency of rare alleles in various populations of *Drosophila melanogaster*, Singh and Rhomberg (1987) concluded that there is a large amount of gene flow among these populations, consistent with the observations of dispersal rates discussed above. A more recent study by Turelli and Hoffmann (1991) monitored the spread of a reproductive parasite, *Wolbachia*, among California *Drosophila simulans*. They found rates of spread an order of magnitude larger than those found in direct measurements of dispersal.

There is not universal acceptance of high levels of gene flow. Agis and Schlötterer (2001) performed an analysis of microsatellite loci on *Drosophila melanogaster* taken from populations along Australia's east coast and concluded that there were low levels of gene flow. In a later study using microsatellite genotyping of a larger number of populations in a broader geographical region, however, Kennington et al. (2003) found high levels of gene flow, confirming the direct observations.

1.6 Evidence for responses to light polarization in Diptera

1.6.1 Large flies

Given the high rates of dispersal that have been observed in flies, we expect that flies must have some mechanism for maintaining a straight course for long periods of time. A mechanism relying on sun position, including a sky polarization-based component, seems a likely candidate, given the importance of this sensory capacity to other insect species. The body of work on the detection of the plane of polarization by flies, while extending over a relatively long period of time, does not coalesce into a unitary understanding. We will attempt to summarize the findings of that body of work here.

Wellington (1953) reported that *Sarcophaga aldrichi* adults, when walking outdoors with their wings clipped, turned when a linear polarizing filter was rotated above them. This behavior persisted when either the compound eyes or the ocelli were covered with paint. This observation was somewhat anecdotal, and no control conditions were reported. Later, Fernández-Moran (1956) concluded from electron microscopy of the eye of *Musca domestica* that cells R1–6 might be responsible for polarization sensitivity.

A series of studies in the fly *Musca* revealed that it also exhibited turning responses when stimulated by white light through a rotating polarizer (Kirschfeld and Reichardt, 1970). By examining the responses to rotating polarized contrast gratings of different spatial wavelengths, these researchers further concluded that the R7/8 system mediated responses to polarization. Gilbert McCann and David Arnett, working at Caltech, performed electrophysiological and behavioral experiments on the visual system of flies (McCann and Arnett, 1972). They reported that the contributions by the R1–6 and R7/8 photoreceptor subsystems could be differentiated by observing responses to moving patterns of stripes of different widths. They inferred that the R7/8 cells have smaller receptive fields and greater acuity than R1–6, based on the smaller diameter of the photosensitive parts (rhabdomeres) of the R7/8 cells. They found that tethered flying *Musca domestica* exert a torque in the same direction as moving large stripes, but that this response inverts for moving smaller stripes (angular width less than 5°). They attributed this inversion to the contribution of only the R7/8 system, and reasoned that it could be used to separate the response properties of this system from those of the R1–6 system. In both electrophysiological and behavioral tests they found slight polarization sensitivity of the R7/8 system to light in the wavelength range 420–550 nm, but not to ultraviolet wavelengths. The segregation of the R1–6 and R7/8 channels based on spatial properties as done in these two studies has since been called into question, however, with consensus now being that the two systems are specialized for different spectral and intensity ranges (Heisenberg and Buchner, 1977; Rister et al., 2007).

Using intracellular recordings in *Calliphora*, Järvilehto and Moring (1974) found

that some R1–6 retinula cells were sensitive to the angle of polarization, but they did not observe any such sensitivity in R7/8 cells. The difficulty of performing this type of recording resulted in small sample sizes, however. Horridge and Mimura (1975) reported polarization sensitivity at the receptor level in cells R1–6 of *Calliphora stygia*, but were primarily interested in interpreting these in relation to the source of the two spectral sensitivity peaks, and did not look for preservation of this information downstream in the visual system. A study with similar goals was conducted by McCann et al. (1977) on *Calliphora erythrocephala*. They reported that in cells R1–6, the average difference in response to monochromatic polarized light of orthogonal e-vector angles was about 10%.

In the early 1980s a series of anatomical and electrophysiological studies on *Musca domestica* and *Calliphora erythrocephala* suggested that in these flies the DRA was specialized to detect the angle of linearly polarized UV light (Wunderer and Smola, 1982a,b; Hardie, 1984). In a behavioral study of tethered walking *Musca domestica*, von Philipsborn and Labhart (1990) concluded that the DRA was responsible for mediating turning responses to rotating the polarization angle of light.

1.6.2 *Drosophila*

The first reports of *Drosophila melanogaster* orienting to plane polarized light were published in the early 1950s (Stephens et al., 1953). The researchers observed individual mutant vestigial-winged flies, which are unable to fly, walking under polarized white light of various intensities and recorded their headings by hand. They reported a tendency of flies to align their body axis with the angle of polarization. They noted, however, that there was no known ethological context for this behavior. They suggested that it may be due to an apparent brightness difference between polarization axes caused by differential transmission of the different axes at the cornea-air interface, instead of true polarization vision in the sense of actually perceiving the angle of polarization itself.

Martin Heisenberg tested for flight responses in *Drosophila* to a rotating drum



Figure 1.5: Schematic of apparatus used by Heisenberg (1972). The fly was placed in the center of one of three drums. A positive control for contrast (left), was half white and half black. In the other two drums (center and right) the two halves were made of linear polarizers. (Note that the stripes depicted here indicate the axis of the polarizers, not actual contrast stripes.) In the experimental condition (center) the two polarizers were oriented perpendicularly. In the control condition (right) they were parallel.

made up of two polarizers, each spanning 180° (Heisenberg, 1972). In the experimental condition one polarizer was oriented with its axis 45° clockwise from horizontal and the other 45° counterclockwise from horizontal (Figure 1.5). In a control condition both polarizers were oriented in the same direction, 45° clockwise from horizontal. He observed wild-type flies exerting a torque in the direction of motion of the experimental drum, but not the control drum. *Opm 2* mutant flies, however, showed almost no turning response to the polarized drum, but a robust response to a rotating contrast pattern. From this he concluded that the “high acuity” R7/8 system was responsible for detecting the polarization angle.

In 1980, Reinhard Wolf and his colleagues in Heisenberg’s lab published a thorough study of polarization sensitivity during flight and walking in *Drosophila melanogaster* (Wolf et al., 1980). Using a flight simulator, they observed changes in the rotatory response elicited by moving stripes when the light making up the stripes was polarized in different directions. They also measured turning responses to rotating the angle of polarization, and conducted the necessary controls to show that these

responses were not due to uncontrolled intensity effects. Given that they saw polarization sensitivity to light in the visible portion of the spectra, and that the angle of polarization affected the R1–6-mediated response to moving stripes, they concluded that polarization sensitivity is mediated by R1–6 in *Drosophila*, and not restricted to the DRA.

In the three decades following the work of Wolf et al., to our knowledge there were only three studies of polarization vision in *Drosophila*. Coombe et al. (1989) recorded intracellularly from cells in the first two optic neuropils, the retina and the lamina, of *Drosophila*. Although the cells were unmarked, they reported that they recorded from R1–6 in the retina and the large monopolar cells in the lamina. They observed sensitivity to the angle of polarization of green light in the retinula cells, but not in the lamina cells. In 1991, Fortini and Rubin published a description of the projections of the R7/8 cells from the DRA through the visual neuropil (Fortini and Rubin, 1991). They used gene fusions to create a histological marker and serially sectioned the preparation to image these projections. This work did not directly address the role that these cells played in polarization-dependent behavior, though. In a developmental study, Wernet et al. (2003), demonstrated the role of *homothorax* (*hth*) in driving the development of the anatomy of the DRA. They showed that *hth* is expressed in the maturing DRA and maintained throughout the life of the adult fly. Furthermore, they demonstrated that eliminating *hth* prevents development of the DRA and misexpression of *hth* induces DRA-like characteristics in other parts of the eye (enlarged rhabdomeres and expression of the same opsin in both R7 and R8.)

Very recently, Wernet et al. (2012) published a genetic analysis of polarization-dependent walking behavior in *Drosophila*. Using Gal4 lines to target specific photoreceptor cell types, they analyzed the effects of driving the heat-sensitive synaptic blocker *shibire*^{ts} or functionally rescuing phototransduction in otherwise blind *norpA* mutants. Based on a population measure of spontaneous orientation, these researchers concluded that R7/8 in the DRA is responsible for detecting the polarization angle of dorsally presented UV light. They also found sensitivity to ventrally presented polarized UV and green light. For these responses they found evidence for the involvement

of both the R7/8 system and the the outer photoreceptors, R1–6. In addition to their genetic manipulations, these researchers conducted a series of electron microscopic studies examining the microvillar arrangement in different parts of the eye. They confirmed that the microvilli of R7 and R8 in the DRA do not show significant twist along their axes, and that they are oriented roughly perpendicular to one another. Additionally, they observed regions in the dorsal eye in which there was reduced microvillar twist in not only R7 and R8, but also in R4–6. From this they concluded that some ventral R8 and R4–6 cells may mediate the observed orientation to ventrally presented polarized green light, and that some ventral R7 and R4–6 cells may underly the response to ventral UV polarization.

In summary, there is a reasonable amount of evidence that dipterans respond to changes in linearly polarized light. Unfortunately, much of this evidence is from extremely unnatural behavioral conditions, or purely electrophysiological studies, and almost none of it has been reproduced in independent laboratories.

1.7 Polarization information in the nervous system

1.7.1 Optic lobes

Little is known about how information about light's polarization is transformed by the fly's nervous system after it is detected by cells in the retina. Aside from the negative result for lamina cells by Coombe et al. (1989), the only electrophysiological data were collected in larger insects, which are more amenable to this type of recording. A distinction can be made between polarization-sensitive neurons, which alter their responses when the e-vector is changed, and polarization-opponent neurons. Polarization-opponent neurons receive antagonistic input from receptors sensitive to orthogonal e-vectors. This cell type seems the most appropriate to mediate true polarization vision, since their response can be independent of stimulus intensity. Cells fitting this description were first observed using intra- and extracellular recordings in the medulla of the cricket (Labhart, 1988). (The medulla is a neuropil in the optic

lobe of insects just downstream of the lamina.) The firing rate of these cells was independent of light intensity above a threshold, and reliably followed the orientation of the polarized stimulus. Labhart observed neurons with preferred e-vector directions in three directions: 35° , 85° , and 155° from the long axis of the animal. If these are the only cell type that relays polarization information to the central nervous system, it seems that the animal would be able to compute the general celestial e-vector direction, but would lack detailed spatial resolution of the exact pattern. This report did not contain any anatomical data about these projections of these cells.

Homberg and Würden (1997) described polarization-sensitive neurons in the optic lobe of the locust, *Shistocerca gregaria*. These neurons showed intensity-independent responses to e-vector direction. The researchers also provided images of the neurons, and written descriptions of their branching patterns: The “neurons had tangential arborizations in the medulla, a few sidebranches in the accessory medulla, and projections to the lamina or to the contralateral optic lobe.” The neurons did not enter the dorsal rim area of the medulla (which is a retinotopically organized structure), leading the researchers to conclude that they received polarization information input from other interneurons.

Later studies reported polarization-opponent neurons in the optic lobe of the desert ant, *Cataglyphis bicolor* (Labhart, 2000). Researchers have also recorded from polarization-sensitive heterolateral neurons in the medulla of a cockroach, *Leucophaea maderae* (Loesel and Homberg, 2001).

1.7.2 Central brain

To date, most of our information about central representations of polarization information downstream of the optic lobes comes from electrophysiological investigations of locusts (for example, Heinze and Homberg, 2009; Träger and Homberg, 2011) and, more recently, monarch butterflies (Heinze and Reppert, 2011). Much of this work has focused on the central complex, a collection of midline neuropils common to all insects (Homberg, 2008; Loesel et al., 2002). In this region numerous cell types have

been identified that show polarization-dependent responses. In addition, there is evidence for representations of other sensory modalities (Ritzmann et al., 2008), as well as studies implicating a role for control of locomotion (Ridgel et al., 2007; Strauss and Heisenberg, 1993) in this region of other species.

Chapter 2

Rigid tether experiments demonstrate *Drosophila* have the capacity to detect the angle of linearly polarized light

"I see how it is," said Fix. "You have kept London time, which is two hours behind that of Suez. You ought to regulate your watch at noon in each country."

"I regulate my watch? Never!"

"Well, then, it will not agree with the sun."

"So much the worse for the sun, monsieur. The sun will be wrong, then!"

—Jules Verne, *Around the World in Eighty Days*

(Translated by George Makepeace Towle)

2.1 Introduction

In our first set of experiments we looked for behavioral responses to artificially polarized light by fruit flies. Flying is a more efficient form of locomotion than walking (Tucker, 1970; Berrigan and Partridge, 1997), as measured by so-called minimum cost of transport—the minimum amount of metabolic energy above baseline required to traverse a given distance per unit mass. We assumed that compass information is most important to an organism traversing relatively large distances, since local cues

are sufficient to direct short-distance movements. Given these considerations, we reasoned that behavioral evidence for orientation to the compass provided by celestial polarization would be most readily observable in flying flies.

A freely flying fly presents significant challenges in terms of experimental design: Tracking individuals is not trivial, and the movement of the individual determines the exact sequence of stimuli it is exposed to. Polarized light has its own unique characteristics that make eliminating confounding factors an important consideration (see Sections 2.2.3 and 2.2.4). As a compromise between simulating real-world orientation behavior and presenting reproducible stimulus conditions, we opted to first use a preparation in which an individual fly was rigidly held in place but was free to flap its wings as it would in free flight. This preparation allowed us to deliver identical stimuli across multiple trials and measure responses while the organism engaged in fictive flight maneuvers. We recorded these fictive maneuvers, as described in Section 2.2.5.

2.2 Methods

2.2.1 Flies

We used flies from a lab stock descended from 200 mated *Drosophila melanogaster* females caught in the wild. The stock was on a 16/8 hour light/dark cycle. Unless stated otherwise, we used male flies in all experiments. Between 3 and 4 days after eclosion, we anesthetized each fly by cooling it to $\approx 4^{\circ}\text{C}$. After positioning it with a fine paint brush, we attached its notum to a 0.1 mm pin made of tungsten using UV-cured glue. We also immobilized the fly's head by gluing it to the pin and thorax. Care was taken in positioning the pin such that the fly was held in a flight posture in the arena, approximately 60° from the horizontal. Following this procedure we prevented the fly from flapping its wings by placing a ≈ 2 mm square piece of tissue paper on its tarsi and gave it at least half an hour to recover. If the fly stopped flapping during an experiment we gently blew on it to motivate continued flight. Any

such periods were excluded in later analysis.

2.2.2 Stimuli

At sunset in the middle latitudes orienting parallel to the primary axis of dorsal skylight polarization would result in the animal facing either north or south, whereas facing perpendicular to it would result in east-west orientation. A priori, there does not seem to be a reason flies would prefer one orientation over another with respect to light polarization (or compass direction). Hence, there is not a clear hypothesis for the behavior of an animal presented with a static field of polarized light. The situation is different, however, when the polarization pattern changes in time. A ubiquitous visuo-motor behavior among motile animals is the tendency to stabilize wide-field rotations of the visual scene by physically rotating with it. This tendency serves to keep an animal on a straight course, since it is more likely that coherent wide-field rotation of the visible world is caused by rotation of the animal rather than rotation of the external world. Because of its prevalence, it is sometimes termed the “optomotor response”, although clearly it is just one of a multitude of optomotor behaviors exhibited by animals. We decided to look for an analogous response to a field of light with rotating polarization angle. If the animal is able to perceive the angle of polarization and use it to direct locomotion, we reasoned that it should attempt to compensate for rotations of that angle. We predicted that an animal able to perceive the e-vector of light should attempt to turn in the same direction as rotation of the polarization angle.

Polarized light is relatively easy to produce in the laboratory by passing ordinary light through a polarizing filter (polarizer). By rotating such a filter, one can rotate the angle of polarization of the transmitted light. In our laboratory experiments, we used arrays of light emitting diodes (LEDs) to produce light of desired color, either UV (peak wavelength 355 nm), blue (peak wavelength 470 nm), or green (peak wavelength 525 nm). This light passed through a linearly polarizing filter whose orientation could be controlled by a computer via a stepper motor and custom control electronics (see

Figure 2.4). In order to make sure that the fly could not see the rotation of the filter itself or its mounting ring, we placed a ground glass diffuser between it and the fly. This diffuser did not disrupt the polarization angle of the light, but did obscure the rotating pieces from the view of the fly. We set the intensity for all wavelengths after passing through these optical elements to be $\approx 1.2 \mu\text{W cm}^{-2}$.

2.2.3 Considerations for light passing through polarizing filters

The above description has neglected one critical effect unique to the interaction of light and polarizing filters. As pointed out in the paper by Wolf et al. (1980), the reflections at the surfaces of such a filter introduce intensity gradients in the transmitted light across the filter's surface that are exactly in phase with the pattern of polarization produced by the filter. The amount of light transmitted at an oblique incidence angle through a polarizing filter depends on the angle between its path and the transmission axis of the polarizer. This difference in the amount of transmitted light is due to the difference in the ratio of reflected to transmitted light of different polarization angles at a surface. Consider linearly polarized light hitting a partially reflective surface. When the electric field vector is parallel to the surface, more light is reflected and less is transmitted than when the magnetic field vector is parallel to the surface. Unpolarized light can be decomposed into equal parts of each of these two axes of polarization. Hence, even when the incident light is not polarized, more light is transmitted through a polarizer when the light's path is in the same plane as the plane containing the surface normal and the transmission axis of the polarizer, compared to when the light's path is out of that plane (Figure 2.1). An alternative, but equivalent, explanation is that the surface of the filter, or any transmitting surface, acts as a weak polarizer when light strikes it at an oblique angle. When the axis of this weak polarizer aligns with that of the polarizing filter, more light is transmitted than when their axes are perpendicular to one another.

When light coming through a flat polarizing filter is viewed from a single point

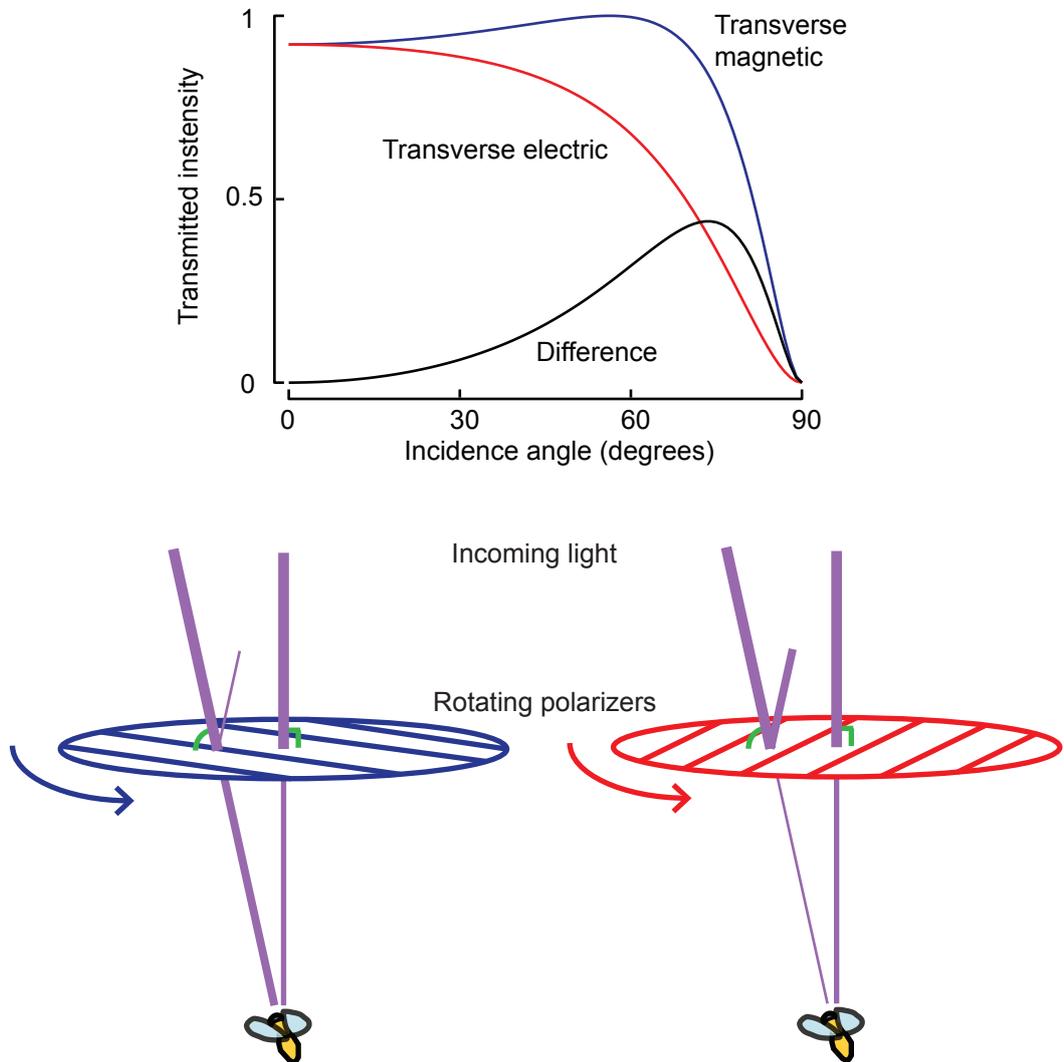


Figure 2.1: Light of different polarizations incident on a surface. In the top panel we show the intensity of light of each polarization direction that is transmitted through two air-surface interfaces and the difference between them. In the bottom panel a schematic shows less light is reflected when the polarization axis is in the same plane as that defined by the surface normal and the incident beam path (left) than when the polarizer axis is normal to that plane (right).

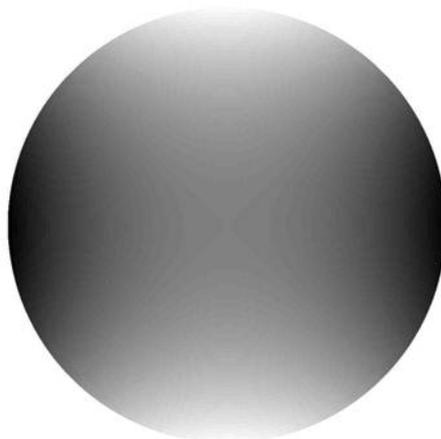


Figure 2.2: Intensity variation pattern across the face of a flat linearly polarizing filter illuminated homogeneously subtending 60° . This image is scaled such that it uses all grayscale values between white and black to demonstrate the intensity pattern expected for a polarizer of the same angular size as that used in our experiments. Note that the differences in transmitted intensity are largest at the outer edge.

in space (as, for instance, when a fly is looking through one), light coming from different places will travel through the polarizer at different angles. We have seen that different incidence angles result in different transmission/reflection ratios. Hence, any polarizer that subtends a large field of view will generate noticeable intensity variation across its surface, and if it rotates this pattern will rotate with it. We calculated the expected intensity variation across the surface of a polarizer by considering only first-order reflections (neglecting multiple internal reflections) and generated Figure 2.2 to represent the type of pattern transmitted by a polarizer of the size used in our experiments.

The intensity pattern depicted in Figure 2.2 is visible to normal eyes, and hence a fly responding to a rotating polarizer that transmitted such a pattern could not be conclusively said to be able to perceive the angle of polarized light. In order to avoid the creation of such a pattern, one could restrict the polarizer to a small angular size, since the greatest effect on intensity is found at larger transmission angles. A smaller stimulus would presumably be less salient for a fly, however. Alternatively, a polarizer that was curved such that it formed a partial sphere with the fly at its center would not introduce intensity patterns across its surface. Unfortunately, curved polarizers

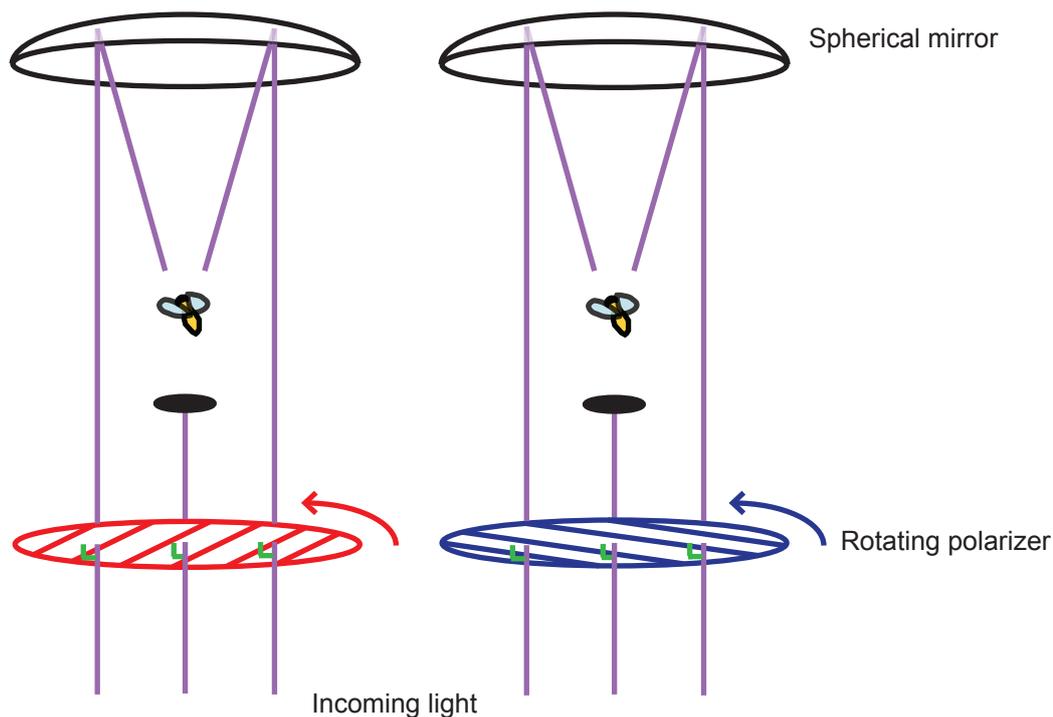


Figure 2.3: Light traveling through a polarizer before being reflected toward the fly by a spherical mirror. As recommended by Wolf et al. (1980), we used a spherical mirror (top) to ensure that all the light reaching the fly (purple lines) had been transmitted at a normal incidence through the polarizer (hashed oval), as indicated by the green right-angle marks. This technique eliminates the effect on intensity discussed in Section 2.2.3. An opaque shield (black oval) blocks the polarizer from direct view of the fly.

are not widely available. The solution we settled on, following Wolf et al. (1980), was to reflect the light transmitted through a flat polarizer off a spherical mirror. When placed at the focal point of such a mirror, all of the light reaching the fly had to travel through the polarizer normal to its surface (Figure 2.3). First surface mirrors preserve polarization, so we are left with a uniform field of linearly polarized light. We used a 25.4-mm-diameter mirror with focal distance of 25.4 mm, resulting in a stimulus with 60° outer diameter as seen by the fly. We blocked the light coming from the polarizer directly from the fly's view with a small opaque ball.

While the reasoning behind this stimulus delivery method appears sound, how can we be certain that it is actually functioning as we expect it to? The critical

control experiment is to unpolarize the light that has traveled through the polarizer and look for behavioral responses. If the animal still responds to the rotation of the polarizing filter in this unpolarized condition, we can conclude that it is responding to some aspect of the light other than its polarization. We accomplished this control by placing a quarter wave plate after the polarizing filter. When aligned correctly, this element transforms the light into circularly polarized light, which should be indistinguishable from unpolarized light to the fly. (There are reports of circular polarization vision in animals, but very little evidence for such vision in insects (Brady and Cummings, 2010; Blahó et al., 2012), and none for flies.) This filter arrangement is termed a circular polarizer. When the stimulus was in the wavelength range visible to humans, we used an achromatic quarter waveplate, effective over this whole range of wavelengths. When the stimulus was ultraviolet light, we used a waveplate effective only for that wavelength, necessitating a bandpass filter to restrict the wavelength range of the stimulus to that which could be effectively circularly polarized by the wave plate.

In addition to the circular polarization negative control, we created a positive control aimed at determining the type of behavioral response that could be elicited by the color, intensity, size, position, angular velocity, etc., of our stimulus. This positive control was a transparent glass filter with alternating quarters covered with black electrical tape (Figure 2.5). This produced a pattern visible to the fly of the same mean intensity and with the same symmetry as the linear polarization pattern. Both this pattern and linearly polarized light repeat after 180° rotations. This type of symmetry is sometimes called “axial” because it is only defined up to a two-way axis through the origin, rather than a directed one-way ray

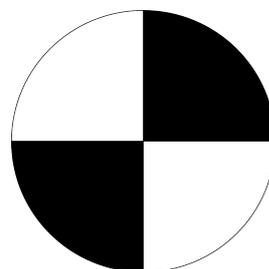


Figure 2.5: The contrast pattern filter used in open-loop rigid tether experiments. Note that the pattern repeats after 180° . This is the same spatial wavelength as a linear polarizer.

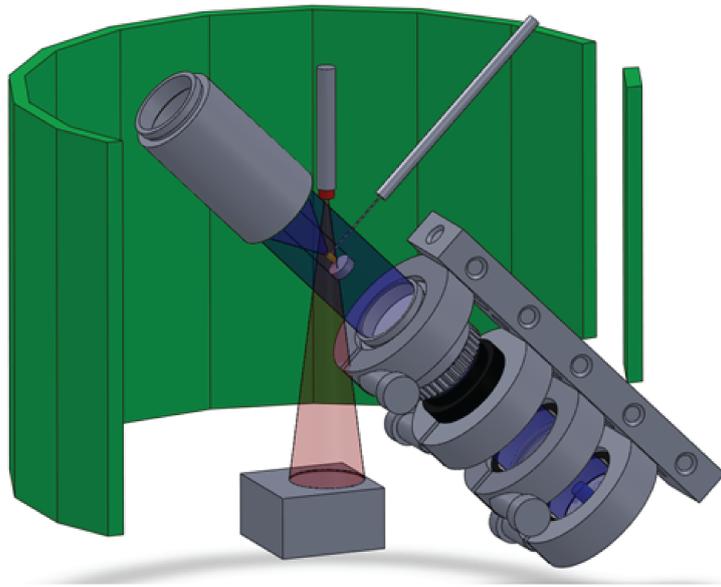


Figure 2.4: Indoor rigid tether arena. The semitransparent red cone shows the infrared beam used to track wingstrokes by the photodiodes pictured as the gray box (bottom center). The semitransparent blue area shows the stimulus light path, from the LED in the bottom right, through the various filters, then reflected back to the fly by the spherical mirror in the gray tube (top left). The fly is held by a gray cylinder emerging from the top right. The surrounding green LED panels in front have been cut away in order to show the interior of the arena.

out from the origin. The insect visual system is thought to be sensitive to the so-called spatial wavelength (angular extent of repeating elements of the visual pattern) when computing visual motion (Clark et al., 2011; Eichner et al., 2011), which is why we designed our positive control to repeat after the same angular displacement as linearly polarized light.

We surrounded the arena with programmable panels of green LEDs, as described by Reiser and Dickinson (2008). Using these we could present standard patterns that elicit known behaviors to ensure that flies were correctly positioned in the arena and begin experiments in reproducible starting conditions. The full behavioral arena is depicted in Figure 2.4.

2.2.4 Considerations for light reflecting off surfaces

A final consideration when using polarized light stimuli in behavioral experiments also has to do with the interaction of light and reflective surfaces. As discussed above, light reflected from a surface at nonnormal angles is partially polarized. If the reflecting material is dark, these surface reflections make up a large proportion of the total reflected light. If, on the other hand, the material is white, more light is reflected overall, diminishing the fraction of partially polarized light from surface reflections. This effect is responsible for a dark shiny car appearing to sparkle more than a white car—the white car’s sparkles are swamped by the larger overall reflection from its paint. This consideration is discussed by Horváth and Varjú (2004). Because of this effect, we covered all surfaces in our behavioral arena with matte white tape or paper.

2.2.5 Monitoring flight behavior

Thus far we have discussed our method for presenting the fly with a uniform field of linearly polarized light, whose polarization angle could be rotated without changing other aspects of the light. We now turn to a description of how we monitored the fly’s behavior during our experiments, which has been described in detail elsewhere (Lehmann and Dickinson, 1997; Götz, 1987). We positioned each fly between an

infrared LED and an array of two photodiodes, each covered by an opaque mask with a window (Figure 2.4). On either side, the fly’s wing interrupted the beam of infrared light, casting a shadow over the window above the photodiode on that side. The windows were shaped such that the portion of the window being covered by the shadow was proportional to the wingstroke amplitude. The signals from these photodiodes thus provided a record of the size of individual wingstrokes. When the wingstroke amplitude on the left was greater than the wingstroke amplitude on the right, the fly produced a torque which in an unrestrained animal would rotate the animal to the left. Hence, the record of bilateral wingstroke amplitudes can be interpreted as a record of the fly’s attempts to turn during fictive flight. These records are the data we will use to analyze fly behavioral responses to various stimuli. Because this optical system uses analog electronics to measure wingstroke amplitude, we will report wingstroke amplitude in volts throughout this chapter.

We call an experiment “open-loop” if the stimulus is independent of the behavior of the fly. This type of experiment is distinguished from “closed-loop” experiments, in which the behavior of the fly is used to alter the stimulus itself. Closed-loop experiments can be used to mimic free-flight behavior, by converting the attempted turns we measure with the photodiodes into motion of a visual stimulus. By moving the stimulus in the opposite direction to the fly’s attempted turn, the arena operates as a virtual reality chamber in which we can measure fly’s tendency to orient toward a particular orientation of the visual stimulus.

2.3 Open-loop experiments

2.3.1 Open-loop methods

We designed our first experiments as simple open-loop trials. We started each trial by rotating the filter to a starting orientation chosen randomly from the set $\{0^\circ, 45^\circ, 90^\circ, 135^\circ\}$, then turning on the LEDs that provided light to be transmitted through the filter. For 10 seconds the filter was held in a fixed position, then we rotated it

at 180° per second either clockwise or counterclockwise for 40 seconds, then stopped it and held it in place for another 10 seconds before turning the LEDs off. Each fly was presented with three blocks of six trials (three trials in each direction of rotation, in random order). The filter was changed between blocks, such that each fly saw all three filters (circular polarizer negative control, linear polarizer, intensity pattern positive control) in random order. We presented a dark stripe under closed-loop control by the fly on the surrounding green LED panels for 10 seconds between trials and while we changed filters. Flies are attracted to these vertical objects, which elicit robust steering behavior to keep the stripe front of the fly. This behavior, often called stripe-fixation, served to ensure that the fly was in a uniform starting condition at the beginning of each trial.

2.3.2 Open-loop results

The goal of our first experiment was to determine if flies responded behaviorally to changing the angle of linearly polarized light. We rotated the polarization angle of blue or UV light and measured an individual fly's attempts to turn during restrained stationary flight. We found that flies attempted to turn in the rotation direction of the polarization angle with roughly half the amplitude elicited by the rotation of an equivalent contrast pattern. (Figure 2.6, center column and right columns). Flies did not attempt such compensating maneuvers when we rotated a circularly polarizing filter (Figure 2.6, left column). We excluded from our analysis any trial in which the fly stopped flying, and we excluded any fly that did not fly continuously for at least one trial of every trial type (filter and rotation direction).

In order to quantify the strength of fly responses to the various conditions, we computed the mean difference in response to clockwise and counterclockwise rotations over the final 15 seconds of filter motion (Figure 2.7). Using this metric, we found that every fly responded to rotating the linear polarizer with larger steering responses than those elicited by rotating the circular polarizer. The circular polarizer effectively controls for unintended contrast gradients that rotated with the filter (such as those

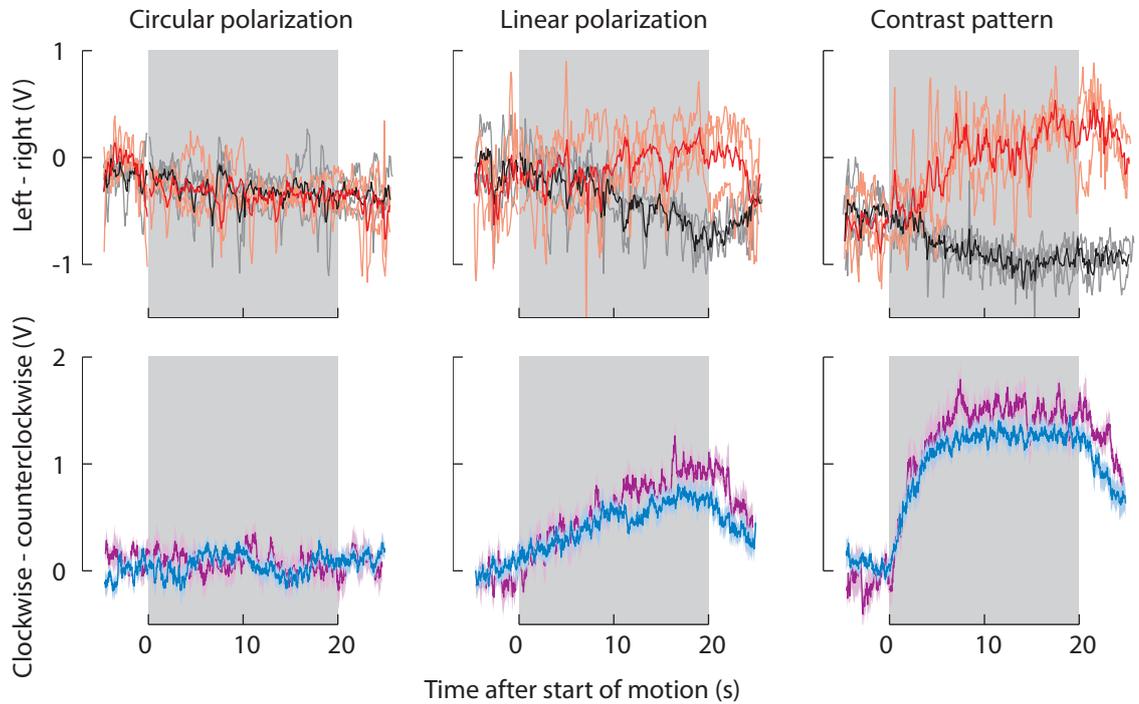


Figure 2.6: Flies attempt to turn in the same direction as polarizer rotation. Responses to the circularly polarizing filter are in the left column. Responses to the linearly polarizing filter are in the center column. Responses to the contrast pattern are in the right column. The filter was stationary until it started moving at a 180° per second at time $t = 0$, then it stopped 20 seconds later. The top row shows responses of a representative individual when presented with blue light. Light lines depict the difference between left and right wingstroke amplitudes for each 40 second trial. Heavy lines show the average response for all three trials of a given filter type and rotation direction. Lines in black represent trials in which the filter rotated counterclockwise, those in red show responses to clockwise filter rotation. The second row contains average responses of all flies in this experiment. We subtracted the average response for each fly to counterclockwise filter rotation (heavy black lines from top row) from the average response for that fly to clockwise filter rotation (heavy red lines from top row). We plot the mean and standard error of those individual fly responses here in bold lines and light patches, respectively. Responses to UV light are in purple ($n = 10$ flies), and responses to blue light are in blue ($n = 14$ flies).

from inhomogeneities in the filter or from the effect described in Section 2.2.3). Hence, we conclude that flies responded to rotation of the angle of linearly polarized light, and not some other stimulus in our arena.

One possible type of “polarization vision” would be to perceive light of different polarization angles as simply lighter or darker than other polarization angles, a false-intensity effect. (A human wearing polarizing sunglasses could be said to possess this type of vision.) Such a false-intensity system could result from a photoreceptor that preferentially absorbed one e-vector angle above all others. In this case, rotating the angle of polarization away from this preferred direction in either the clockwise or counterclockwise direction would result in identical diminishing responses in the photoreceptor. Hence, such a system would not be able to disambiguate clockwise from counterclockwise rotations in our experiments, where the fly was held in a fixed position. Since the flies in our experiment did respond to different directions of rotation differently, this logic allows us to conclude that the flies in this experiment were responding to the *angle* of polarization, and not just exhibiting a false-intensity effect.

Flies responded to linearly polarized light with steering responses that were roughly half as large in amplitude as those elicited by the rotating contrast pattern. Without a direct opponency experiment it is impossible to conclude how this translates into relative saliency of these two types of vision. We can speculate, however, that this system most likely would be of secondary relevance to ordinary contrast-based vision. In addition, it is consistent to surmise that it acts on a longer timescale, based on the slower rise times in the responses shown in Figure 2.6. These aspects of polarization vision seem reasonable when considering the utility of this modality as a long-distance navigational aid, perhaps unsuited to the rapid escape-response and collision-avoidance algorithms implemented by the contrast-sensitive visual system.

It is noteworthy that the responses to blue light are of smaller amplitude than those to a matched-intensity UV light, although this difference is not statistically significant. We cannot conclude which photoreceptors are responsible for mediating polarization-dependent behavior based on this evidence alone, but it suggests that

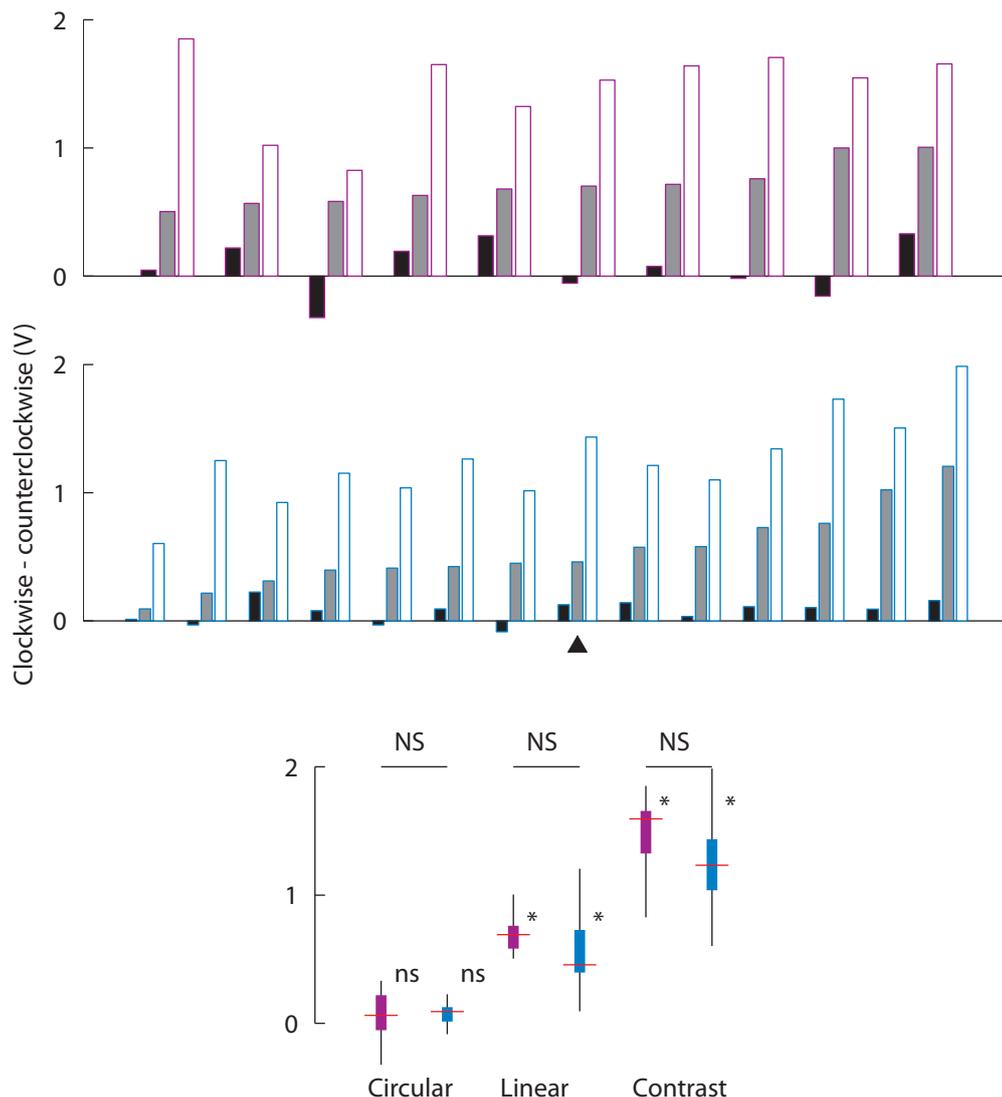


Figure 2.7: Responses to linearly polarized light are consistent and statistically significant. The values plotted here are averages of the traces in the second row of Figure 2.6 during the last 15 seconds of filter motion. In the bar charts (first two rows) the responses of an individual fly are grouped. The bar groups are ordered by increasing response to the polarized filter condition. The left bar (black fill) in each group shows the fly's response to the circular polarizer. The center bar (gray fill) shows the fly's response to the linear polarizer. The right bar (white fill) shows the fly's response to the contrast pattern. The top row contains data from all flies exposed to UV light. The middle row shows data from flies in blue light. The group of bars marked with a filled triangle contains data from the same fly as the traces in the top row of Figure 2.6. The bottom row contains box plots summarizing the data in the first two rows (UV in purple, blue in blue). At the $p < .001$ level, all of the responses to linear polarization and the contrast pattern were statistically different from zero (shown with *), whereas the responses to circularly polarized light and the differences in responses to the same filter in different colors are not statistically significant (shown with ns and NS, respectively), as evaluated using the student's t-test.

those photoreceptors are more sensitive to UV light than to blue light.

2.3.3 Experiments to control for effect of reflections

In the experiments described in Section 2.3.2 we controlled for possible intensity patterns introduced by the polarizing filter with our circular polarization control. We neglected a second possible source of confounding information, though—reflections off surfaces visible to the fly. As discussed in Section 2.2.4, polarized light reflected from glossy surfaces can produce unexpected intensity patterns. In addition to attempting to cover any such surfaces in our arena, we performed an experiment designed to directly test for such an effect. In this experiment we covered the mirror with a piece of white paper. This eliminated the polarized light information reflected from the mirror, but left all other possibly reflective surrounding surfaces unchanged. Hence, if the flies were receiving information about the direction of rotation of the filters from somewhere other than the mirror, we would expect to see turning responses to filter rotation in this experiment. We only tested flies in UV light for this experiment, since their responses to UV were slightly stronger when we did not cover the mirror.

Figure 2.8 displays data from the covered mirror experiment. Using the same analysis as we conducted for the uncovered mirror experiment, we did not observe statistically significant turning responses to rotating any of the filters. From this negative result we concluded that unexpected reflections were not responsible for the effects we observed in our first set of experiments. This experiment also controls for the possibility that the flies could see the diffuser directly (below and behind them, see Figure 2.4), verifying that it is indeed blocked from their view in our arena.

In addition to this control experiment, we conducted another with female flies in which we removed the mirror completely, leaving its mechanical mounting brackets and the rest of the arena in place. This manipulation also completely abolished the turning response to rotating the polarization angle of UV light.

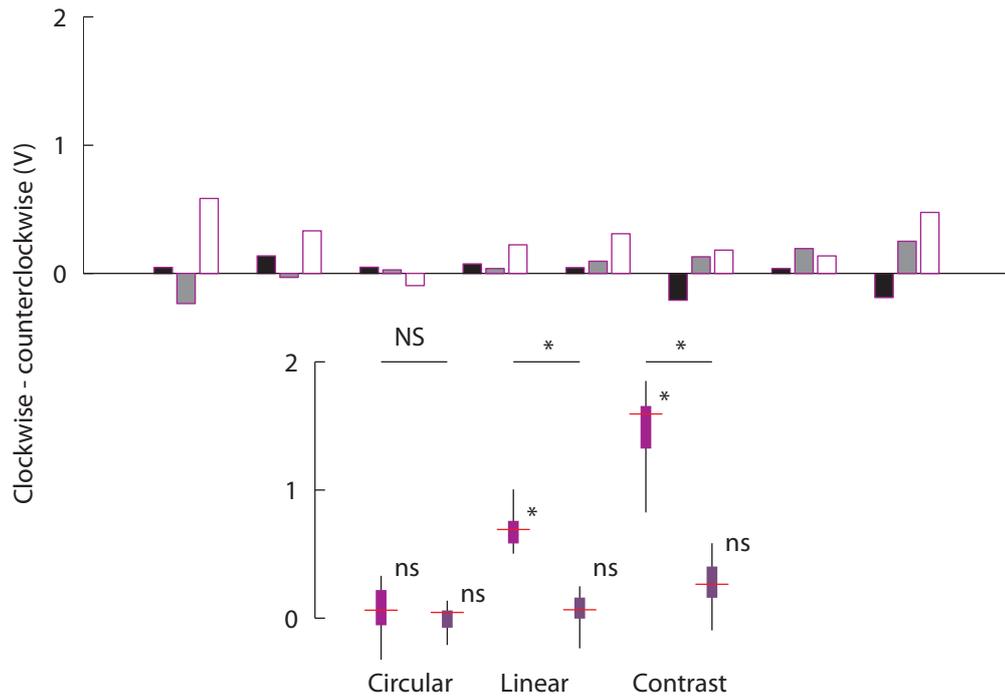


Figure 2.8: Flies do not respond to rotation of the polarizer when the mirror is obscured by opaque white paper. The top row contains individual fly responses, plotted using the same analysis and scale as those in Figure 2.7. The darker boxes in the bottom row show the combined data from all the flies ($N = 8$ flies total). We have reproduced the boxes from Figure 2.7 to the left of each control box for comparison. We used the same method to assess statistical significance, the student's t-test at $p < .001$ level.

2.3.4 Experiments comparing males and females

We looked for a difference between how male and female flies responded to linearly polarized light. This would have practical implications for our experiments, since we would ideally run behavioral tests on the sex that shows the largest polarization-dependent effect. A difference between the sexes would also have interesting biological significance, considering the different evolutionary pressures imposed on the two sexes. There is evidence for sex-biased dispersal in many taxa (Prugnolle and de Meeûs, 2002; Greenwood, 1980), including relatively recent reports in insects (Ortego et al., 2011; Hardy et al., 2008; Lagisz et al., 2010; Sundström et al., 2003), although such bias is by no means universal (e.g., Bouyer et al., 2010). Given our hypothesis of polarization vision's role in long-range dispersal, a sex-based difference in response to polarized light could suggest the existence of a difference in tendency to disperse. Although clearly a within-individual experimental design is impossible in this case, we took pains to make sure males and females experienced identical conditions prior to and during testing. We alternated flies of each sex both while preparing (mounting) the flies, and while running the experiments.

Figure 2.9 contains data from the sex difference experiment. The data were analyzed in exactly the same manner as described above (Section 2.3.2). Females responded to rotating the angle of polarized light in a manner similar to males. The population average of the females was slightly lower than that of males, but this difference was not statistically significant ($p = 0.06$).

There was one difference in the way we conducted this experiment that should be noted. We reversed the order of the filter and the diffuser in our arena. We wanted to test if the circular polarizer control was still at baseline in this condition, even without the diffuser blurring out possible inhomogeneities. We found no difference in the responses of males between this filter arrangement and the original arrangement, indicating that either order functions satisfactorily.

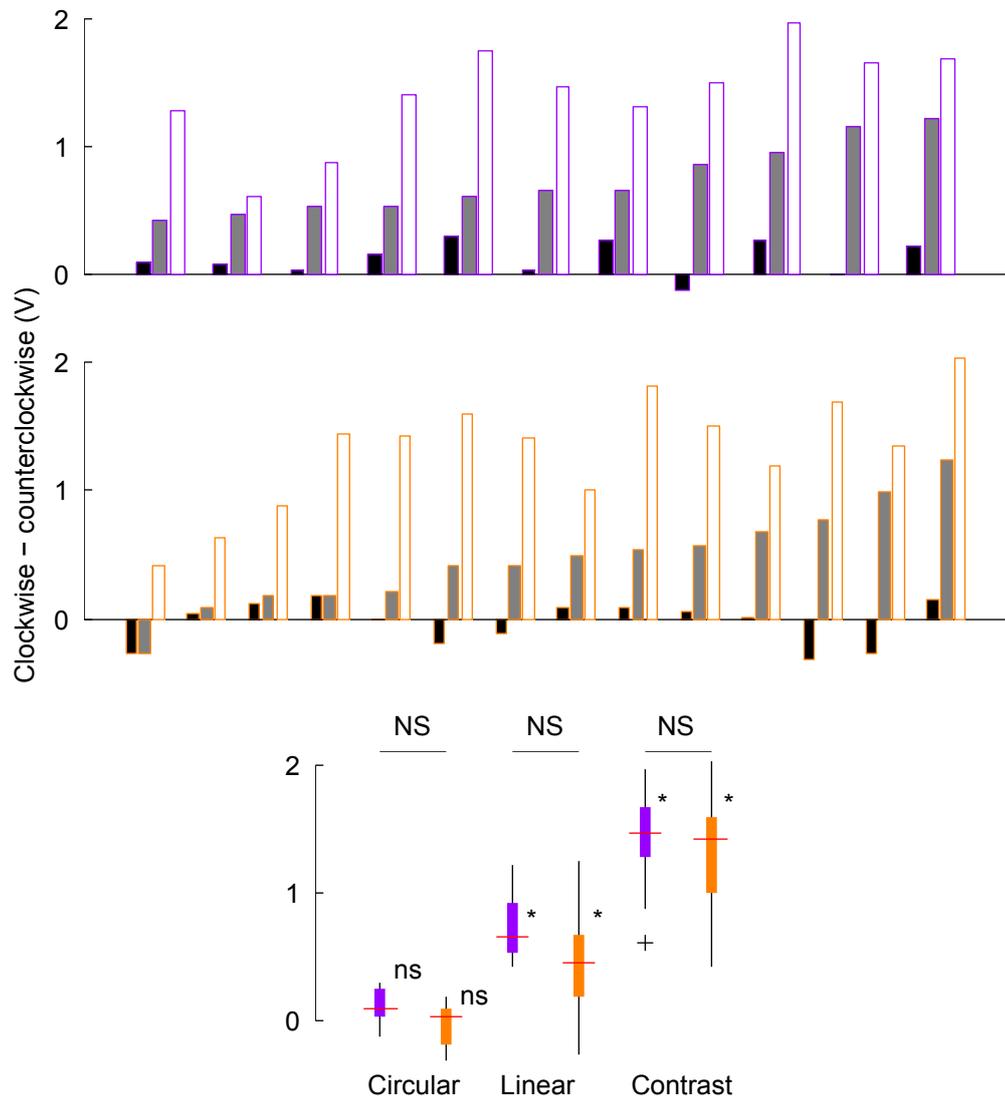


Figure 2.9: Male and female responses to rotating polarized light. The top two rows contain responses of individual flies to rotations of the three filters (black: circular polarizer, gray: linear polarizer, white: contrast pattern). The top row (purple bars) represents data from 11 male flies, while the middle row (orange bars) contains responses of 14 female flies. The bottom row compares the population responses to the different filters. Females responded to rotating linearly polarized light with statistically significant turning responses. We did not observe any statistically significant differences between males and females in any conditions. (Evaluated using student's t-test at $p < .001$ level.)

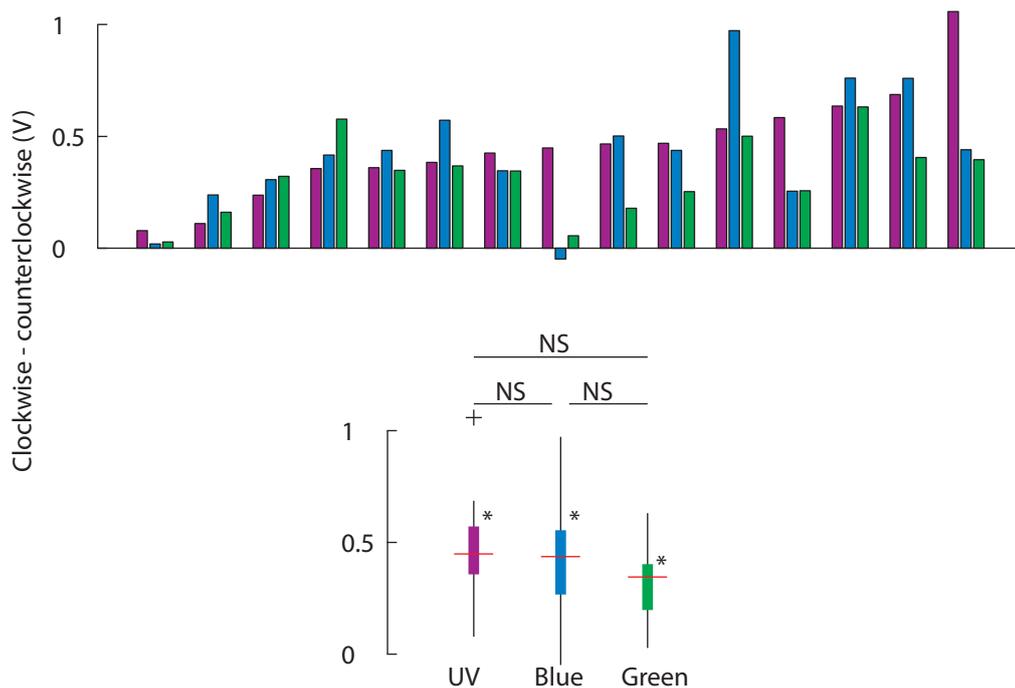


Figure 2.10: Flies respond to linearly polarized UV, blue, and green light. In the top row each bar group represents a single fly ($n = 15$ flies total). We ordered the fly responses by increasing response to UV light. Bar color corresponds to stimulus color (from right to left, UV, blue, green). In the bottom row we compare population responses to one another. We did not observe statistically significant differences between the responses to the different stimulus colors (student's t-test).

2.3.5 Experiments comparing responses to light of different wavelengths

We conducted one final comparison, this one aimed at discovering what range of wavelengths elicit behavioral responses to shifting the polarization angle. Each fly was exposed to three clockwise and three counterclockwise trials of linear polarized light of each color (UV, blue, and green) in random order. We analyzed the data as described above. The results of this experiment are presented in Figure 2.10.

Although we observed the highest population average of fly turning behavior in response to polarized UV light and the weakest such response to green light, there was not a consistent ranking on an individual fly basis. The top row of Figure 2.10

shows that we observed every possible ranking of response amplitude to different colors except green, UV, blue from highest to lowest, and that there was considerable variation across the response amplitude of different individuals. This variability prevents us from making any solid conclusions concerning which wavelength is most salient for polarization responses. Because we saw the strongest effect in response to UV light, we may say that these data do not contradict the role of UV-sensitive photoreceptor mediating polarized light sensitivity. We will discuss the implications of our experiments with respect to which receptors mediate these responses in Section 4.3.

2.4 Closed-loop experiments

2.4.1 Closed-loop experimental design

The open-loop experiments described above were adequate to confirm the ability of flies to detect and respond to linearly polarized light in our arena. These experiments were limited, though, in that they put the fly in extremely artificial conditions. In these open-loop experiments the fly could not alter its sensory environment with its own actions. In free flight, an attempt to turn is translated by aerodynamic interaction of the wings with air into an actual turn. This turn affects the information picked up by the fly's sensory systems. For instance, during a yaw turn to the right, the visual system perceives rotation of the visual scene to the left. In our next set of experiments, we mimicked this type of closed-loop sensory-response sequence. Instead of setting the rotation of the polarizing filter to a constant value and leaving it to rotate at that rate for the duration of the experiment, we started each experiment with the filter stationary. When the fly's wingstrokes were symmetrical (the left and right wingstroke amplitudes were equal) the rate of rotation of the filter was zero. As soon as our automated wing tracking system detected a difference in left and right wingstroke amplitudes, however, it sent a command to rotate the filter in the opposite direction of the fly's intended turn, thus simulating the effect of an

actual free flight turn on the visual field. Specifically, we multiplied the difference in wingstroke amplitude by a constant negative gain and used the result to set the angular velocity of the stepper motor controlling the filter position. The computer running this control loop ran at approximately 160 Hz. This experimental design not only verified the results of our open-loop experiments, but allowed us to address different questions, such as which particular polarization orientation a fly preferred, and how that preference changed over time.

A few changes in our analysis should be taken into consideration at this point. Since the rotation of the filter now depends on the behavior of the animal during the experiment, flies placed in the exact same starting conditions will nonetheless experience different stimulus conditions throughout the experiment. Hence, we cannot use fly response as a primary result metric, since the stimuli they were responding to were different. Instead, the rotation rate of the polarizer, as the variable directly under control of the fly, provides a record of the fly’s behavior. If, during the course of a closed-loop experiment, the polarizer was most often in an orientation α , we can conclude that the fly preferred to steer in that direction relative to the polarization direction. As mentioned above, polarization stimuli are axial—while an arbitrary visual pattern will have 360° rotational symmetry, meaning that steering in direction β will be equivalent to steering in direction $\beta \pm 360^\circ$, a pattern of uniformly linearly polarized light will have 180° rotational symmetry (direction α is equivalent to direction $\alpha \pm 180^\circ$.) This axial symmetry provides us with a useful internal control—an animal responding to linearly polarized light should display a preference distribution with axial symmetry.

2.4.2 Closed-loop results

In our first set of closed-loop experiments, we again tested for a difference between individual fly responses to linearly polarized and circularly polarized UV light. We tested individual male flies with either a circular or a linear polarizer for as long as they would fly. We subsequently analyzed only the first 12 minutes of each trial. We

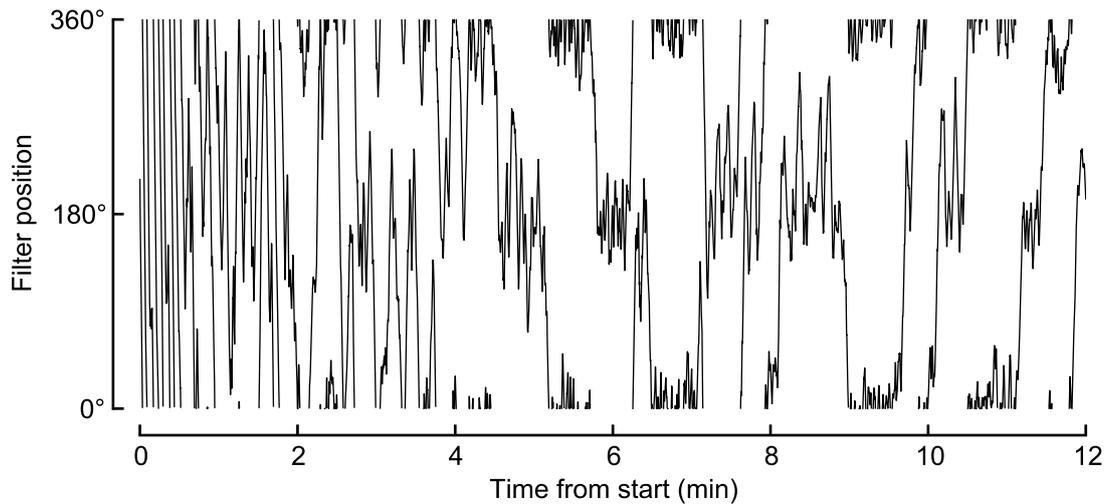


Figure 2.11: Time course data from a closed-loop experiment. Zero degrees corresponds to the the polarization axis perpendicular to the long axis of the body of the fly. Angles increase counterclockwise.

chose this duration for several reasons:

1. A reasonable number of flies flew for at least this long, making statistical conclusions possible.
2. In looking at the raw data, fly behavior appeared to settle into a steady state several minutes into the trial, leaving plenty of representative data after initial conditions ceased to have an obvious role.
3. This was the total trial time of our open-loop experiments, and the duration of the experiments in Chapter 3.

To be included in our analysis, we required that each animal flew a minimum of 11 minutes and did not stop more than an average of once per minute over the course of the experiment.

Figure 2.11 displays data for a single fly controlling the rotation rate of the linear polarizer. By the fourth minute the fly held the polarizer predominantly in the horizontal orientation (filter position 0° , 180° , and 360°). The high-frequency oscillations

and balance between left and right turns indicate that the dynamics and balance of the system were not too artificial to evoke relatively natural flight behavior.

Given that rotating the angle of linearly polarized light evoked attempted turns in the direction of rotation under open-loop conditions, we expected flies to stabilize the linear polarization pattern in closed-loop conditions. On the other hand, since flies did not attempt to turn in response to rotating a circularly polarizing filter, we expected that flies would not stabilize such a filter in closed-loop experiments. Our first intuition was that this would be apparent in a lower average angular speed of the filter during closed-loop trials with the linear polarizer compared to trials with the circular polarizer.

Figure 2.12 displays data on the median angular speeds in the two conditions. We used the median as our measure of central tendency because we wanted to look for higher overall turning, which would indicate less stabilization of the filter. The median, since it is less influenced by extreme values, would not be as strongly influenced by infrequent large-amplitude turns. In order to calculate the angular speed, we first smoothed the complex representation of the angular position of the filter with a 5 second flat sliding window, then calculated the angular speed from this smoothed record. Using this analysis, we did observe higher average angular speeds of the circular polarizing filter than the linear polarizer (significant at the $p < .05$ level using one-tailed student's t-test). This difference, however, did not capture the clear difference in the data between the two

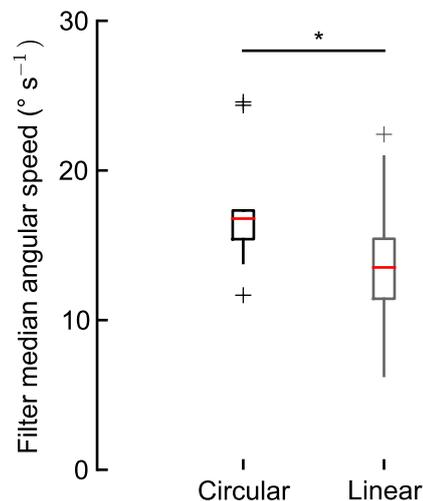


Figure 2.12: The angular speed of the linear polarizer (right, $n = 19$ flies) under closed-loop control is lower than that of the circular polarizer (left, $n = 9$ flies). (* indicates $p < .05$ one-tailed t-test.)

conditions that was apparent while running the experiments.

Although our analysis of median angular speed did indicate that flies stabilized the linear polarization pattern, we required a more refined analysis to look for true navigation based on polarization. By navigation, we refer to the ability to hold a given course, not just avoid uncontrolled turns. In our closed-loop experiments, the hallmark of holding a course would be not simply lower average angular speed, but stabilization of a consistent angle of polarization. Such a consistent angle of polarization would result in a bimodal distribution of polarizer orientations with peaks separated by 180° , since linear polarization is axial, as discussed above. To look for this type of distribution of filter orientations, we computed the axial angular variance, V (following Mardia (1972)):

$$V' = 1 - \frac{1}{n} \sqrt{\sum_{i=0}^n (\sin^2 p\alpha_i + \cos^2 p\alpha_i)} \quad (2.1)$$

$$V = 1 - (1 - V')^{1/p^2} \quad (2.2)$$

where α_i is the filter orientation at time-point i , n is the total number sample points, and $p = 2$, since the data are axial. The axial angular variance lies in the interval $[0, 1]$, with one indicating maximal variance and zero indicating minimal variance about the mean angle. Figure 2.13 shows circular histograms of filter positions for four flies from our experiment, including the individual in Figure 2.11. We have included values of V for those four individuals to indicate sample distributions corresponding to different values of V . We ordered the individuals from left to right in order of increasing strength of fixation on a polarization orientation—it is clear that the individual on the right stabilized the polarizer axis horizontally, and the next individual to the left preferred an orientation just clockwise from vertical. The fly whose data are shown on the left, however, did not show a strong preference for any particular angle over the course of the trial. The V values correspond well to this ranking—higher fidelity fixation results in lower axial variance. Note that this measure is independent of the fixation angle itself, it only matters how tightly the

samples are grouped about that angle.

We show the population axial angular variance, V , values in the second row of Figure 2.13. When we put the flies in closed-loop control of the linear polarizer, they would often stabilize a particular polarization angle, as shown by the relatively low average axial variance in this condition. They were unable to stabilize the circular polarizer, indicated by a significantly higher average axial variance of filter position. These results verified our findings in the open-loop experiments: flies could detect the angle of linearly polarized light, and they responded to changes in it. Furthermore, the results of these closed-loop experiments demonstrated that flies not only attempted to stabilize the rotation of polarization angle, but actually fixated on specific orientations of that angle. Generalizing to free-flight behavior, this indicated that *Drosophila* at least have the prerequisite abilities necessary to hold a flight heading relative to linearly polarized light at the timescale of these experiments.

In walking flies it has been reported that flies spontaneously orient their bodies along the axis of polarization (Wernet et al., 2012). We looked for any population preference in our flying arena (Figure 2.14). For each trial we calculated the fly's preference angle θ using the following formula:

$$\theta = \frac{1}{p} \arctan 2 \left(\sum_{i=1}^n \sin p\alpha_i, \cos p\alpha_i \right) \quad (2.3)$$

where

$$\arctan 2(s, c) = \begin{cases} \tan^{-1} \frac{s}{c} + \frac{\pi}{2} \left(1 - \frac{c}{|c|} \right) & c \neq 0 \\ \pi \left(1 - \frac{s}{2|s|} \right) & c = 0 \end{cases} \quad (2.4)$$

and α_i is the filter orientation at time-point i , n is the total number sample points, and $p = 2$, as before. We did not observe a clear grouping of these data, indicating no population preference for any particular e-vector angle. It is possible, however, that such a preference could be obscured in this analysis by flies in our experiment that did not exhibit a strong preference for any e-vector angle. We eliminated those flies by restricting our analysis to the 50% of flies whose axial angular variance for the trial

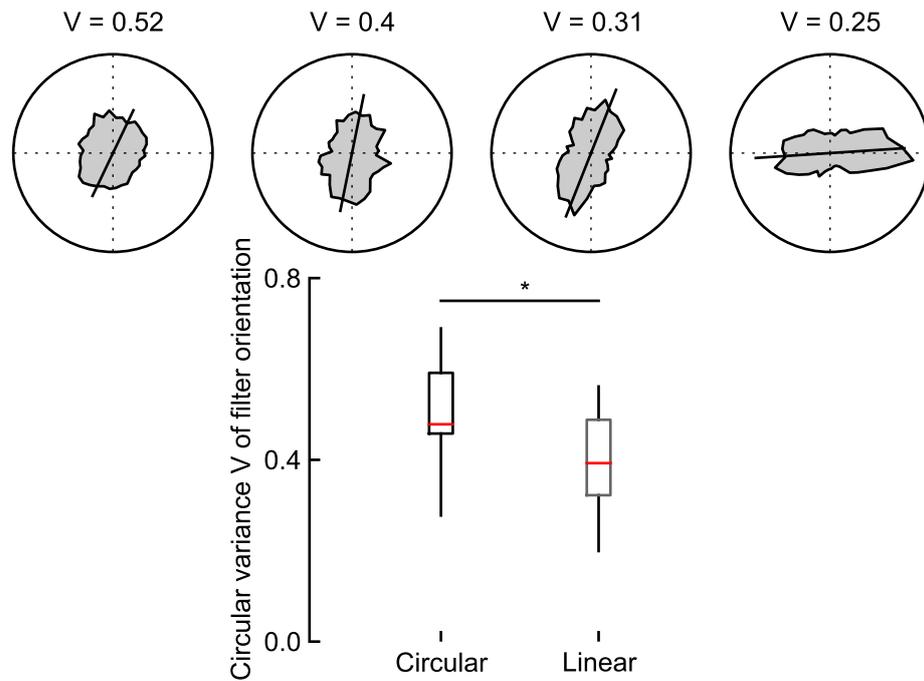


Figure 2.13: Axial angular variance is lower for the linear polarizer position than for the circular polarizer position in closed-loop experiments. The top row shows circular histograms of linear polarizer orientations for four different flies. The data on the far right are the same as those shown in Figure 2.11. The number above each is the axial circular variance for those data. The black line through the origin shows the mean angle (equation 2.3) and its length is proportional to $1-V$. The second row contains data from all the flies in this experiment. The average axial circular variance for flies with the linear polarizer (right, $n = 19$ flies) was significantly lower than that for flies with the circular polarizer (left, $n = 9$) at the $p < .01$ level (one-tailed student's t-test).

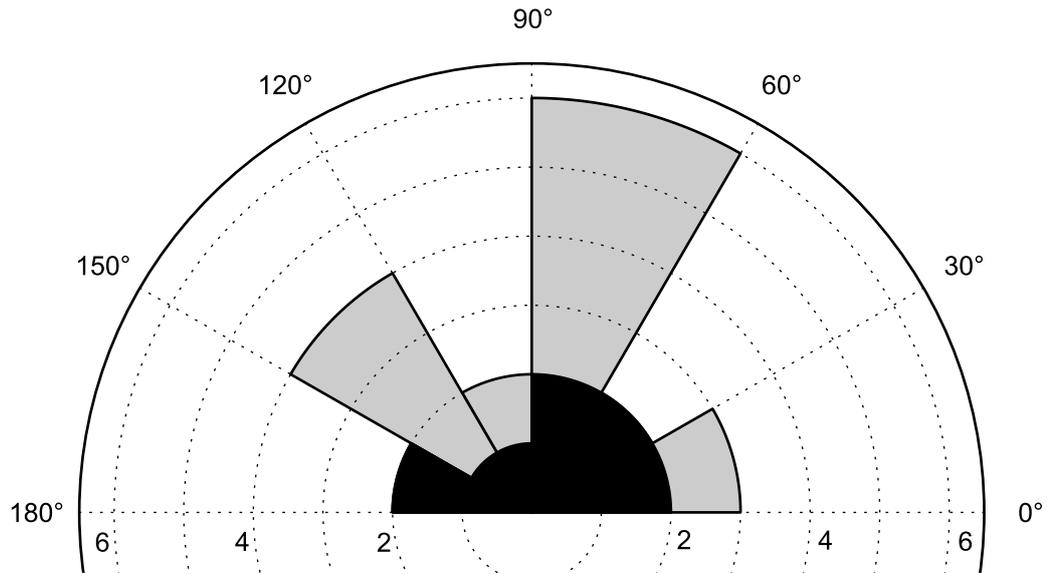


Figure 2.14: Preferred headings of flies in closed-loop experiments. We calculated the average e-vector angle stabilized by each fly for the 12 minute closed-loop experiments. This circular histogram shows the number of flies that stabilized each e-vector angle (gray). In black we show only the 10 flies who had axial angular variance of headings below or equal to the population median axial angular variance. These are the flies who showed a more pronounced e-vector preference.

was below or equal to the population’s median axial angular variance (Figure 2.14, black). This half of our sample showed more robust fixation of e-vector orientation. In these flies we see even less evidence for any preferred polarization orientation.

2.4.3 Long-duration closed-loop results

Since the celestial polarization pattern is the most obvious source of behaviorally relevant polarized light in nature, and this pattern is determined by the sun’s location in the sky, it is natural to think of polarization vision as a source of compass information to the fly. A compass is primarily useful in maintaining a heading over long duration trips. With this in mind, we designed a set of experiments to determine how the preference angles of individual flies shift over the course of many hours. Inspired by the work of Götz (1987), we tested individual flies in our closed-loop flight arena for half hour periods with linearly polarized UV light. Between these periods we fed the flies

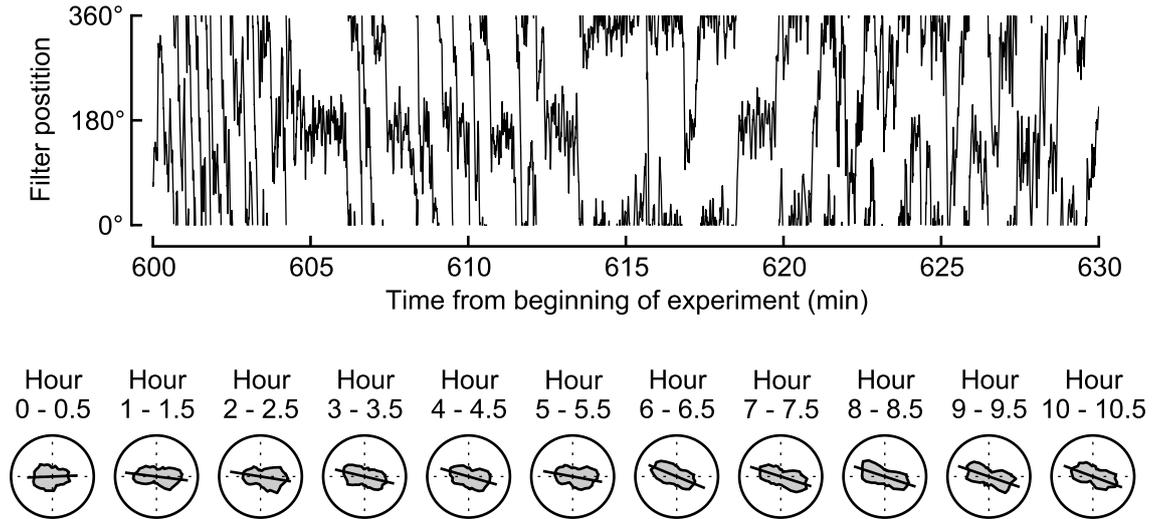


Figure 2.15: An example fly stabilizes the same polarization angle for over ten hours. The top row shows the orientation of the filter over the course of the final half-hour trial. The bottom row contains circular histograms of filter position for all of the trials from this fly. The black line through the origin shows the mean angle for that trial. Its length is proportional to $1-V$, where V is the axial angular variance during that trial.

by placing a piece of tissue paper that had been soaked in sucrose solution against their tarsi. This allowed them to feed to satiety and rest (they would automatically stop flying upon tarsal contact). After half an hour, we would re-initiate fictive flight by gently blowing on the fly. We repeated this until the fly would not fly despite repeated attempts to re-initiate flight. For each trial we calculated the fly's preferred angle using equation 2.3.

Several flies maintained extraordinarily constant preference angles over the course of the experiment. The data from one such fly are presented in Figure 2.15. The preference angles (θ in equation 2.3) for this fly for all of the trials are within 25° of one another, and the fly displayed clear stabilization over the course of the experiment.

For each fly that flew for at least four trials we have plotted the preference angles for each trial over the duration of the experiment in Figure 2.16. There is not an immediately obvious effect on preference angle by time of day or duration of flight—the lines appear to have negligible slope. The polarization angle is only defined over

a range of 180° , and here we chose the interval $(-90^\circ, 90^\circ]$. There is some ambiguity as to where to plot points lying near $\pm 90^\circ$, since -90° and 90° actually represent the same polarization angle. Therefore it is somewhat misrepresentative to plot these points far from one another. It was this consideration that led us to choose the interval $(-90^\circ, 90^\circ]$ for these plots, even though it represents a shift from the convention in Figure 2.15, where we opted for angles increasing from 0° to 360° . Because most flies preferred angles near 0° in our long-duration experiment, the range $(-90^\circ, 90^\circ]$ allowed us to avoid having many flies' preferences cross the discontinuity in our coordinate system. One fly, however, displayed preference angles separated by more than 90° in consecutive trials (leftmost red trace in Figure 2.16). We decided that it was most intuitive to interpret this shift as a shift in the opposite direction by less than 90° , an identical polarization angle that we simply plot in a different location on our axes. This technique is sometimes termed "unwrapping". This type of artifact of plotting and analyzing directional data as if it were linear can often lead to errors, highlighting the importance of choosing a plotting technique appropriate to the data set.

Upon closer examination of the behavior of all the flies in this experiment, we noticed a suggestive trend. Often a fly's preference over the first several hours would progressively shift clockwise, then stay constant for the remainder of the day. Figure 2.17 shows data from a fly exhibiting this type of behavior. For the first six trials the fly's preference angle shifted more or less consistently in a clockwise direction.

In designing this experiment we were looking for a consistent shift over the course of the day. This type of shift in the animal's preferred heading with respect to polarization angle would allow it to compensate for the apparent movement of the sun in the sky. On average, the sun moves 15° clockwise every hour in the northern hemisphere (where our fly stock was collected). It has been reported (Kalmus, 1956) that bees correctly compensate for this change in the sun's position over the course of the day, but are unable to correct for the reversal to counterclockwise movement when transported to the southern hemisphere. In Figure 2.18 we have replotted the data from Figure 2.16 with the first four trials in red to highlight the trend we noticed. We have included a line indicating the slope of ideal average compensation in the

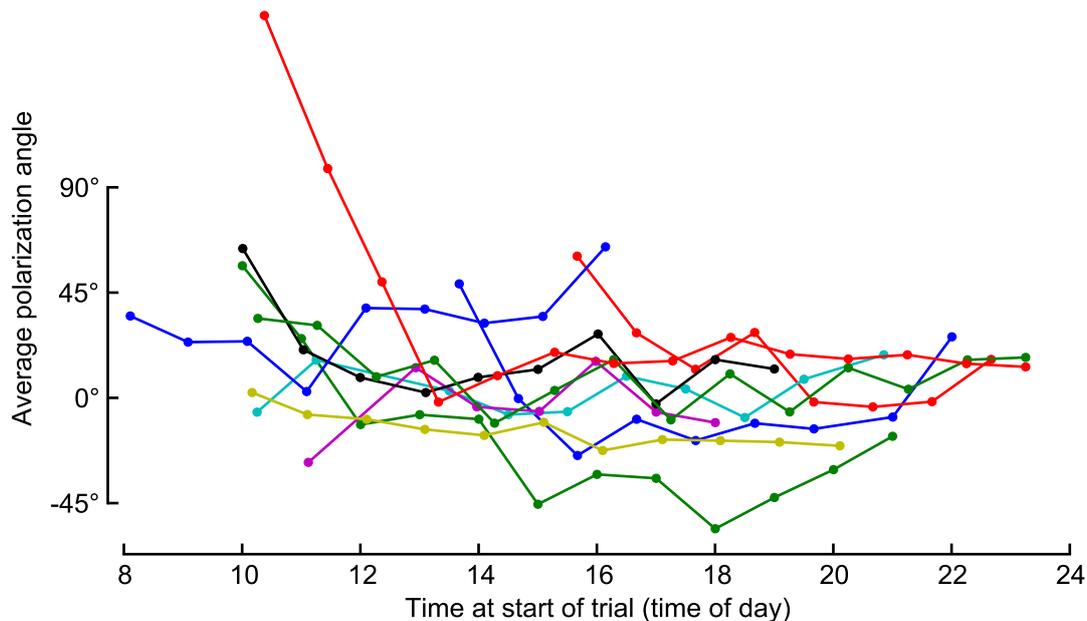


Figure 2.16: Average polarization angle for each half-hour trial in the long-duration closed-loop experiment. Different colors represent different individual flies. When a fly's preference changed by more than 90° we "unwrapped" the next trials—changing subsequent trial averages to their 180° complement.

northern hemisphere.

This analysis is still somewhat coarse, since we are treating each half-hour trial as a single sample point, and eliminating all trials after the first four. We do not have a clear understanding of why time compensation should diminish after four hours, and more experiments are required to examine this behavior. With this data set, we were able to perform a quantitative analysis of fly behavior during the first four hours (Figure 2.19).

We aim to discover how each fly's preference angle changed over time for the eight flies that we tested for four consecutive hours. As a first attempt, a linear regression comes to mind. This would be inappropriate for our axial data, however, since the difference between axial angles cannot be treated as the difference between linear samples¹: A linear regression would penalize a best fit line that predicted a preference of -89° for a sample time whose true value was $+89^\circ$, even though these two

¹Unfortunately, several published studies in the field have used standard linear regression techniques on axial data (for example, Heinze and Homberg, 2007, 2009; Träger and Homberg, 2011).

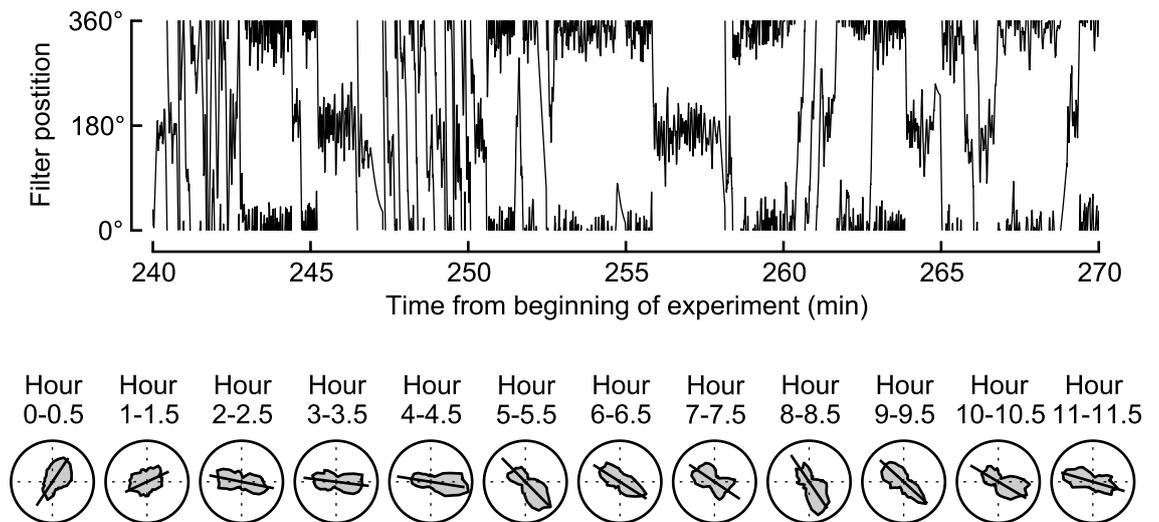


Figure 2.17: An example fly shows shifting preference angle for several hours, then holds a constant preference. The top row shows filter position during the fifth trial, when the fly held the filter roughly horizontal. The circular histograms in the second row show that the fly's preference shifted from 45° clockwise from horizontal to 45° counterclockwise from horizontal over the first six trials, then stayed roughly constant for the rest of the day.

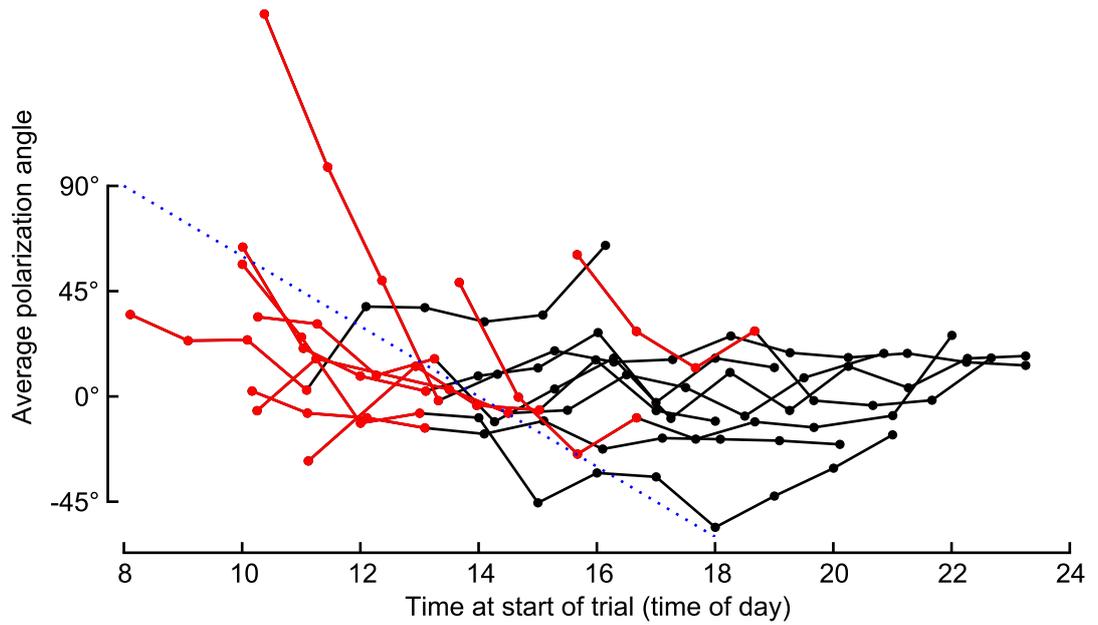


Figure 2.18: Some flies consistently shift their preference angle in the direction expected to compensate for earth's rotation in the first four hours. The data presented here are the same as those in Figure 2.16. The first four trials are highlighted in red for each fly. The slope of the dotted blue line shows the average compensation necessary to adjust for the apparent motion of the sun in the northern hemisphere sky.

angles are in reality only 2° apart. To avoid this, we constructed a best fit algorithm based on the angular difference δ_i between sample point α_i and the line at that time point β_i where

$$\delta_i = \frac{1}{p} \arctan 2 (\sin p(\alpha_i - \beta_i), \cos p(\alpha_i - \beta_i)) \quad (2.5)$$

and `arctan2` is defined as in equation 2.4. We used the `leastsq` function in Python's SciPy module to numerically minimize the sum of the squares of each fly's polarization angles and a straight line through the angular mean (equation 2.3) of all its samples. The results of running this analysis on the first six hours of data from the individual shown in Figure 2.17 are demonstrated on the left side of Figure 2.19.

In the left panel of Figure 2.19 we plotted two copies of the polarization angle data along the vertical axis, one translated up by 180° . This is a technique that visually reminds the viewer that plotting angular data on a linear scale can introduce warped impressions. In the case of these data, however, we feel that the angular-difference based best-fit algorithm is capturing a real trend in the data.

Looking at the entire data set of the first four trials of all eight flies, we do see a tendency of flies to shift their preference in the clockwise direction using this best-fit algorithm. This tendency is demonstrated by the preponderance of negative slopes in the right panel of Figure 2.19. We are hesitant to draw firm conclusions based on these data alone, however. The idea to only look at the first four hours was suggested to us by these data, so the experiment should be replicated before evaluating if there is enough evidence to support the existence of time compensation in polarization preference by *Drosophila*.

2.5 Discussion

The experiments reported here indicate that flying *Drosophila* have the capacity to detect the angle of polarization of light, and can use it to hold a steady course in a restricted laboratory setting. The open-loop experiments were critical to show that

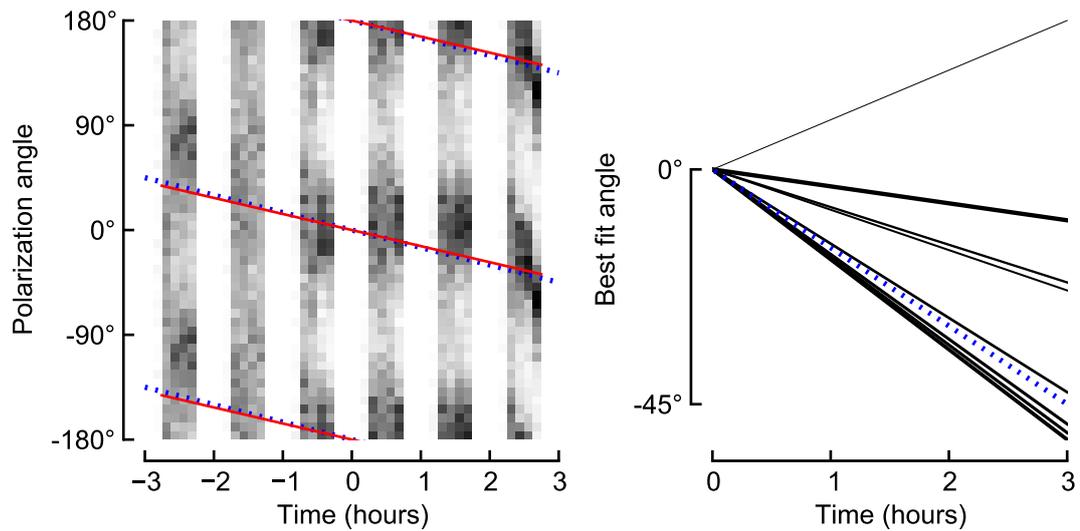


Figure 2.19: Angular difference-based least-squares algorithm to find a trend in each fly's preference shift over time. In the left panel we have plotted a grayscale heat map of filter orientations over time for the first six trials of the individual fly shown in Figure 2.17. The darker regions show longer filter residence at that angle. We have reproduced the data above and below to underscore that axial data repeats after 180° , making a regular linear regression impossible. The red line shows the result of our best-fit algorithm. The dotted blue line shows the slope of ideal compensation based on the sun's average 15° per hour clockwise shift in location in the northern hemisphere. On the right are shown the results of the best-fit algorithm applied to the data from all eight flies. The line width is proportional to the root-mean-squared error of the best-fit line. The blue dotted line is the same as in the left panel. In both panels angles increase counterclockwise.

flies could detect the *angle* of polarization: An alternative hypothesis is that the animals have receptors that are simply more sensitive to one e-vector angle than another, making that preferred angle appear subjectively brighter. This could not be said to be true polarization vision, however. Under this hypothesis rotating the e-vector away from the “bright” direction would result in the stimulus dimming, it wouldn’t give a directional response. Since the animals in our open-loop experiments correctly inferred the direction of rotation of the e-vector, they must have perceived a directional signal. A possibility is that the flies really only had this type of unidirectionally sensitive receptors, but their preferred axes are arrayed across the eye such that rotation of the uniform e-vector field stimulates them sequentially—a sort of matched-filter for uniform e-vector rotation. This, arguably, indicates a measure of the e-vector direction (encoded by location of maximum excitation in the eye), and so we still can conclude that the flies detect that direction.

We found that flies were most sensitive to polarization angle changes in the UV, and that males responded with slightly stronger responses than females. In the case of males, the response to rotating the e-vector elicited a turning response roughly half the amplitude of that elicited by a rotating contrast pattern of matched mean luminance, size, and speed.

In closed-loop experiments we confirmed that the dynamics of the sensory response to this type of stimulus were adequate to stabilize the flies’ flights. Furthermore, flies not only stabilized global rotation, but reliably steered in a preferred direction, and this preferred direction differed between flies. When tested over many hours this preference stayed relatively constant, but we observed some evidence for time-compensation in the first few hours.

Chapter 3

Outdoor loose tether experiments demonstrate flying *Drosophila* orient to natural sky polarization

The sun every morning came up astern; every evening it went down ahead. I wished for no other compass to guide me, for these were true.

—Joshua Slocum, *Sailing Alone Around The World*

3.1 Introduction

The responses to artificially polarized light in a laboratory flight arena described above could not indicate to what use an organism in more natural circumstances would put this sensory modality. It is possible that the ability to detect the e-vector angle is a vestigial trait, unused by *Drosophila* in the wild. In order to approach a more direct measure of the behavior of flies in their native environment, we undertook a series of experiments using the natural sky as a stimulus. As discussed earlier, much of the fly eye receives light from the dorsal hemisphere. We reasoned that we could measure flight responses to changes in skylight (specifically, its e-vector angle) by restricting the fly's view to a window in the dorsal hemisphere. The rest of the visual surround was devoid of directional information such as landmarks or intensity gradients. This is analogous to the experiments on dancing bees mentioned in the introduction. Our flies, however, were not motivated by any training or food cues; we had to rely on

observation of the flies' innate behavior. Given the apparent utility of keeping a straight course, we hoped that this motivation would be sufficient to observe steering responses to changes in the sky's natural polarization pattern. Our first experiments were designed to test for gross changes in the behavior of flies over the course of minutes when viewing a naturally polarized sky versus viewing a natural sky without polarization information (Section 3.2). After this, we refined the experiments in order to look at acute changes in flight behavior when we shifted the polarization angle midflight (Section 3.3)¹.

3.2 Rotating arena

3.2.1 Methods

Animals

We used animals from the same stock and reared under the same conditions as those in the indoor experiments. After anesthetizing each fly, we positioned it with a fine paint brush and attached its notum to a 0.1-mm-diameter by 10-mm-long steel pin with UV curing glue. When vertical, the pin held the fly in a flight posture, approximately 60° from the horizontal. Following this procedure we prevented the fly from flapping its wings by placing a ≈ 2 mm square piece of tissue paper on its tarsi and gave it at least half an hour to recover. If the fly stopped flapping during an experiment, we waved above the arena to re-initiate flight. Any such periods in which a fly was not flying were excluded in later analysis. Any flies that stopped flying more than 4 times during an experiment or longer than 60 seconds total were discarded. All experiments were conducted on cloudless days (unless stated otherwise) in Pasadena, California (34° 8' N, 118° 7' W) between one hour prior to sunset and one hour after sunset. The wall of the arena blocked the sun from the fly's view. We shaded the arena from direct sunlight with a piece of cardboard positioned outside the arena low

¹Much of the work described in this chapter has recently been published (Weir and Dickinson, 2012).

enough to be out of sight of the fly while the sun was above the horizon.

Arena

To examine fly behavior under a natural sky, we modified the magnetic tether arena developed by Bender and Dickinson (2006). An axially symmetric magnetic field held the fly in place, but it was free to rotate in the yaw direction (Figure 3.1). A 25.4-mm-tall by 12.7-mm-diameter cylindrical magnet was fixed in the center of a 152.4-mm-diameter, 6.4-mm-thick disk of glass by another 12.7-mm-diameter, 21.2-mm-tall cylindrical magnet. Below, a V-shaped aperture held the pin in place above a 25.4-mm-outer-diameter, 12.7-mm-inner-diameter, 25.4-mm-tall ring magnet. The walls and floor of the arena were matte gray, except for white plastic covering the ends of both magnets closest to the fly. No dark glossy surfaces, which can act as polarizers, were visible to the fly (see Chapter 34 in Horváth and Varjú, 2004 or Section 2.2.4). When in the arena, a fly could view the sky through a ring-shaped window (measured from vertical: outer diameter = 58.5° , inner diameter = 30.6°), encompassing the view angles of approximately 17% of the fly's ommatidia (Heisenberg and Wolf, 1984). In experiments using optical filters, we placed the filter directly above this window. We recorded videos (Straw and Dickinson, 2009) of the fly from below through the hole in the ring magnet, at either 290 or 130 frames per second. An infrared LED (peak wavelength = 850 nm) provided illumination through the same hole. Wavelengths emitted by this LED were such that it was not visible to the fly. The fly's heading was later calculated by custom machine vision analysis routines written in Python. The entire arena could be manually rotated on a bearing at its base, which was equipped with a spirit level to ensure a consistent upright orientation.

Experimental design

We placed each fly in the arena and filmed its heading for 12 minutes. Every three minutes we rotated the arena 90° . Although we attempted to make the interior of the arena radially symmetrical, this rotation controlled for any subtle intrinsic features of the arena that the flies could orient to independent of the exterior sky as well

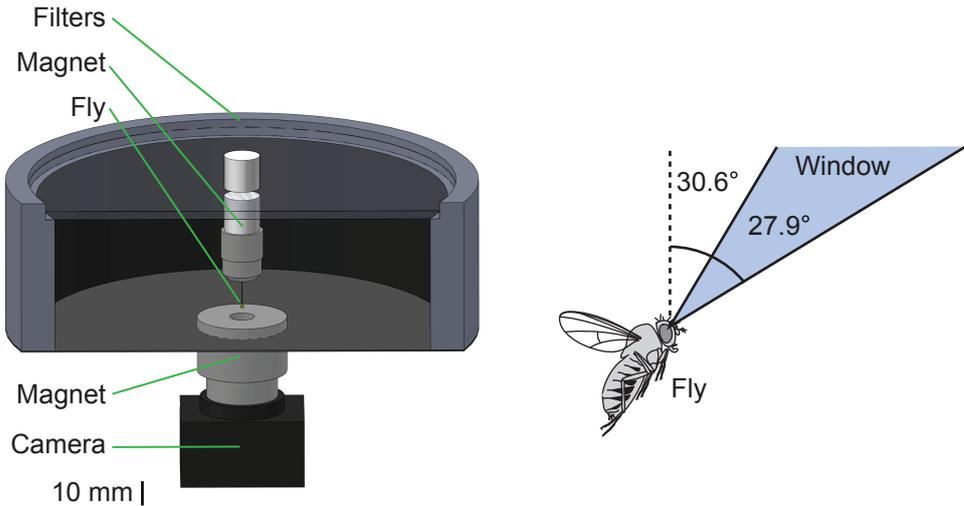


Figure 3.1: The rotating arena used in the first outdoor experiments. The fly was glued to a steel pin and suspended between two magnets. It could view the sky through a glass window (spanning the region 30.6° to 58.5° elevation from vertical, right panel), above which various filters were placed. A camera below the fly recorded its azimuthal orientation. The entire arena was rotated about its vertical axis by 90° every 3 minutes.

as radial inhomogeneities of the magnetic field. Each experiment was conducted in one of five conditions. In the first condition, there was no filter and only the glass window separated the fly from the sky. In the second condition, we placed a circular polarizing filter (Left Handed PFC Circular Polarizer, Aflash Photonics, Hollywood Park, TX) above the window, thereby effectively eliminating the linear polarization information from the sky. This filter also blocked wavelengths shorter than 400 nm and attenuated over half the intensity transmitted in the rest of the spectrum. In the third condition, we used a blue bandpass filter (Roscolux #74: Night Blue, Rosco Laboratories, Stamford, CT) that was more restrictive both in wavelengths and total intensity transmitted. In the fourth condition, we used a gray neutral density filter (Roscolux #398: Neutral Grey, Rosco Laboratories, Stamford, CT) to block the same amount of total intensity as the polarizing filter but without restricting the wavelengths. Spectra of skylight transmitted through these filters are shown in (Figure 3.2). In the fifth condition, we tested flies indoors in total darkness,

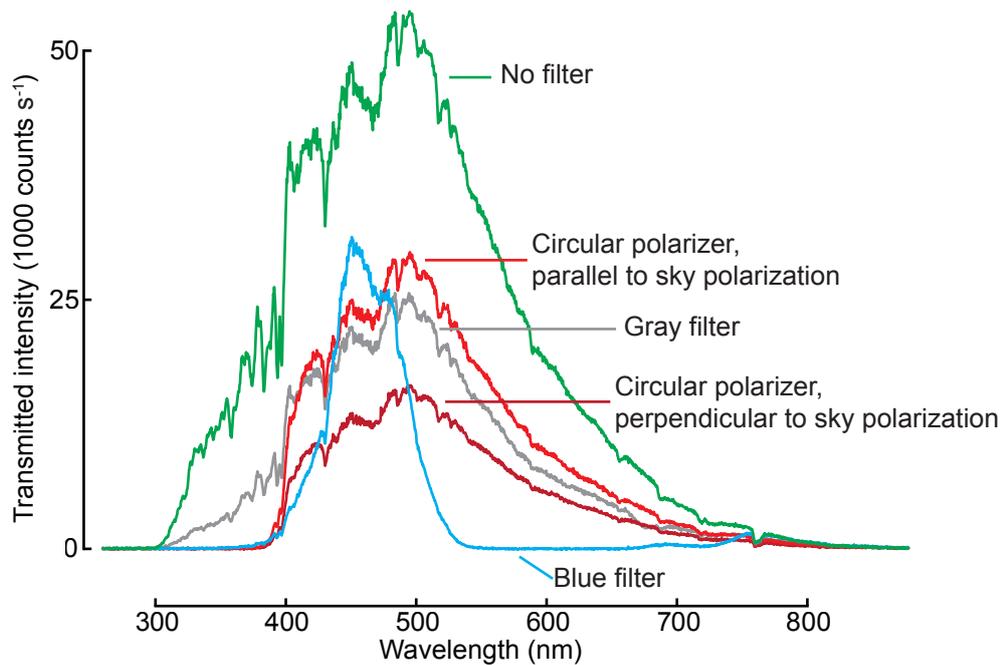


Figure 3.2: Transmission spectra for filters used in the first outdoor experiments. The two spectra for the circular polarizer were obtained by aligning its transmission axis parallel or perpendicular to the primary axis of celestial polarization. They are bounds on the amount of light transmitted during the circular polarizer condition in our experiments. Other filters did not affect transmitted polarization. In the second set of outdoor experiments we used the same polarizing filter, but flipped over, such that it acted as a linear polarizer instead of a circular polarizer.

by covering the arena with a dark cloth.

Analysis

We implemented all analyses in the Python computer programming language. In order to calculate confidence intervals for the mean change in heading after the rotations in our first outdoor experiments, we used the bootstrapping method described by Fisher (1993). Briefly, we constructed 1000 bootstrap samples by randomizing the order of 1000 copies of the original sample, then ranking the estimated means of the bootstrap samples. The confidence bounds are found by indexing into this ranked list at the desired percentiles.

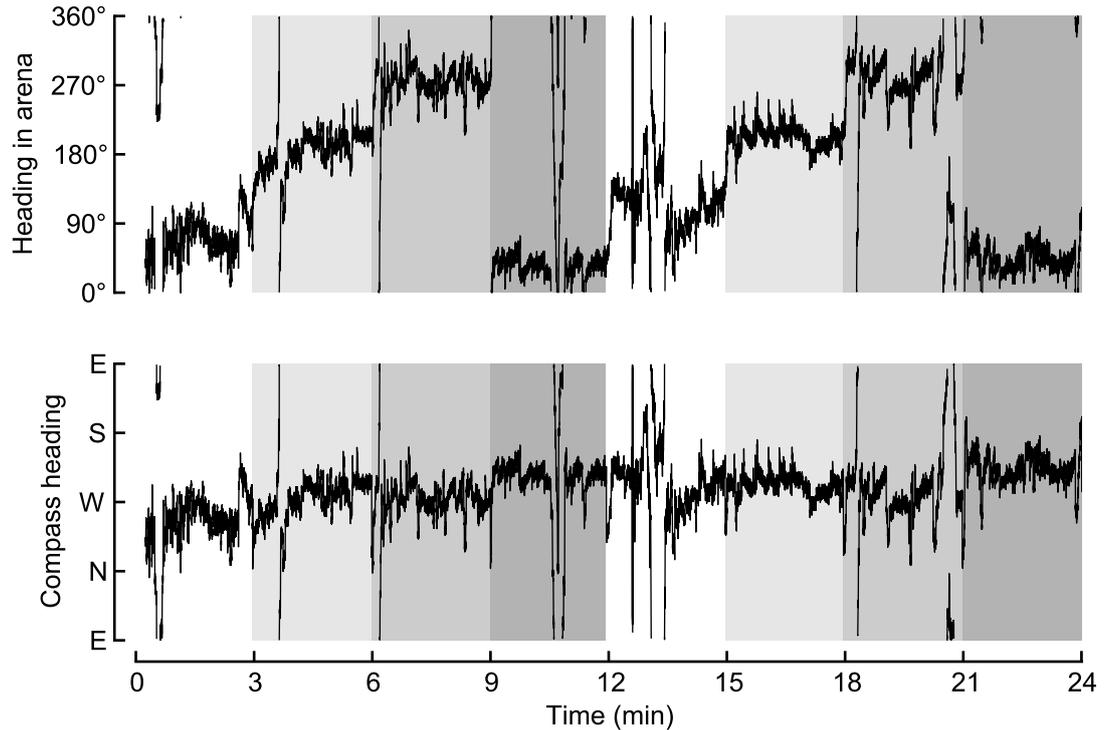


Figure 3.3: An example trace showing 24 minutes of flight orientation in arena coordinates (above) and outside world coordinates (below). Changes in background grayscale level indicate when we rotated the arena by 90° . Only the first 12 minutes were used in subsequent analyses, in order to increase the rate of data collection.

3.2.2 Results

To observe flight orientation of *Drosophila* under a natural sky, we tethered wild-type flies within a portable magnetic arena (Bender and Dickinson, 2006) with a clear ceiling equipped with a digital video camera for automatically tracking flight heading (Figure 3.1). During the hour before and the hour after sunset, we recorded the headings of flies relative to arena coordinates for 12 minutes (Figure 3.3). To test whether flies oriented using celestial cues rather than some unaccounted-for feature of the arena itself, we rotated the arena by 90° every three minutes.

When the skylight reaching them was not altered by optical filters, some flies compensated for rotations of the arena, thereby maintaining a consistent heading in world coordinates (Figure 3.3, bottom trace). To quantify the flies' response to

the rotation of the arena, we computed the circular mean of each animal's relative change in heading after each of the three rotations of the arena (Figure 3.4). The population circular mean of these individual averages was significantly shifted in the direction opposite to the arena rotation, as expected for an animal that corrected for the disturbance by maintaining a real world heading. In order to determine which features of the sky flies used to accomplish this compensation, we covered the arena with a circularly polarizing filter, which eliminates the natural linear polarization pattern. In this condition, flies' headings did not shift significantly with respect to the arena upon rotation. One caveat associated with use of a circular polarizer is that it decreases the total light intensity reaching the fly and severely attenuates ultraviolet frequencies (Figure 3.2). We tested whether these effects could explain the flies' lack of orientation under a circular polarizer by covering the arena with two control filters: a blue bandpass filter that restricted the range of wavelengths reaching the fly (even more so than the circular polarizer) and a neutral density (gray) filter that diminished total light intensity by roughly the same factor as the circular polarizer. Under these conditions, flies compensated for the rotations in a manner similar to flies under unfiltered skylight, although not quite as effectively (Figure 3.4). Not surprisingly, when we conducted the same experiment indoors with the arena covered by an opaque black cloth, flies were completely unable to compensate for the physical rotation of the arena.

We examined the flies' behavior for the entire duration of the experiment by computing fictive trajectories for each fly assuming an arbitrary constant forward flight speed of 0.5 meters per second and integrating the headings in world coordinates (Figure 3.5). Inspection of these calculated trajectories indicates that flies under the circular polarizer followed more circuitous routes, tending to end the experiment at a shorter calculated distance from the fictive "release point." We quantified this effect by computing the total distance traveled under our constant flight speed assumption (Figure 3.5, bottom right). Flies with access to polarized skylight ended the trial significantly "farther" from where they started than flies covered by the circular polarizer. The fictive distances traveled by flies navigating under the blue bandpass

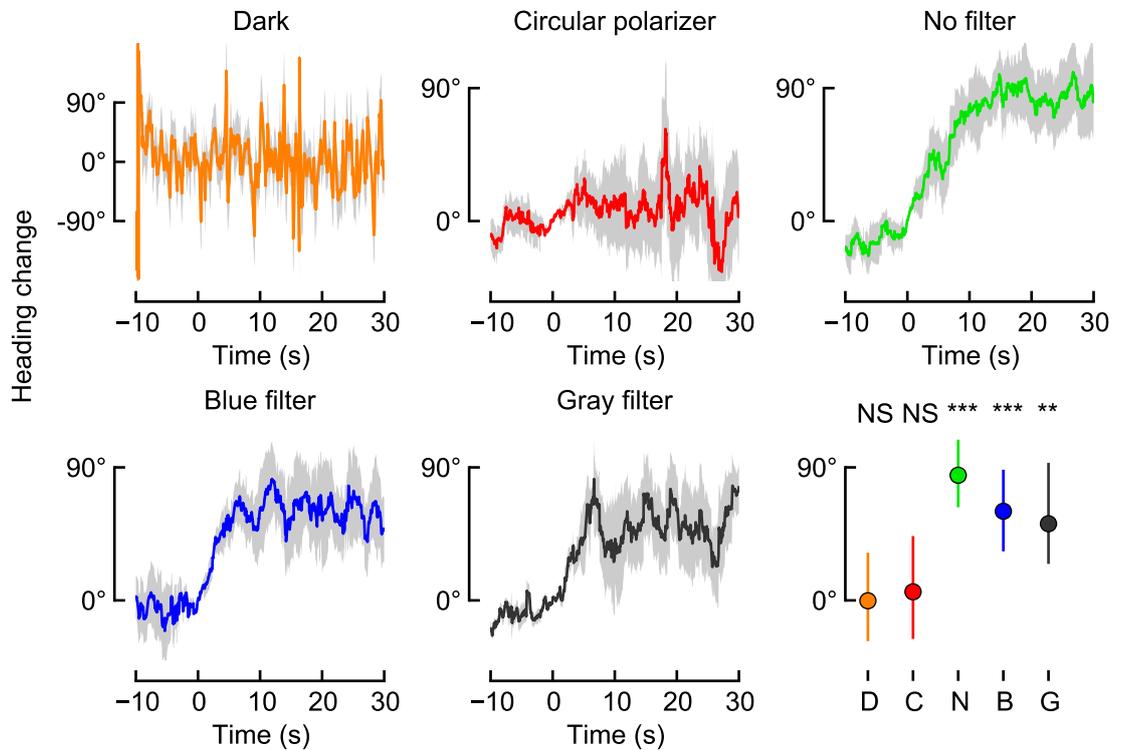


Figure 3.4: Circular mean (colored line) and circular variance (gray patch) of the change in heading with respect to arena after a rotation at time $t = 0$. A change of 90° would indicate perfect compensation for the external rotation. For each fly, we calculated a single response by averaging its responses to all three rotations during the experiment. The mean and variance of these single fly responses are displayed. Different experimental conditions and sample sizes (n , the total number of individual flies tested), were as follows: orange, complete darkness (experiment conducted indoors) $n = 18$; red, arena covered with circular polarizer, $n = 21$; green, only glass window between the fly and the sky, $n = 21$; blue, blue bandpass filter above glass window, $n = 19$; gray, neutral density filter above glass window, $n = 12$. Bottom-right plot: Circular mean of change in heading between 10 and 30 seconds after rotation of arena. Bars indicate 95% confidence intervals as computed by bootstrap method by Fisher (1993). Asterisks indicate with what confidence mean is different from zero ($***p < 0.001$, $**p < 0.01$, NS $p > 0.05$). Ninety-five-percent confidence intervals include 90° for no filter and gray filter conditions, and 99% confidence interval includes 90° for blue filter condition.

filter and neutral density filter were indistinguishable from the unfiltered condition. The fictive distances traveled by flies in the dark serve as baseline measurements for the performance expected in our arena in the complete absence of visual cues.

To evaluate individual fly performance, we calculated the mean heading during 24 30-second segments for each fly. We used the Rayleigh test for uniformity (Fisher, 1993) at the $p < 0.05$ level to determine whether an individual managed to hold a straight course for the duration of the experiment. Twelve out of 21, 13 out of 19, and seven out of 12 flies showed stable courses in the no filter, blue filter, and gray filter conditions, respectively. Only four out of 21 flies under the circular polarizer and two out of 18 flies in the dark showed significant directional preferences under the same analysis.

The results discussed thus far were collected on cloudless days. When clouds were visible to the flies, they appeared to be able to use them to correct for the external rotations in this experiment (Figure 3.6). Although we only have data from 10 flies with the circular polarizer and clouds present, they indicate that flies can partially stabilize their course using these features visible in the sky. The behavior of flies with no filter or a gray filter when clouds were visible is not qualitatively different from their behavior in the absence of visible clouds. Two aspects of these results should be noted: First, the ability of flies to use visible clouds to correct for external disturbances is not unexpected, since they can use other visual landmarks to accomplish course stabilization. Second, we did not control for the proportion of the visible sky covered by clouds and the homogeneity of those clouds. At one extreme, uniform deep cloud cover could eliminate the polarization information without providing any additional contrast landmarks. At the other, a few visible clouds could provide additional directional cues without disrupting the general polarization pattern. More systematic experiments are needed to evaluate the roles of these possible effects.

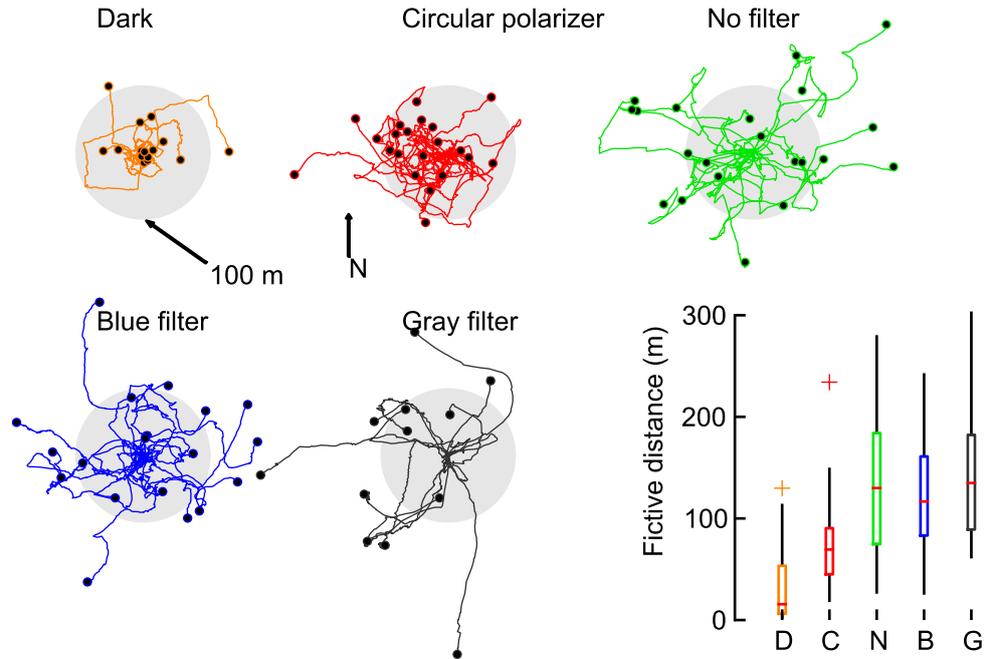


Figure 3.5: Fictive trajectories assuming constant forward flight speed of 0.5 meters per second in world coordinates. Gray background circles indicate a radius of 100 m. Black circles indicate the position at the end of the experiment for each fly. Colors and sample sizes are the same as in Figure 3.4. Bottom-right panel: Fictive distance traveled at the end of 12 minute experiment (the distance from the origin of the black dots in other panels). A fly orienting perfectly in one direction would “travel” 360 meters. Median indicated by horizontal red line, box extends from lower to upper quartile values. Vertical black lines extend to most extreme data point within 150% of the interquartile range. Outliers, defined as any points outside the range of the black lines, are shown as crosses. The N, B, and G samples are significantly greater than the C sample at the $p < 0.05$ level as computed by the Bonferroni-corrected one-tailed Mann-Whitney U test.

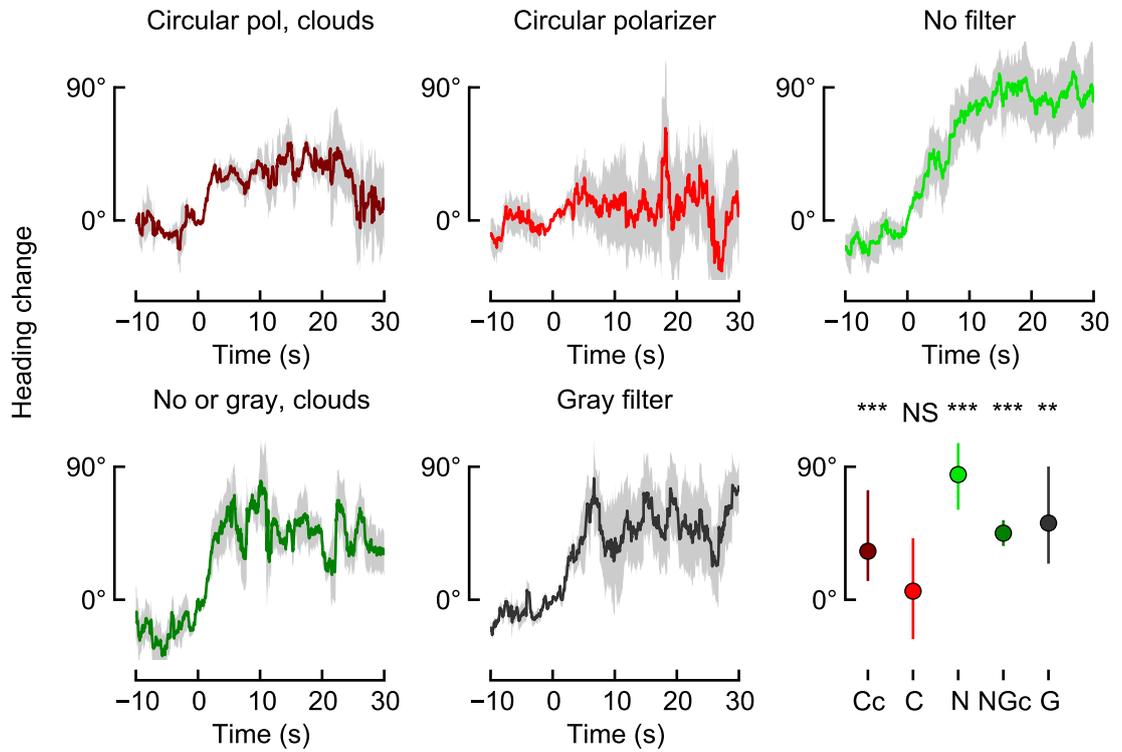


Figure 3.6: Change in heading with respect to the arena after a rotation of the outdoor arena when clouds were visible to the fly. This figure was constructed using the same method as Figure 3.4. The data for the circular polarizer, no filter, and gray filter conditions are the same as in that figure. The top-left panel shows data from experiments with the circular polarizer when clouds were visible to the fly (dark red, sample size $n = 10$ flies). The bottom-left panel (dark green) shows data from experiments with no filter or the gray filter when clouds were visible (these two conditions were combined in order to produce a reasonably large sample size; there were only six experiments with clouds and the gray filter, and 10 with clouds and no filter).

3.3 Switching arena

3.3.1 Introduction

Although the results in Section 3.2.2 suggested that flies can use polarization cues from the sky to stabilize heading, we desired a more direct test to determine whether flies will reorient when only the pattern of polarization, and no other celestial feature, changes. For these experiments we used an optoelectronic polarization switcher (Figure 3.7), which rotates the plane of polarization of transmitted light by 90° when in the active, switched state. In the passive, unswitched state, the polarization of the transmitted light is not altered. In either mode, other parameters of the transmitted light such as intensity, color, and degree of polarization are unchanged by transmission through this device. We set the mode of the switcher by applying a voltage across the device. To a human, who is unable to detect the polarization angle of light, the device appears as a clear glass window in both the switched and unswitched states. We first tested flies outdoors with a diffuser to block clouds or other visual features in the natural sky but with a polarizer above the switcher to polarize the transmitted light (results on page 79) and then tested flies with only the switcher (results on page 82).

3.3.2 Methods

Arena

When partially polarized light passes through a polarizing filter, the intensity transmitted depends on the orientation of the filter. Because skylight is partially polarized, this resulted in changes in the global intensity pattern when we rotated the first outdoor arena with the circular polarizer. We aligned the filter with its transmission axis approximately 45° to the main celestial polarization direction to alleviate this problem, but some intensity change was inevitable. We designed a second portable arena to ensure complete isolation of the effect of celestial polarization (Figure 3.7). As in the first outdoor experiment, we used a loose (magnetically) tethered fly enclosed in

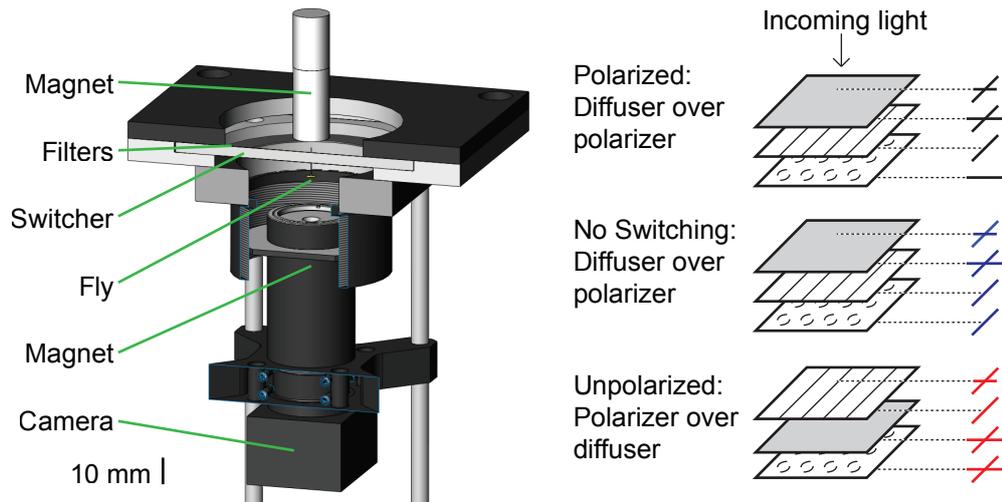


Figure 3.7: Polarization switching arena. As in the previous outdoor experiments, the fly was suspended between two magnets and free to rotate about its yaw axis while being filmed from below. The glass window was replaced by a polarization switcher, which can rotate the polarization angle of transmitted light by 90° depending on the voltage applied across it. In both switched and unswitched states, it does not change other properties (intensity, color, or degree of polarization) of the light. The exterior angle of the transparent window is 58.4° , roughly the same as in the previous outdoor experiments, and the interior angle is 24.6° . Second panel: Schematic of the three experimental conditions. The colored bars on the right indicate the polarization state of the light at each level.

an arena. In this arena, however, the window above the fly was an optoelectronic liquid crystal polarization rotator (Crystal Vision, Borlänge, Sweden). This device either leaves the transmitted light unchanged or it can rotate the plane of polarization by 90° (we call this mode “switched” in order to avoid confusion with a physical rotation). Changing modes does not alter the wavelength, intensity, or degree of polarization of the transmitted light.

Figure 3.8 demonstrates the operation of this device by displaying transmission spectra of skylight passing through it in both states when between two linear polarizers. There is some deviation from perfect 90° rotation of the polarization angle for wavelengths different from 500 nm: when the switcher is between polarizers with their transmission axes aligned some light still gets through in the switched state. This implies that the e-vectors for those wavelengths have not been rotated by ex-

actly 90° . We see that more light is transmitted in this state when the polarizers are perpendicular to one another, indicating that the e-vectors have indeed been rotated significantly even in these wavelengths.

For experiments with the optoelectronic switcher, we used the same size magnets as the first arena (except the ring magnet's inner diameter was 6.35 mm) but in a slightly different configuration. The two top magnets were in contact and both were above the window. The fly tether directly contacted the window, with no bearing. We found that the magnetic field was sufficient to keep it centered in place. The resulting outer diameter of the visible window was the same as before (58.3° outward from vertical), but the inner diameter was smaller: 24.6° , viewable by approximately 19% of the fly's ommatidia (Heisenberg and Wolf, 1984). The interior of the arena was painted entirely white, and its interior diameter was 50 mm. The fly was illuminated by four infrared LEDs (peak wavelength 850 nm) below an infrared pass filter painted white on top.

Experimental design

In the first set of experiments with the polarization switcher, we covered the window of the arena with a sheet of diffusing paper that eliminated the linear polarization pattern of the transmitted light (Figure 3.9). In the first condition, we placed a linearly polarizing filter below the diffuser, such that light reaching the fly was artificially polarized, and its polarization angle could be rotated by the polarization switcher (Figure 3.7). We switched the polarization every 60 seconds for 12 minutes. In the first control condition, the filter configuration was the same, but we did not switch the polarization. In the second control condition, we placed the diffuser below the polarizer, such that unpolarized light reached the fly, to control for effects of changing the switcher's state.

In the second set of switching experiments, we used only the natural polarization pattern in skylight—only the polarization switcher was between the fly and the sky. The first control was again with no filter but without switching the polarization. The second control was to cover the arena with the diffusing filter, eliminating polarization

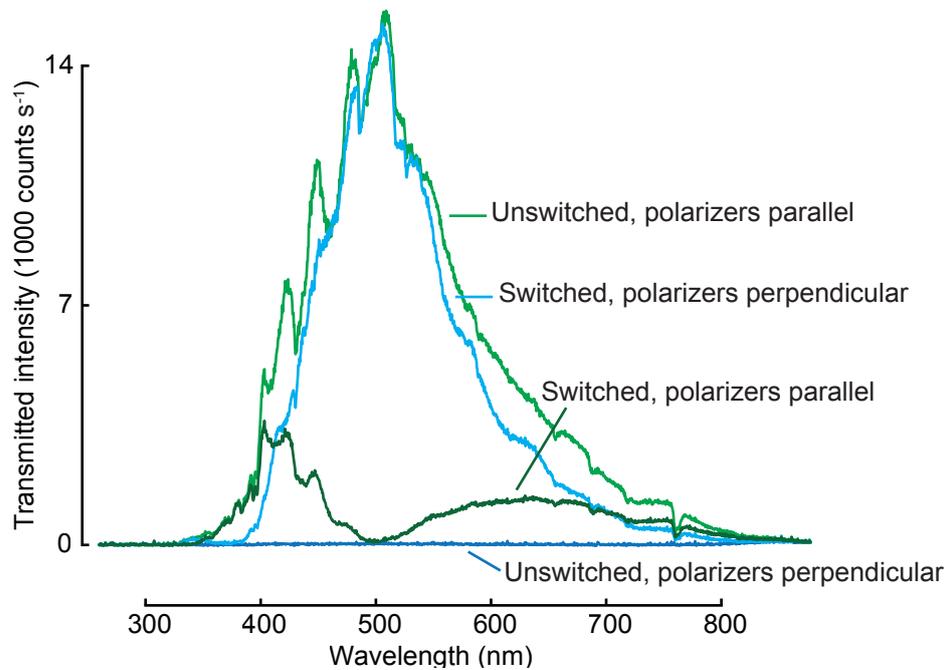


Figure 3.8: Transmission spectra demonstrating the performance of the polarization switcher. These spectra were collected using the natural sky as a light source and linear polarizers above and below the switcher. An ideally operating polarization switcher would transmit no light (the spectrum would be zero for all wavelengths) when in the active, switched state between polarizers with parallel transmission axes or in the inactive, unswitched state between perpendicular polarizers. The spectra transmitted by such a switcher would be indistinguishable from that transmitted by two parallel polarizers when it was in the unswitched state between parallel polarizers or the switched state between perpendicular polarizers. We see that there is some minor deviation from this ideal performance of our switcher when it is in the active state: it does not rotate the polarization of all wavelengths by precisely 90° .

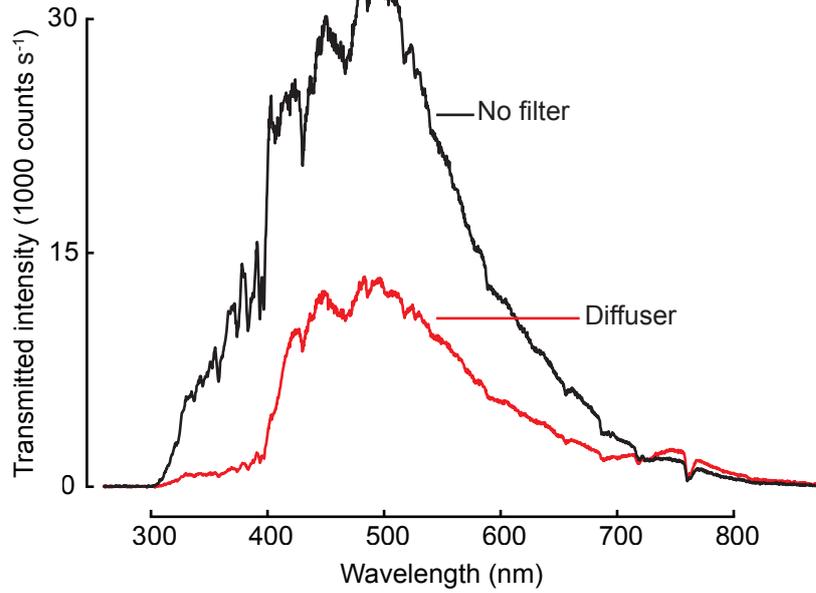


Figure 3.9: Transmission spectra for the diffusing filter used in both experiments with the polarization switching arena. A spectrum of unfiltered skylight is included for comparison. Short wavelengths are partially attenuated by the diffuser.

in the arena and controlling for effects of switching (Figure 3.13).

Analysis

We calculated autocorrelations f for the data in the switching experiments by transforming the angular observations α into their representation in the complex plane a , then taking the real part of the autocorrelation of these complex points, calculated using standard procedures:

$$a = e^{ip\alpha} \quad (3.1)$$

$$f = \text{Re}(a \star a) \quad (3.2)$$

In order to calculate angular speeds, we smoothed the complex orientations, a , (in this case $p=1$ for both) with a 0.1 second flat sliding window, then took the absolute angular difference between neighboring data points multiplied by the sampling

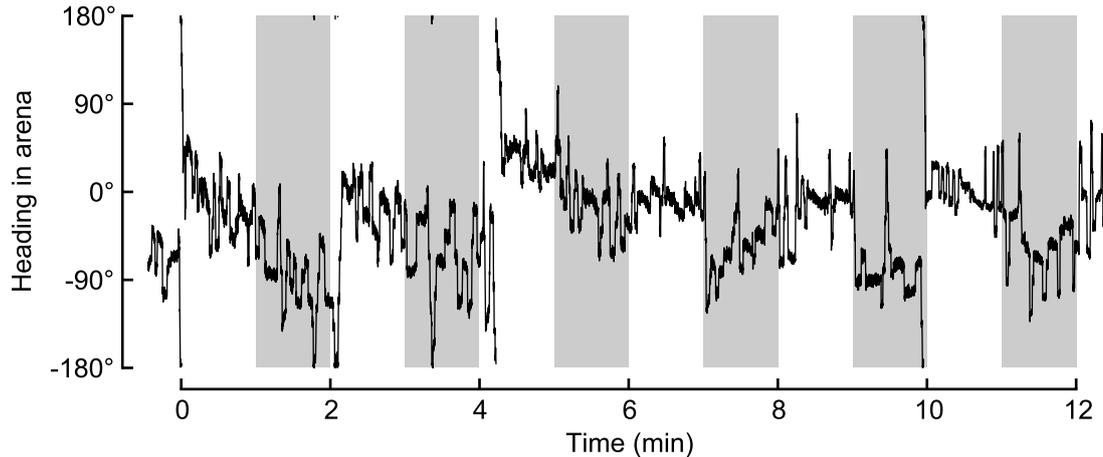


Figure 3.10: An example trace showing heading of a fly for 12 minutes in the polarized condition of the switching arena experiment, during which the polarization was unaltered for six 1-minute blocks (white background) and rotated by 90° for six 1-minute blocks (gray background). Zero degrees corresponds to flying parallel to polarization axis.

frequency.

3.3.3 Results

Results of switcher experiments with artificially polarized skylight

Because there is twofold symmetry of artificially polarized light, such as that produced by the polarizer in the first set of switching experiments, we treated the headings in this experiment as axial in subsequent analyses ($p = 2$ in, Equations 2.4 and 3.1). Flies exhibited course adjustments when we switched the polarization, compared to control flies for which the polarization was unswitched. We examined the behavior of the flies using several different analyses, each highlighting a different aspect of the flies' responses. First, examination of the raw heading data for some flies showed clear dependence on the state of the polarization switcher (data from one such individual are shown in Figure 3.10).

In order to look for more subtle responses in all of the flies in our experiments, we examined the average autocorrelation of the time series data from all flies. This

was required, as opposed to a simple average heading, since flies were not motivated to head in any specific direction. Furthermore, we wished to see any coherent response to changing the angle of polarization, and we could not assume flies would always turn in the same direction to a 90° shift in the polarization angle. The average autocorrelation of the time series data from all flies shows marked periodicity at the switch frequency of 0.5 cycles per minute (Figure 3.11), indicating that the flies' headings were more highly correlated with past headings when comparing times with the same polarization state than when comparing times with different polarization states. This periodicity was absent in control experiments in which the polarization was not switched. Hence, it did not result from some source independent of the intended stimulus. This periodicity in the autocorrelation of fly headings was also absent when the polarizer was placed above the diffusing paper, ensuring that only unpolarized light reached the fly. This control excluded the possibility that the periodicity was the product of some unexpected effect of switching the state of the polarization switcher. The individual shown in Figures 3.10 and 3.11 reliably altered course in response to switching the polarization, leading to a large oscillation amplitude in its autocorrelation at the switch frequency. Other flies contributing to the average in Figure 3.11 showed weaker responses, resulting in a smaller average oscillation amplitude. Possible reasons for this variation across individuals are discussed below.

The influence of the rotation of the polarization angle is also manifest by a change in the angular speed averaged over all flies: immediately following the 90° rotation of the polarization angle, the flies' angular speed increased (Figure 3.12). This increased angular speed indicates a turning response. By contrast, the averaged response of the flies in both control conditions showed no significant change in angular speed.

Note that in these experiments, we would not expect to observe the same change in mean heading that we measured in the first experiment, because for a fly, interpreting the instantaneous shift of polarization by 90° as a clockwise or a counterclockwise rotation is equally valid. In order to examine the responses of individual flies, we calculated the mean heading during ten 6 second segments within each trial and compared these samples between trials for which the polarization was switched or

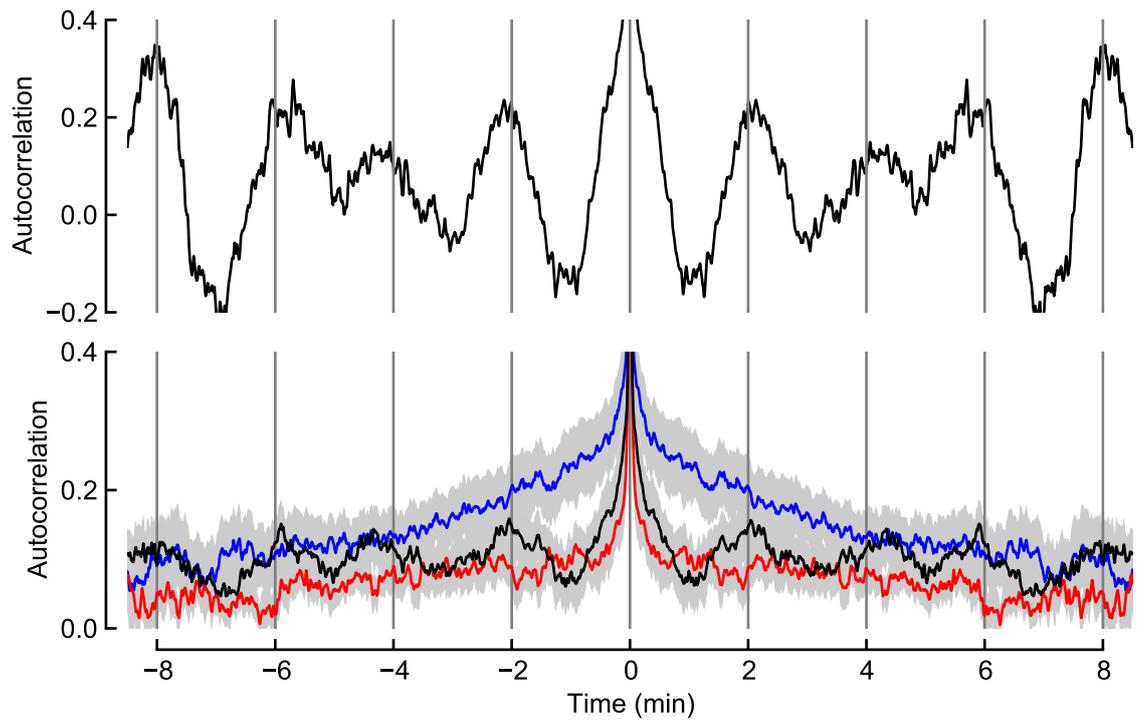


Figure 3.11: Autocorrelation plot of headings from the same fly as in Figure 3.10 (top panel) and the average response for all flies (bottom panel). The time axis is shared between both panels. Vertical gray lines depict the lag corresponding to the switching cycle during our experiments. Trials in which the polarization was switched are shown in black, sample size $n = 13$ flies; polarization was not switched (shown in blue), $n = 14$; polarization switcher state changed, but diffuser below polarizer, eliminating polarization (shown in red), $n = 13$. Mean autocorrelations are plotted as lines, SEM in gray.

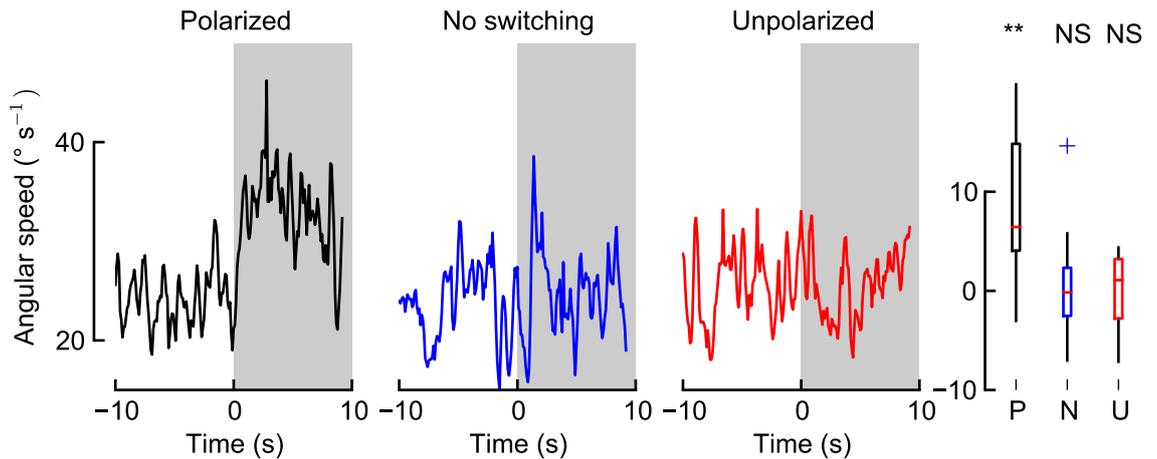


Figure 3.12: Average changes in angular speed after switcher state was changed with artificially polarized natural light. The fly's mean angular speed for 10 seconds before each switch was subtracted from the fly's mean angular speed for 10 seconds after that switch. The mean of these differences for each fly are shown in the box plots. Box plots were constructed as in Figure 3.5. The change in angular speed is significantly different from 0 for the polarized condition at the $p < .01$ level (**), but not significant even at the $p < .05$ level for the other two conditions (NS) (evaluated using a two tailed t-test).

unswitched. Using the Watson test for equal means (Fisher, 1993), at the $p < 0.05$ level, six out of 13 flies showed differences between the trial types when the polarization was switched, as opposed to only one out of 14 when the polarization was not switched and one out of 13 when the diffuser was below the polarizer so that the incident light was unpolarized.

Results of switcher experiments with naturally polarized skylight

In the experiments described above, the presence of the diffuser served to even out gradients across the natural sky, providing a homogeneous field of light, which passed through a linear polarizer before reaching the fly. Those results indicated that flies can orient using artificially polarized natural light, but it does not directly demonstrate the ability to orient using sunlight that is naturally polarized by the atmosphere. In order to test flies' ability to react to a change in the orientation of naturally polarized skylight, we repeated the experiments using the optoelectronic polarization switcher

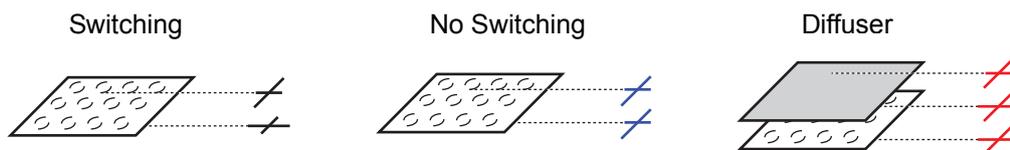


Figure 3.13: Schematic of experiments using the switching arena and naturally polarized light. There were three conditions in these experiments: In the switching condition only the polarization switcher was between the fly and the sky and we activated it for six 60 second periods interspersed with 60 second passive periods. In the no switching condition the switcher was left in one state for the entire experiment. In the diffuser condition we covered the arena with a diffuser, which eliminated the natural polarization, but we switched the state of the polarization switcher in the same way as the switching condition.

but without the diffuser and polarizer. We performed one set of control experiments in which we placed a diffuser over the arena to remove polarization cues and another in which we simply did not switch the rotator on and off (Figure 3.13). Most flies responded to the 90° rotations of the polarization angle of natural skylight with course adjustments in a manner similar to that under artificially polarized skylight (example of raw data in Figure 3.14). Flies made no such adjustments when either the polarization was not switched or when the light was not polarized because of the diffuser.

We observed the 2 minute periodicity in the autocorrelograms characteristic of behavioral dependence on trial type (Figure 3.15). (Note that here we did not treat the angles as axial, because in this case other cues, principally spectral and intensity gradients, were present in the skylight to disambiguate angles separated by 180° .) The individual fly in Figures 3.14 and 3.15 showed a strong response to switching the polarization and maintained a very consistent course, resulting in a larger autocorrelation of its heading compared to the population average.

In another analysis of the data from these experiments, we observed that flies increased their turning rate in response to switched polarization (Figure 3.16), but not in the control conditions. We found that the average angular velocity of flies increased by approximately 10° per second in the 10 seconds following change of the polarization switcher state. When the polarization switcher was not changed there

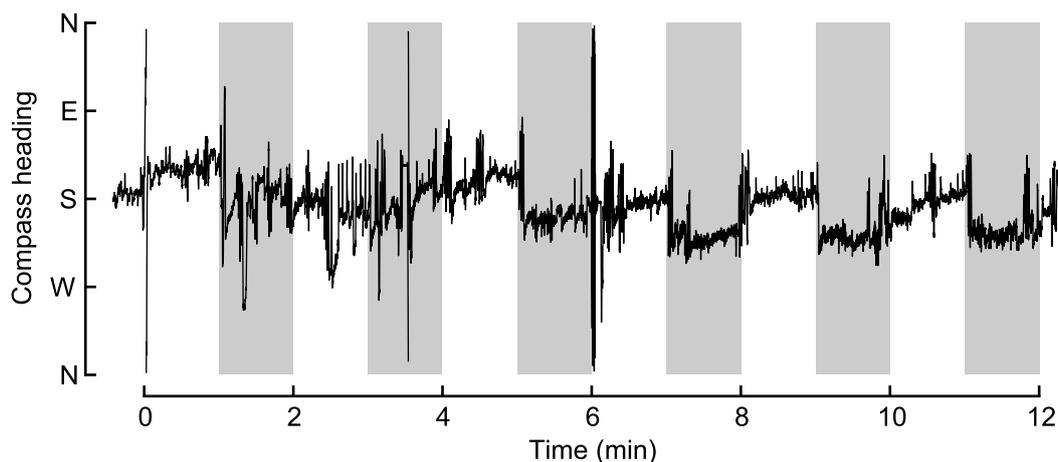


Figure 3.14: An example trace showing a fly’s heading in world coordinates for 12 minutes with naturally polarized skylight, during which the polarization was unaltered for six 1-minute blocks (white background) and rotated by 90° for six 1-minute blocks (gray background)

was no average change in turning, nor was there when the diffuser scrambled the sky’s polarization (although in this final case the flies appeared to have a higher baseline turning rate, perhaps resulting from less-oriented flight in this condition.) Finally, we performed the same statistical tests as above to look for individual fly responses, and we found that at the $p < 0.05$ level, 11 out of 16 flies showed differences between the trial types when the polarization was switched, as opposed to only three out of 12 when the polarization was not switched and two out of 11 when the light was unpolarized (the diffuser was above the fly). This result was surprising, given the plethora of other cues present in skylight that the flies could potentially use to navigate, suggesting that polarization vision is an important component of the course control system in flies under a natural sky.

The data from our two experiments using the polarization switcher indicate that although some flies unambiguously altered their heading in response to the shift of the polarization angle, there is a large variability in the response across flies. Whereas some flies exhibited a robust reaction, others showed no obvious response to the experimental change in polarization angle. Such behavioral variation might arise from a number of factors. Although we took efforts to perform experiments under

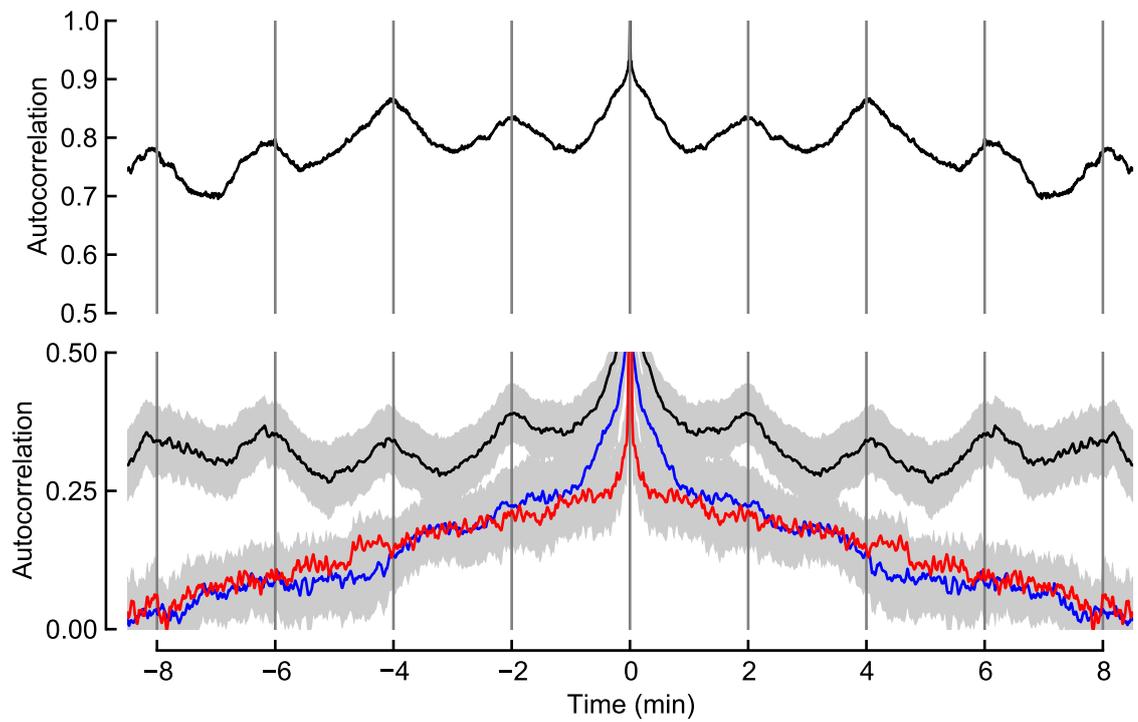


Figure 3.15: Autocorrelation plot of headings in the switching arena experiment with naturally polarized skylight. In the top panel is the autocorrelation of headings from the same fly as Figure 3.14 and in the lower panel the average response for all flies are shown. The time axis is shared between both panels. Vertical gray lines depict lag corresponding to the switching cycle during our experiments. Trials in which the polarization was switched are shown in black, sample size $n = 16$ flies; polarization was not switched (shown in blue), $n = 12$; polarization switcher active, but diffuser above arena, eliminating polarization (shown in red), $n = 11$. Mean autocorrelations plotted as lines, SEM in gray

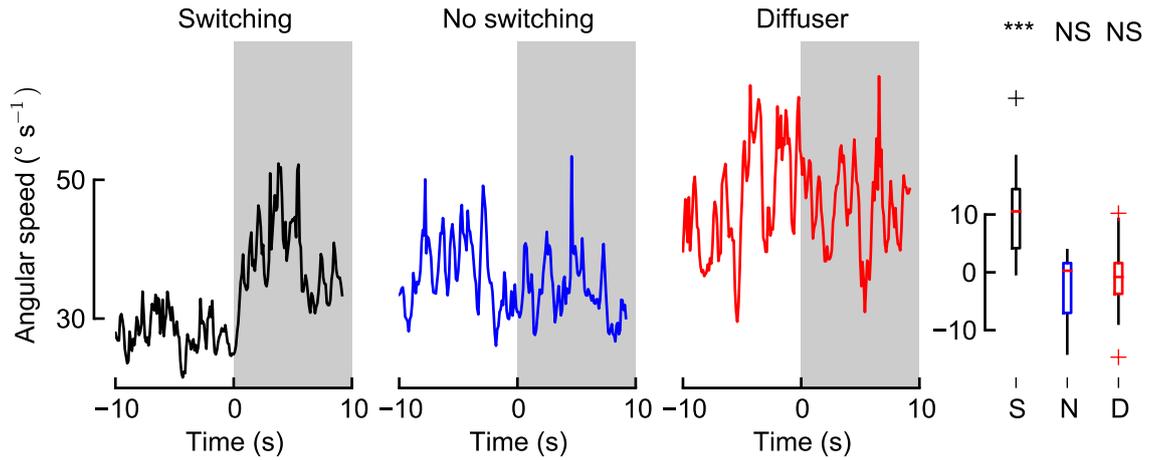


Figure 3.16: Flies turn in response to changing the angle of naturally polarized skylight. The mean of the flies' angular speeds after polarization was switched at time $t = 0$ is plotted in the first three panels. A single average response was determined for each fly by averaging its responses to all 12 switches during the experiment. The mean of these single fly responses is shown. The gray background indicates time after switch. In the right panel we show the average changes in angular speed: An individual fly's mean angular speed for 10 seconds before each switch was subtracted from that fly's mean angular speed for 10 seconds after that switch. The mean of these differences for each fly are shown in the box plots. Box plots were constructed as in Figure 3.5. The change in angular speed is significantly different from 0 for the polarized condition at the $p < .001$ level (***) , but not significant even at the $p < .05$ level for the other two conditions (NS) (evaluated using a two tailed t-test).

comparable atmospheric conditions by restricting our studies to within a 2 hour time window each day, the intensity of light reaching the flies, the degree of polarization of that light, chromatic gradients, and other aspects undoubtedly varied from trial to trial. Thus, the actual experimental conditions in each experiment were different, an inherent consequence of using a natural stimulus such as skylight. Second, unlike with studies of long-distance migrants such as monarch butterflies or locusts, we have no guarantee that our subjects were actually motivated to fly straight, and some individuals may have been operating in a local search mode in which they ignored celestial cues. Third, the genetic diversity within our lab stock, descended from 200 wild-caught females, may have contributed to the differences among flies. Finally, it is worth noting that because of the physical restriction of our flight arena, the area of sky visible to the flies was rather small, extending over roughly 35% of the dorsal rim area of the compound eye—the region thought to mediate polarization vision in insects (Labhart and Meyer, 1999; Wehner and Strasser, 1985)—and less than 20% of their entire visual world (Heisenberg and Wolf, 1984). Given these experimental constraints, together with the statistical significance of the response in population averages and in roughly 60% of all individual flies, we are confident that our results demonstrate that *Drosophila* can navigate using skylight polarization.

3.4 Discussion

Collectively, our results indicate that *Drosophila* possess the optic and neural machinery to navigate, if in a rudimentary fashion, using the pattern of natural skylight polarization. They can hold a straighter course when provided with a natural polarization pattern than they can when this signal is scrambled by a circular polarizer (Section 3.2). When an artificial pattern of linear polarization (but naturalistic in terms of color and intensity) was shifted instantaneously by 90° , flies changed course accordingly (Section 3.3, experiments with polarizer). When the unaltered polarization pattern of skylight was shifted by 90° without changing its other features, flies also responded with course adjustments (Section 3.3, experiments without polarizers).

Central place foragers such as bees and desert ants have been the subject of intensive investigation into the role of a celestial compass in insect navigation. Among other topics, the important concepts of time compensation (Dyer and Dickinson, 1994; Wehner, 1984), path integration (Collett et al., 2006; Wittlinger et al., 2006), and multisensory integration (Dyer, 1996; Müller and Wehner, 2007) have been examined in detail in these organisms. A small specialized region of the eye called the dorsal rim area is thought to be critical for these behaviors in many species (Labhart and Meyer, 1999; Wehner and Strasser, 1985), although the evidence in flies is somewhat contradictory. Flies possess a dorsal rim area, which has been implicated in polarization responses (von Philipsborn and Labhart, 1990), but prior experiments using a tethered flight arena suggest that the rest of the eye may play a role in responses to polarized light (Wolf et al., 1980). Our results do not bear directly on this discrepancy, because our sky stimulus was visible to ommatidia both within and outside the dorsal rim area. Within the dorsal rim area, photoreceptors R7 and R8, which have been proposed to underlie polarization vision, both express an opsin with a peak sensitivity in the ultraviolet. Thus, our observation of polarization-dependent responses to wavelengths longer than 400 nm provides further indirect evidence for the role of other photoreceptors besides R7 and R8 within the dorsal rim. We cannot, however, rule out their involvement because it is possible that they exhibit some small but functional sensitivity to the wavelengths used in our experiments. The possible existence of alternate, spectrally distinct pathways for detecting polarized light may have contributed to the variability we measured in experiments in which UV light was attenuated by filters.

Through studies of migratory insects such as monarch butterflies and locusts, the neural circuitry that underlies polarization vision and its influence on motor behavior has begun to be elucidated. Researchers have traced the polarization vision pathway from the eye to the central brain to neurons arborizing in the thoracic ganglion (Heinze and Homberg, 2009; Heinze and Reppert, 2011; Homberg et al., 2011; Mouritsen and Frost, 2002; Stalleicken et al., 2006; Träger and Homberg, 2011). This electrophysiological evidence suggests that the central complex, a series of unpaired

neuropils of the central brain, plays a key role in processing polarized light. The ubiquity of this brain region along with the relevance of polarization vision to the life history of a variety of species suggests that orientation responses using polarized light may represent a rather ancient component of insect behavior (Homberg, 2008; Loesel et al., 2002). At first glance, the fruit fly, which is neither a central place forager nor known as a seasonal migrant, seems to be a strange choice of species in which to study polarization vision. Because long-distance directed flights, either for migration or homing, have not been directly observed in flies, one cannot rely on innate motivation to navigate to a specific location when designing experiments. Nonetheless, a fly (or any insect for that matter) that finds itself in a resource-poor area, without observable attractive cues, faces a critical challenge. Maintaining a straight path ensures that it does not waste limited resources repeatedly traversing the same ground. Indeed, evidence suggests that several species of fruit flies, including *Drosophila melanogaster*, could fly over 10 kilometers without access to food or water (Yerington, 1961; Coyne et al., 1982, 1987). Given the energy resources of even a well-fed fly (Götz, 1987; Lehmann and Dickinson, 1997; Wigglesworth, 1949), this feat would only be possible by maintaining a straight heading. Because the sun is often obscured by clouds, masked by local features, or below the horizon, an alternative source of compass information—such as that available from skylight polarization—would be extremely useful for animals attempting to maintain a heading relative to global coordinates. An intrinsic compass preference would not be necessary, simply the ability to choose a heading and maintain it. Our experiments were designed to mimic this situation, and we observed that flies did indeed use skylight polarization to help maintain a steady course. The fruit fly, too often thought of without reference to its evolutionary history, thus displays another of the almost implausibly complex behaviors found in the insect world. The wealth of behavioral, physiological, and genetic tools available in *Drosophila* make it an ideal system in which to examine the open questions surrounding this behavior. Our observation of flies using celestial polarization to hold a course is a step in this direction.

Chapter 4

Conclusion

Flight is but momentary escape from the eternal custody of earth.

—Beryl Markham, *West with the Night*

The previous sections described a series of quantitative behavioral experiments designed to examine *Drosophila* flight responses to changes in the polarization of light. Where possible we have discussed the behavior of flies in terms of their natural history, attempting to place it in the context of an animal in the natural environment. Here we will summarize the major findings of this work, discuss methodological advances, relate it to the question of which receptors mediate polarization vision, put it in the context of the broader field, and propose future directions for research on related topics.

4.1 Summary of findings

In the Chapter 2 we reported responses of rigidly tethered flies to rotating the angle of polarization of light. These responses confirmed that *Drosophila* perceive the e-vector of light and can steer with respect to it. In both open- and closed-loop experiments flies exhibited steering responses consistent with attempts to stabilize the rotation of the polarization angle. In closed-loop experiments flies displayed individual preferences for particular e-vector orientations, but we did not observe any consistent preference across the population. There is some evidence for a shift

in individual flies' preferred direction with respect to polarization over the first few hours of a long flight consistent with compensation for the sun's apparent movement in the sky. This phenomenon needs further work before it can be taken as hard evidence for time compensation. Otherwise, the preferences of flies in long-duration flights remained stable for the most part.

Because of the limits on interpreting laboratory experiments with artificial stimuli in terms of natural behavior, we conducted a series of outdoor experiments, reported in Chapter 3. When flies were allowed a view of the natural sky they sometimes followed remarkably straight paths, even in the presence of external disturbances. When they were in darkness, however, or deprived of the polarization information from the sky, their paths were not as straight and the flies were less able to correct for external disturbances. Furthermore, when the angle of artificially polarized skylight was shifted by 90° , without changing the light's intensity, color, etc., the flies turned in response. Finally, when the same shift was applied to the e-vector of naturally polarized skylight, *Drosophila* changed course accordingly. To our knowledge, this is the most direct demonstration of flies using celestial polarization to hold a course.

4.2 A new technique for studying biological polarization sensitivity

There are many pitfalls to avoid when conducting biological experiments using stimuli to which humans are insensitive—the experimenter cannot evaluate the stimulus parameters directly, so he is forced to rely on the readings of sensors. We have discussed several potential confounds when using polarized light stimuli (Sections 2.2.3 and 2.2.4), such as the effects on intensity when this type of light is partially reflected by surfaces. The polarization switcher we used in Section 3.3 helps avoid some of these sources of error. Only recently has this type of device begun to be used in biological experiments. To our knowledge, only a small number of studies on marine organisms have used a similar liquid crystal apparatus to examine responses to light polarization

(Ortiz-Gutiérrez et al., 2003; Glantz and Schroeter, 2006, 2007; Pignatelli et al., 2011; Temple et al., 2012), and they all used an artificially polarized light source. The use of such a system to modify natural sources of polarized light is to our knowledge novel, and represents the most controlled method for distinguishing polarization-dependent behavior in the field from other types of behavior. These devices are readily available, and it is our hope that future investigators will take advantage of them for answering biological questions.

4.3 Implications for receptors involved in the detection of the polarization angle

As alluded to in Section 1.6, there has been a history of debate regarding which photoreceptors contribute to the detection of polarized light by flies. In *Drosophila melanogaster*, Heisenberg first concluded that polarization-dependent behavioral responses were mediated by the inner cells R7/8 (Heisenberg, 1972), but later attributed them to the outer R1–6 system (Wolf et al., 1980). The recordings by Coombe et al. (1989) showing polarization sensitivity in R1–6 cells support the latter view. Most recently, Wernet et al. (2012) reported contributions by both systems (R1–6 and R7/8) to polarization responses. While we did not perform any direct interventions on photoreceptor systems aimed at addressing this question, our experiments using different wavelength stimuli can give some insight.

One model of polarization vision holds that it is mediated entirely by photoreceptors R7/8 in the DRA, both expressing rhodopsin *rh3*. When experimenters ectopically expressed this rhodopsin in cells R1–6 in otherwise blind flies, they observed a single peak of sensitivity to UV wavelengths in electroretinograms (extracellular recordings from the fly retina.) This sensitivity peak falls off rapidly, and approaches baseline for wavelengths longer than approximately 400 nm (see Figure 1.3). We saw responses to rotating the polarization angle of not only UV and blue light (peak wavelengths 355 and 470 nm), but also green light with peak wavelength 525 nm (Figure

2.10), well outside of the reported sensitivity range of *rh3*. One possible explanation for this is that *rh3* actually has a wider sensitivity peak than was previously believed, yielding some residual sensitivity to the wavelengths in our study. A mechanism not present in the somewhat artificial circumstances of ectopic expression in R1–6 cells could be responsible for underestimating the sensitivity range of this rhodopsin in its native R7/8 cellular environment in the DRA. We know that the curves in Figure 1.3 are not entirely accurate: Fingerman and Brown (1953) observed phototactic responses to red light and concluded that the upper limit of the visual system is above 675 nm. More recently, Hanai et al. (2008) demonstrated that entraining the circadian clock of flies to red light of wavelength longer than 600 nm requires *rh1* or *rh6*, indicating that these rhodopsins are sensitive to those wavelengths, even though the work of Salcedo et al. (1999) (in Figure 1.3) suggested that these rhodopsins were insensitive to wavelengths above 600 nm. Given that the sensitivity ranges of *rh1* and *rh6* were underestimated, it is possible that the range of *rh3* is also larger than was reported. Supporting this explanation is our observation that the response to UV light was stronger than that to green wavelengths, and it is almost indisputable that the peak of *rh3* sensitivity is in the UV, regardless of possible sensitivity in the visible spectral range. Our outdoor experiments also indicated that depriving the fly of UV light resulted in a small decrease in its ability to hold a course based on skylight polarization (Figure 3.4).

Turning this argument around, however, we should note that the outer cells R1–6 express *rh1*, which is sensitive to both a wide range of visible wavelengths (blue, green, and some red) and to UV light. Thus, postulating a polarization sensitivity mediated by cells R1–6 throughout the eye is entirely consistent with our data.

4.4 Impacts on related work

Most studies of the neural control of flight by flies have been conducted in the laboratory. There is a long history of using both tethered and free flight studies to examine sensory capacities and algorithms used by the nervous system (for example,

Reichardt and Poggio, 1976; Maimon et al., 2008; Straw et al., 2010), and these studies have yielded many insights into motion processing and neural algorithms, among other topics. One of the most identifiable flight maneuvers observed in such studies is the “body-saccade”—a rapid, high-amplitude turn that occurs frequently during flight (Bender and Dickinson, 2006). Recently, researchers have studied the statistics of these turns in order to argue that *Drosophila* employ a so-called Lévy-flight strategy while foraging in the absence of cues (Reynolds and Frye, 2007). This strategy consists of straight flight segments punctuated by saccades at intervals drawn randomly from a heavy-tailed distribution. It is hard to reconcile this type of strategy with the findings of long-range dispersal discussed in Section 1.5.1, since a fly who periodically makes high-amplitude turns could not manage to travel the distances they have been observed to travel. Nor is it reasonable to think that the assumptions of the Lévy flight model were violated—it is unlikely that the flies were following an appetitive sensory cue the entire time, especially since flies were observed to disperse in different directions and even when released near food. The work presented here suggests that even in the relatively cue-poor desert environment, flies could use celestial polarization to hold a steady course, and not be forced to turn at random.

4.5 Future directions

A number of unanswered questions remain surrounding the perception of light polarization by flies. Further behavioral experiments would be useful in determining how individual flies form and maintain a preference for a particular e-vector orientation. This could serve as a model of decision making in general. Are individuals genetically predisposed to a given polarization angle? Does climate or time of year influence preferred directions? Any evidence of seasonality would be extremely exciting, given the prevalence of migration in the animal kingdom. If *Drosophila* could serve as a model for this behavior, its genetic basis might become accessible to study. Other possible mechanisms for choosing a heading relative to polarization angle would be equally interesting. Does the time directly preceding flight initiation play a role, or

does a given heading preference “crystallize” once the fly has already been flying for a period of time?

From a sensory control of flight perspective, it would be useful to know how this system interacts with other sensory modalities. Behavioral experiments could be designed to examine the interaction of polarization vision with contrast-based vision or other sensory modalities. Presumably information about the e-vector of light is somehow weighted when combined with information from other sensors. What affects the relative weight of this modality? Does it differ based on species? It is tempting to suppose that desert species, evolved to travel long distances across inhospitable terrain between food resources, may place a greater importance on polarization information than cosmopolitan species. If this is true, examination of these related species could serve as a model for how evolution acts on the nervous system to adapt it to diverse environmental constraints.

The genetic tools available in *Drosophila* also make it an attractive species in which to study the neural basis of sky polarization-based navigation. Recently, researchers have produced large collections of driver lines that express Gal4 in fairly restricted sets of neurons. It is possible to screen for the function of the cell types each driver line expresses in by using so-called genetic actuators: Genetically encoded calcium indicators, temperature-sensitive activators, and inward rectifying channels to inactivate cells are just some of the reagents that are readily available. By imaging, activating, and inactivating genetic cell types in the optic lobes of flies during behavioral experiments like those described here, it may be possible to identify which cells carry polarization information to the central brain (A comprehensive review of the techniques available for this type of analysis is provided by Simpson, 2009). These cells can then be targeted for electrophysiology (Maimon et al., 2010).

The central complex is a reasonable place to look for more central representations of polarization information in the insect brain. The same type of genetic techniques can in principle be used here to identify cell types important for processing polarization cues. It is probable that this level of the nervous system integrates multiple sources of information from different sensory modalities. This potentially makes anal-

ysis of cell type function more difficult, but also more rewarding. Eventually sensory information, combined with information about the current behavioral state of the animal, must be used to drive an appropriate motor output, be it initiating flight, turning, landing, or any of the other multitude of behaviors open to an individual. How the fly's minuscule brain manages to produce correctly coordinated behavior in response to a huge variety of circumstances is one of the great open questions that will doubtless take much work to answer.

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