

The magnetic sense of honey bees - analyses of underlying mechanisms and potential function

**by
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Abstract

I studied a potential function and underlying mechanism(s) of the magnetic sense in honey bees, *Apis mellifera*.

A waggle-dancing bee informs hive mates about a food source. Directional information pointing to the food source relative to the sun's azimuth is encoded in the angle between the straight segment of her waggle dance and a reference line such as gravity or the local geomagnetic field (LGMF). Neither cancelling the LGMF nor shifting its declination affected the recruitment success of waggle-dancing bees, implicating gravity as the reference line for the dance alignment.

To study the underlying mechanism(s) of the bees' magnetic sense, I analyzed lyophilized and pelletized bee tagmata by a Superconducting Quantum Interference Device. A distinct hysteresis loop for the abdomen but not for the thorax or the head of bees indicated the presence of magnetite in the abdomen. Magnetic remanence of abdomen pellets produced from bees that I did, or did not, expose to an NdFeB magnet while alive differed, indicating that magnet-exposure altered the magnetization of this magnetite in live bees. Following exposure of live bees to the same magnet, magnetized bees, unlike sham-treated control bees, failed to sense the presence of a magnetic anomaly, demonstrating a functional connection between magnetite in the abdomen and the magnetoreceptor, and temporary or permanent disablement of the receptor through magnet-exposure.

To test whether bees sense the polarity of a magnetic field, I trained bees to associate a magnetic anomaly with a sugar water reward. I then presented trained bees with a sugar water reward in two separate watch glasses, placing one reward in the center of the anomaly that I either kept the same as during bee training (control experiment) or that I altered by reversing its polarity (treatment experiment). That bees continued to recognize the magnetic anomaly when its polarity was kept unaltered, but failed to recognize it when its polarity was reversed, indicates that bees have a polarity-sensitive magnetoreceptor.

To increase the detectability of magnetite in bee tissues, I lyophilized samples to reduce water content, maximized the signal amplitude by pelletizing samples, and accounted for sample dimensions in data analyses.

Keywords: Honey bees; Communication; Magnetoreception; Magnetite-based magnetoreception; Behavioural two-choice experiments; SQUID measurements

TO MY PARENTS:
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List of Acronyms

SPM	Superparamagnetic
PM	Paramagnetic
SD	Single domain
EMF	Earth magnetic field
LGMF	Local geomagnetic field
SQUID	Superconducting quantum interference device
CRY	Cryptochrome
Oe	Oersted
AWG	American wire gauge
NdFeB	Neodymium ion boron

Glossary

Coercivity	The resistance of a magnetic material to changes in magnetization, equivalent to the field intensity necessary to cancel out the remanent magnetization.
Cryptochrome	Class of Flavoproteins that are sensitive to blue light. They are found in plants and animals. Cryptochromes are involved in the circadian rhythms of plants and animals and possibly also in the sensing of magnetic fields in a number of species.
Declination	Magnetic declination is the angle on the horizontal plane between magnetic North and true North at a particular location.
Diamagnetism	Diamagnetic substances have very small negative values of magnetic susceptibility. The magnetic dipole moments that are induced in diamagnetic substances tend to align in the sense opposite to that of an applied magnetic field and hence tend to reduce the magnetic field slightly. They have a zero net angular momentum and therefore no permanent magnetic moment.
Ferromagnetism	Permanent magnetic materials can be magnetized by an external magnetic field, and will remain magnetized after the external field is removed. Ferromagnetic materials exchange interactions and couple adjacent Bohr magnetons into alignment.
Flavoproteins	Flavoproteins are proteins that contain a nucleic acid derivative of riboflavin: the flavin adenine dinucleotide FAD or flavin mononucleotide (FMN).
Flux lines	Magnetic field lines.
Helmholtz coils	Helmholtz coils produce a region of nearly uniform magnetic fields near their midpoint. It consists of two circular coils separated by a distance equal to their radius.
Hysteresis	Hysteresis is the dependence of the state of a system on its history. For example, a magnet may have more than one possible magnetic moment in a given magnetic field, depending on how the strength of the ambient magnetic field changed in the past.
Inclination	Magnetic dip angle. The resulting angle of the vertical and the horizontal components of a field.
Paramagnetism	Magnetic materials consist of atoms that have a net magnetic dipole moment, which can align slightly with an external field. Paramagnetic materials have unpaired Bohr magnetons that are not exchange coupled.

Remanence	The remanent magnetization is the residual magnetization in ferromagnetic material after an external magnetic field is removed.
Superparamagnetic	Magnetite particles smaller than 30 nm in size typically exhibit superparamagnetic behavior. These particles cannot stabilize their own magnetization unless an external magnetic field is applied. Superparamagnetic particles have a larger thermal energy than anisotropic energy.
Susceptibility	Is the ratio of magnetization to a magnetizing field.

Chapter 1.

Introduction

Magnetoreception is widespread in the animal kingdom (Wiltschko and Wiltschko, 1995). In most cases studied thus far, animals sense the earth's magnetic field (EMF) and use it to accomplish diverse tasks including (i) navigation and orientation (migratory animals), (ii) the search for anaerobic conditions (magnetotactic bacteria), (iii) the calibration of the circadian rhythm (vinegar flies), and (iv) engaging in fewer random walks (bacteria and magnetotactic protists).

My thesis is about magnetoreception in honey bees, *Apis mellifera*. It is well established that honey bees can sense the EMF, but the biological functions and underlying mechanisms of their magnetic sense are largely unknown. The only known biological functions are in the context of comb alignment (De Jong, 1982) and time sense (Lindauer, 1976). Bees building combs in a new hive orient them according to the comb alignment in their parent hive by making reference to the EMF. This is advantageous in that thousands of comb-building bees have to “agree” on one alignment for their new combs. As bees nest in sheltered, dark areas such as hollow trees or stone cavities that lack any celestial or sun-related cues, they may rely on the EMF as an alternative reference. One might wonder, however, whether this is the only biological function for the magnetoreceptive ability of bees.

Research aimed at characterizing the magnetoreceptor of honey bees is limited in quantity and scope. It has long been known that honey bees can sense exceedingly small magnetic anomalies (0.6% of the EMF) (Walker and Bitterman, 1989b). It is also known that there are magnetic nanoparticles in the abdomen of bees that have been speculated to be part of the bees' magnetoreceptor(s) (Gould et al., 1978; Hsu and Li, 1993; Kuterbach and Walcott, 1986; Desoil et al., 2005). However, these magnetic nanoparticles have never been linked unequivocally through rigorous and extensive experimentation to a magnetoreceptive function.

In this introductory chapter, I will (i) describe aspects of magnetic fields and magnetic materials pertinent to my research, (ii) describe equipment I used for

experiments, (iii) introduce the current models of magnetoreception, (iv) outline how animals exploit EMF-derived information in a compass and map sense, (v) explain the concept of a polarity compass and an inclination compass, and (vi) summarize current knowledge about magnetoreception in honey bees.

1.1. Background on magnetic fields and magnetic materials

1.1.1. Properties of the earth's magnetic field

This subsection of my introductory chapter briefly describes the EMF and properties that animals might use as cues for orientation and navigation.

The EMF can crudely be viewed as the field produced by a powerful (but fictitious) bar magnet located near the center of the earth. Field lines emerge from the earth's surface in the southern hemisphere and re-enter in the northern hemisphere (Figure 1.1) (Winklhofer, 2010). Strictly speaking, the geographic North pole is the north pole a compass needle points toward and therefore corresponds to the South pole of the fictitious bar magnet mimicking the EMF. Nonetheless, to avoid confusion and to facilitate understanding of various phenomena, I will henceforth refer to the geographic and magnetic poles using the same nomenclature.

The intensity and the inclination of the EMF with respect to the Earth's surface vary with latitude. The geomagnetic field is most intense near the poles ($> 60 \mu\text{T}$) and least intense near the equator ($< 35 \mu\text{T}$). Its inclination ranges from $\pm 90^\circ$ (vertical) at the magnetic poles to 0° (horizontal) at the magnetic equator (Winklhofer, 2010).

While the density of the flux lines and therefore the strength of the EMF varies across the earth's surface, the field strength averages about 0.5 Gauss (G). Gauss refers to the cgs (centimeter–gram–second) unit of measurement of magnetic flux density, named after the German mathematician and physicist Carl Friedrich Gauss. The SI-unit for magnetic flux density is Tesla (T), where 1 T equals 10,000 G. Both units are used to describe magnetic field strengths.

True North (geodetic north) is usually referred as the direction along the earth's surface towards the geographic North pole. The locations of the geomagnetic poles and the geographic poles do not correspond precisely, and the locations of the magnetic

poles differ from year to year. This phenomenon gives rise to a non-zero magnetic declination, which is the angle on the horizontal plane between magnetic North and true North at a particular location.

Abnormalities (local deformations) of the EMF are referred to as magnetic anomalies and are caused by magnetizable substances like iron in the earth's crust. They are thought to play a role in the orientation of bees within their home range (Kirschvink and Kobayashi-Kirschvink, 1991).

The intensity, declination and inclination of the EMF are subject to daily changes. Changes in the declination and inclination range within only a few tenths of one degree. Diurnal changes in EMF intensity range between 25 to 50 nT (Liboff, 2013), and happen only during the photophase. Hitchman (1998) postulated that they occur because the sun's ultraviolet radiation ionizes atoms in the upper atmosphere (100 km above the earth's surface). The charged particles that are generated are then driven by thermal convection, creating large scale currents. These currents in turn produce magnetic fields of their own, which modify the EMF on a diurnal basis.

In addition to these regular changes of the EMF, magnetic storms, which are irregular fluctuations of the EMF occur in connection with sun spot activity, solar wind and shock waves.

1.1.2. Magnetic nanoparticles

Magnetic nanoparticles come in different sizes and have specific physical properties. They respond differently to applied magnetic fields, as I will explain below.

Paramagnetic, superparamagnetic and ferromagnetic particles

Magnetite particles < 30 nm in size typically exhibit superparamagnetism (SPM). SPM particles cannot stabilize their own magnetization for a net magnetization to exist; external magnetic fields must be applied.

When an external magnetic field is applied, SPM crystals show a higher susceptibility (magnetization in response to a magnetizing force) than paramagnetic (PM) crystals (Figure 1.2). This means that in the presence of an external field the spins of the unpaired electrons are better aligned in SPM particles than in PM particles. The

susceptibility of both types of particles will rise in a linear function with the increasing strength of an external magnetic field, until all unpaired electron spins are parallel to the applied field and the particles are “saturated”. In field-cycling experiments, SPM and PM particles in test samples are subjected to an external magnetic field. The intensity of the magnetizing force is first increased in multiple steps and then reduced to zero. Subsequently, the direction of the force is reversed, and the force intensity is increased again in multiple steps to the same intensity as before, after which it reduced to zero. The magnetic response of SPM and PM particles results in a sigmoid curve with saturation magnetization at both ends of the curve (Figure 1.2). When the external magnetic field is set to zero in cycling experiments, the interaction energy of SPM and PM particles is too small to maintain the alignment of magnetic moments. With no external magnetic field, the alignment of unpaired electron spins randomize, and thus the particles have no remaining magnetization. For this reason, the exposure to large magnetic fields has no lasting effects on SPM or PM particles (Paul A. Tipler section 27-5).

Stable single domain (SD) particles are ferromagnetic particles ≥ 30 nm in size. This size enables the particles to establish a stable net magnetization, which is independent of an external magnetic field and therefore those particles become permanently magnetic. Previously magnetized SD particles will maintain the alignment of unpaired electron spins because of the strong ferromagnetic interaction of dipole moments with each other. This residual magnetization is referred to as the remanent magnetization. In field cycling experiments, the characteristic response of ferromagnetic particles is also observed as a sigmoid curve, with saturation magnetization occurring on both ends of the curve. However, there is a difference. Over one full cycle the magnetization curve traces out a loop, known as a hysteresis loop. As the external magnetic field is first reduced to zero, the remanent magnetization persists. The field direction has to be inverted, in order to force the magnetization to zero. The field strength required to force the magnetization to zero is known as the coercive force, or coercivity (Figure 1.2). The same process then repeats itself as the magnetic field is ramped to its maximum value in the opposite sense and brought back to zero, leaving a remanent magnetization in the opposite sense.

When magnetic fields, like the EMF, act on isolated ferromagnetic SD particles, their magnetic moments tend to track the direction of the magnetic field and align

themselves like a compass needle with the external magnetic field. Furthermore, their magnetic properties are sensitive to large magnetic fields, as the alignment of unpaired electron spins will change after exposures to large magnetic fields.

Diamagnetism

Diamagnetic substances have very small negative values of magnetic susceptibility. The magnetic dipole moments that are induced in diamagnetic substances tend to align in the sense opposite to that of an applied magnetic field and hence tend to reduce the magnetic field slightly. They have a zero net angular momentum and therefore no permanent magnetic moment.

The behavior of diamagnetic substances in field cycling experiments is such that they create an induced magnetic field in a direction opposite to an externally applied magnetic field (Figure 1.2 d). Diamagnetism presents a challenge for the magnetic characterization of biological samples because water exhibits diamagnetic effects. Weak paramagnetic or ferromagnetic signatures can be masked by the diamagnetic response of large quantities of water (Hautot et al., 2005).

1.1.3. Instruments and equipment used for studying the presence and type of magnetic nanoparticles in honey bees and the effect of magnetic fields on behavioral responses of bees.

Superconducting Quantum Interference Device (SQUID)

The presence and type of magnetic nanoparticles in (biological) test samples can be determined by SQUID (Superconducting Quantum Interference Device) magnetometry. A SQUID moment magnetometer is a very sensitive magnetometer, which detects very small changes in magnetic flux through superconducting coils. With a proper calibration, signals detected by the SQUID can be interpreted in terms of magnetic moments. It is a versatile instrument, which measures magnetic moments of test materials under varying temperature conditions and external magnetic fields.

For my research, I used a SQUID to determine the presence and type of magnetic nanoparticles in tagmata of honey bees. Applying external magnetic fields of increasing and decreasing strength to bee tagma samples, I obtained indicative hysteresis measurements. I further used a SQUID to measure induced magnetic

remanence of bee tagmata, setting the external magnetic field to zero. In this setting, the contributions only of the SD ferromagnetic particles are measured, as other magnetic particles require an external magnetic field to reveal magnetic remanence.

Helmholtz coils

A Helmholtz coil pair is capable of producing a uniform magnetic field along its axis, near the center of the volume it encloses. Multiple Helmholtz coil pairs can be combined into a system capable of manipulating components of the magnetic field along multiple axes at the same time. Helmholtz coil pairs and coil systems are routinely used to create specific magnetic fields, or to cancel or manipulate ambient magnetic fields, such as the Earth's magnetic field.

We used a tri-axial Helmholtz coil system (Figure 1.3), which consisted of six octagonal coils of wire, or three pairs of coils. The two coils in each pair were identical, and were mounted on a supporting wooden structure, placed symmetrically along a common axis, and separated by a distance equal to the radius of the coil, with each coil carrying a the same electric current in the same sense. The three pairs were then arranged so as to be orthogonal to one another. Depending on the direction of the current flowing through the two coils in any one pair, the strength of a particular component of the EMF can be enhanced, decreased or nulled (Figure 1.3).

Magnetic anomalies experimentally generated by a two-choice table

Experimentally generated magnetic anomalies are great means to test the magnetoreceptive ability of experimental animals or insects in field experiments. Generally, to produce a magnetic anomaly, a current is driven through a wire, which is arranged as a coil. The number of times the wire is wrapped around the coil (or the number of 'turns') affects the strength of the magnetic anomaly. For my studies, we produced magnetic anomalies with three co-planar coils of stranded 16 AWG wires that were wrapped in such a way that a magnetic anomaly could be generated in the vicinity of one lateral coil or the other, while leaving the field in the vicinity of the other relatively unperturbed (Figure 1.4). The strength of the magnetic anomaly that was generated exceeded that of the EMF by a factor of 3 or 15, depending on the experiment that was performed. Detailed characteristics of the specific magnetic anomalies designed and tested in the course of my research are described in Chapter 3 and Chapter 4.

1.2. Models of magnetoreception

While it has long been known that animals sense the EMF as well as magnetic anomalies and use the information to accomplish diverse tasks (see above), the underlying mechanisms of the magnetic sense are largely unknown. In this section, I will describe current models of magnetoreception. Both the properties of the EMF and the characteristics of magnetic nanoparticles were considered in the design of current magnetoreceptor models. As many animal species of diverse taxa sense, and behaviorally respond to the EMF, the magnetoreceptor models are correspondingly diverse. Below, I will outline the models of magnetoreception currently described in the literature, with a primary focus on honey bees.

There are two main types of magnetoreceptor models. Mechanical receptor models are based on interactions between magnetic nanoparticles and the EMF; chemical receptor models are based on chemical processes. Below, I will first describe mechanical magnetoreceptor models and then move on to chemical receptor models, which are more pertinent to birds.

1.2.1. Magnetoreception based on SPM particles

The first mechanical magnetoreceptor model is inspired by findings that honey bees possess SPM particles that are arranged as a rod and a ring beneath the second abdominal ganglion (Schiff and Canal, 1993). The corresponding magnetoreception model proposes that the rod and the ring track horizontal and vertical gradients, respectively, of the EMF through electromagnetic induction while bees cross magnetic flux lines during their flight. According to the model, these SPM particles induce very small electric fields in the dendrites of the second abdominal ganglion, while neuronal integration over space sums and enhances the neuronal signal. The magnetic induction and voltage gradient over cellular membranes then mediate the opening of ion channels, thus causing a depolarization of the nervous system (Figure 1.1Figure 1.5a).

The next two mechanical magnetoreceptor models (Hsu et al., 2007; Davila et al., 2003) are based on attraction or repulsion of membrane-bound SPM particles and consequential deformations of cell membranes. By bending cell membranes, mechano-sensitive ion channels can open and neuronal responses ensue.

The model by Hsu et al. (2007) proposes that SPM iron granules in trophocytes of honey bees contribute to the bees' magnetoreceptive ability. Oriented side-by-side, the SPM particles repel each other and expand the iron granules. But oriented end-to-end, the SPM particles attract each other and contract the iron granules. As SPM particles align themselves with the external magnetic field, the direction of the EMF relative to the iron granules creates an expansion or a contraction of iron granules. Consequently, the cytoskeleton being associated with the trophocytes expands or contracts with the iron granules, acting as a signal, magnified by gap junctions that trigger the release of synaptic vesicles, thus initiating neural responses (Figure 1.5B).

1.2.2. Magnetoreception based on single-domain ferromagnetic particles

Yet another mechanical magnetoreceptor model is based on single-domain ferromagnetic particles. When an external magnetic field, like the EMF, acts on single domain (SD) ferromagnetic particles (≥ 30 nm in size), these particles track the direction of the magnetic field independent of an animal's orientation to the EMF. This mechanism is comparable to the movement of a compass needle responding to the EMF.

According to this model, SD ferromagnetic particles are arranged in a chain that is embedded in a cellular membrane and rotates freely within cells (Figure 1.5D). Cytoskeleton filaments are attached to the chain and coupled to ion channels in the cell membrane. The specific local arrangement of chains within cells makes the chains more or less sensitive to certain directions of external fields. A chain will experience maximum torque when it is oriented perpendicular to an external magnetic field. Once a chain aligns itself with the field, the torque is transmitted to the cytoskeleton filaments, ion channels open and the cell membrane depolarizes (Kirschvink et al., 1992; Binhi, 2006; Michael M. Walker, 2008). Like a compass needle, this type of receptor is sensitive to magnetic polarity. It occurs in magnetotactic bacteria which possess magnetosome chains of 15 to 20 magnetite crystals that act together like a compass needle, allowing these bacteria to orient according to the geomagnetic fields in search for anaerobic environments (Blakemore, 1975; Frankel et al., 1979).

In a slightly different model of SD ferromagnetic-based magnetoreception, relatively large and slightly elongated SD ferromagnetic particles are wrapped with an

organic insulator such as myelin, and rotate freely in the thickened membrane of a nerve cell (Kirschvink, 1981) (Figure 1.5E). While an SD particle aligns itself with an external magnetic field, the membrane resistance changes as a function of the angle between a particle and the membrane, thereby “passively depolarizing the membrane (Kirschvink, 1981). This model is indifferent to the polarity of the EMF, and is not sensitive to alternating magnetic fields.

All models outlined above are discussed in recent reviews (Wajnberg et al., 2010; Winklhofer & Kirschvink, 2010).

1.2.3. Chemical-based magnetoreception

The chemical magnetoreception model is based on photon-induced productions of radical pairs in photosensitive flavoproteins such as cryptochrome (CRY) (Figure 1.6), and is proposed for birds (Schulten, 1978; Ritz et al., 2000). The model assumes that photosensitive proteins absorb photons of specific wavelength, and function as electron transfer agents. The electron spin of a radical pair has a small magnetic moment that is affected by the EMF and that creates two radical states, the singlet state and the triplet state. The EMF alters the rate and the proportion of these two biochemically distinct states depending on the orientation of the radical molecule within the EMF (Ritz et al., 2000).

Photon absorption is a crucial first step toward magnetoreception. The absorption is wavelength dependent, with green and blue light having the appropriate energy for the magneto-receptive process (Wiltschko et al., 1993, 2001; Thalau et al., 2005). With CRY residing in the eyes of birds, the birds are thought “to see” the EMF.

The proportion of singlet and triplet states is dependent on the alignment of radical pairs relative to the EMF, rendering the receptor sensitive to the inclination of the EMF but insensitive to the polarity of the EMF. Therefore, animals that possess a chemical magnetoreceptor can discriminate between poleward and equatorward (Ritz et al., 2000, 2010).

Oscillating magnetic fields (7 MHz) can be applied to isolate the chemical magnetoreceptor and render it dysfunctional (Thalau et al., 2005). The effect can then

be tested (confirmed) in behavioral bioassays. Magneto-acoustic exposure may have similar effects on ferromagnetic materials.

1.3. Magnetic map and compass principle

Magnetoreceptors enable animals to sense different parameters of the EMF, thus facilitating orientation and navigation within home and migratory ranges. Below, I will introduce the concept of the map and compass principle, which will help explain how animals may use the EMF.

Various animals sense and make use of the EMF, exploiting it as a compass cue during long-distance navigation. Some animals do not migrate over long distances but can still sense the EMF. Among these are amphibians (Phillips, 1977), chickens (Wiltschko et al., 2007), mammals (Marhold et al., 1997), mollusks (Lohmann and Willows, 1987), crustaceans (Lohmann et al., 1995), and insects (Wajnberg et al., 2010). These animals might use magnetic information about their environments to build magnetic maps for orientation.

Geomagnetic parameters such as the inclination angle and the field intensity provide a sense of “magnetic latitude” (map) (Wiltschko and Wiltschko, 2003), allowing migrating animals to gauge their current position relative to their final destination (compass sense) (Lohmann et al., 2008). For example, animals migrating north from the magnetic equator to the magnetic pole encounter progressively steeper inclination angles or progressively stronger magnetic fields, allowing them to derive some kind of positional information from one or both parameters (Lohmann and Lohmann 1994, 1996; Phillips et al., 2002). Furthermore, the ability of some animals including European robins (Wiltschko and Wiltschko, 1972), African mole rats (Marhold et al., 1997), cetaceans (Klinowska 1985, 1988; Kirschvink, 1986;) and bees (Walker and Bitterman, 1989b) to sense subtle deviations from “regular” geomagnetic field characteristics allows them to learn the “magnetic topography” of their home range, which may facilitate orientation towards specific resources such as nesting or foraging sites (Lohmann et al., 2012; Thalau et al., 2007).

All parameters of the geomagnetic field provide a wealth of directional and positional information to both migrating and non-migrating animals and thus may

overwhelm the capacity of a single receptor system. Therefore, it was suggested that some animals, such as birds, rely on two separate receptors (“a gaussmeter in the beak and a compass in the eye”) that are based on different sensory mechanisms (Wiltschko and Wiltschko, 2006)

Furthermore, a “magnetic map” within the home range of an animal can facilitate local orientation and function independently from visual orientation cues such as specific landmarks, the position of sun or star patterns, which are not always detectable. The magnetic map is thought to be an alternative representation of the landscape, cataloguing differences in magnetic properties such as intensity and inclination. Magnetic anomalies represent landmarks within these maps.

While animals might use the EMF as a compass for long-distance orientation, as a map for within-home-range navigation, or for both, their magnetoreceptors could read and be sensitive to different types of information associated with the EMF. While some animals are not sensitive to the polarity of the EMF, they are sensitive to the inclination of the EMF, which is the angle of EMF flux lines with respect to ground. I will explain below how these data were collected and interpreted.

1.3.1. Polarity compass vs. inclination compass

The question whether migrating animals sense the inclination of magnetic field lines (inclination compass) or the polarity of the horizontal field component (polarity compass) was first and most rigorously studied with birds (Wiltschko and Wiltschko, 1972, 2002). Conclusive evidence that birds sense the inclination of magnetic field lines, rather than polarity, was generated by testing migratory birds in experimental settings where components of the magnetic field could be manipulated, typically by Helmholtz coil systems. Both the reversal of the horizontal field component (which reverses polarity) (Figure 1.7c), and the inversion of the vertical field component (which changes the angle of the inclination with respect to ground) (Figure 1.7d), caused the same adjustment of the birds’ migratory course, indicating that the avian magnetic compass does not perceive the polarity of the geomagnetic field (magnetic north or south). Instead, it recognizes “polewards” as the direction along the Earth’s surface in which the angle between the magnetic field vector and gravity is smallest (Wiltschko 1980; Figure

1.7), and “equatorwards” as the direction along the Earth’s surface in which the angle between the magnetic field vector and gravity is largest (Figure 1.7).

Inverting the horizontal component, however, alters the migratory direction or orientation behavior of those animals that possess a polarity sensitive compass or an inclination sensitive compass. Any manipulation of the vertical component does not have a behavior-modifying effect for animals that possess a polarity sensitive compass. Supporting evidence for polarity sensitivity can then be obtained by shifting the horizontal component of the field, and observing a corresponding shift in orientation behavior. On the basis of these types of experimental manipulations it has been demonstrated that sockeye salmon, *Oncorhynchus nerka* (Quinn, 1980), African mole rats (Marhold et al., 1997), and spiny lobsters (Lohmann et al., 1995) possess a polarity compass.

1.3.2. Magnetoreceptive vertebrates and invertebrates

Magnetoreception is best known for birds that use the EMF as an orientation cue during migration. However, there are many non-migratory animals including insects that are highly sensitive to magnetic fields. Table 1 summarizes current knowledge about magneto-sensitive taxa, their type of magnetoreceptors, and the biological function of their magnetoreceptive ability.

Table 1-1 Summary of magneto-sensitive taxa, their type of magnetoreceptor [magnetite-based (Mag.) or chemical-based (Chem.); polarity-sensitivity (Pol.) or inclination-sensitivity (Incl.)], dependence or not on light, and their biological function (e.g., orientation) if known.

Taxa	Species	Mag./ Chem.	Light dependence	Pol./ Incl.	Function	Ref.
Bacteria	Aquaspirillum magnetotacticum	Mag.	No	Pol.	Orientation	1
Insects	Vinegar flies	Chem.	Yes		Circadian rhythm	2
	Monarch butterfly	Chem.	Yes	Pol.	Orientation	3
	Ants Solenopsis sp.	Mag.	No	Pol.	Orientation	4
	Honey bees	Mag.	No	Pol.	Orientation/ navigation	5,6
Crustaceans	Spiny lobster	Mag.	Yes/No	Pol.	Homing behavior	7
Elamobranch	Sharks	?	No	?	Orientation	8
Amphibians	Red-spotted newt	?	Yes	Pol./ Incl.	Orientation	9
Reptiles	Sea turtle	Mag./ Chem.	No	Incl.	Orientation/ Navigation	10,11
Fish	Salmon	Mag.	No?	Pol.	Spontaneous Orientation	12,13
	Rainbow trout	Mag.	No	Pol.	Navigation/ Orientation	14,15
	Eels	?	?	Pol.?	Orientation	16
Birds	European robins	Chem.	Yes	Incl.	Orientation	17
	Pigeons	Chem.	Yes	Incl.	Navigation	18
	Chicken	Chem.	Yes	Incl.	Orientation	19
	Australian silveryeye	Chem.	Yes	Incl.	Orientation	20
Mammals	Brown bat	Mag.?	No	Pol.	Hanging position	21
	Mole rat	?	No	Pol.	Nest orientation	22
	Cetaceans	?	No	?	Orientation	23

1 = (Blakemore, 1975); 2 = (Yoshii et al., 2009); 3 = (Guerra et al., 2014); 4 = (Sandoval et al., 2012); 5 = (Lambinet et al., 2017 and Lambinet et al., 2017); 6 = (De Jong, 1982); 7 = (K Lohmann et al., 1995); 8 = (Kalmijn 1982) 9 = (Phillips, 1986; Phillips and Borland, 1992); 10 = (Lohmann, 1991; Lohmann and Lohmann, 1996); 11 = (Lohmann and Lohmann, 1993) ; 12 = (Quinn, 1980); 13 = (Quinn et al., 1981); 14 = (Diebel et al., 2000); 15 = (Walker et al., 1997); 16 = (Souza et al., 1988); 17 = (Wiltschko and Wiltschko, 1972); 18 = (Walcott and Green, 1974); 19 = (Wiltschko et al., 2007); 20 = (Wiltschko et al., 1993); 21 = (Wang et al., 2007); 22 = (Burda et al., 1990; Marhold et al., 1997); 23 = (Klinowska 1985)

1.4. Magnetoreception in honey bees

In this last section of my introductory chapter, I will focus on honey bees. I will report - in some detail - what is known about their magnetic sense, summarizing studies on magneto-sensitive behavior and describing the magnetic nanoparticles that were found in honey bees.

1.4.1. Honey bees sense magnetic fields

Honey bees exhibit sensitivity to the EMF in diverse contexts during their daily routines, including information conveyance. A forager bee communicates a potent food source to her hive mates by waggle dancing on a vertical comb within the hive. The directional information pointing to the location of a food source is encoded in the angle between two lines, the waggle run line (the straight portion of her dance) and gravity (K. V. von Frisch, 1948). The directional information encoded in this angle may be 15° to 25° off the true location of the food source, a phenomenon termed (residual) misdirection (K. V. von Frisch, 1948). The cause for misdirections is not known but there is evidence that misdirections are correlated with various parameters of the EMF (Lindauer and Martin, 1968; Martin and Lindauer, 1977a). For example, misdirections disappear when the EMF is experimentally reduced to $\leq 5\%$ (2.3 μT), or when the waggle run line accords with the inclination of the EMF (Lindauer and Martin, 1968). Furthermore, the number of sound pulses produced by a waggle dancing bee during her waggle run line encodes the distance to a potent food source; over the course of a day, variations in sound pulses that encode the distance to a specific food source correlate with both the time when misdirections appear and the diel increase of the EMF's intensity (Kilbert, 1979).

Furthermore, as already mentioned above, deprived of any external cues other than the ambient EMF, freshly swarmed bees align their combs in the new hive according to the comb alignment in the parent hive (De Jong, 1982). They also adjust comb alignments according to changes in the declination of the EMF. Moreover, bees are sensitive to directional information provided by the EMF, and make reference to magnetic directions rather than visual landmarks when put in conflict and when reference to the sun's position is not possible (Frier et al., 1996).

Evidence that honey bees recognize magnetic fields during foraging was shown in an elegant series of two-choice experiments (Walker and Bitterman, 1985; Walker and Bitterman, 1989a, b). In these experiments, bees were trained to associate a magnetic anomaly with a sucrose solution, offering them two visually indistinguishable feeding (sucrose) stations and alternating the location of the anomaly between these stations in consecutive replicates. The experiments revealed (*i*) that moving bees, but not stationary bees, are capable of sensing magnetic anomalies (but see Liang et al., 2016 for a contrasting conclusion) with a minimum intensity of 260 nT (Walker and Bitterman, 1985; Walker and Bitterman, 1989a,b), and (*ii*) that magnetic particles in the anterodorsal abdomen of bees may function in magnetoreception (Walker and Bitterman, 1989a). Subsequent experiments showed further that bees are more sensitive to vertical than to horizontal magnetic field lines, and that some bees in a T-maze learned to associate magnetic north as a directional cue for exiting the maze and for returning to the hive (Kirschvink and Kobayashi-Kirschvink, 1991). Exposure of (three) bees to a 1-ms, 1000-G magnetic pulse apparently impaired the bees' magnetoreceptor and reversed their directional magnetic preference for exiting the maze (Kirschvink and Kobayashi-Kirschvink, 1991).

Behavioral evidence for a magnetic-based magnetoreceptor in bees was shown, using alternating magnetic fields with adjustable intensity and frequency (Kirschvink et al., 1997). Up to a frequency of 10 Hz, SD ferromagnetic particles physically track the applied magnetic field (or the EMF), but beyond 10 Hz the viscosity of cell fluid inhibits particle movement and the perception of magnetic fields subsides (Kirschvink et al., 1997). If the frequency increases beyond 10 Hz, the field intensity must increase for magnetic perception to occur. Recognition of a 60-Hz magnetic field requires a field intensity of 430 μ T (10 times the earth magnetic field) (Kirschvink et al., 1997).

1.4.2. Honey bees possess magnetic particles

Honey bees are good model organisms for locating and characterizing a magnetoreceptor because they (*i*) use the geomagnetic field for orientation during foraging (Frier et al., 1996) and for alignment of their combs (De Jong, 1982), (*ii*) detect small magnetic anomalies relative to the geomagnetic background (Walker & Bitterman, 1985), (*iii*) distinguish between magnetic anomalies in behavioral experiments (Kirschvink and Kobayashi-Kirschvink, 1991; Walker and Bitterman, 1989a, 1989b;

Walker and Bitterman, 1985; Walker et al., 1989), and (iv) can readily be obtained from large hives for laboratory analyses and field testing. At least five research groups have been working on magnetoreception in honey bees over the last four decades, in the process producing a vast amount of detailed information on magnetic nanoparticles in bees (for a summary see Wajnberg et al., 2010). Pioneering SQUID magnetometry experiments in the late-seventies with live and dead honey bees revealed that bees possess magnetic nanoparticles with natural and inducible remanence (Gould et al., 1978). The ongoing quest to “find the needle in the haystack” (Johnsen and Lohmann, 2008), the needle being the magnetoreceptor of honey bees (or other animals), generated evidence that honey bees have magnetic nanoparticles of different sizes in their bodies.

These nanoparticles include 15 million SD ferromagnetic particles (particle size: 100 nm) (J L Gould et al., 1978), 2×10^8 SPM particles (particle size range: 30 -35 Å) as well as other nanoparticles ranging widely in size (Gould et al., 1980). Subjecting iron granules, extracted from bee trophocytes to hysteresis measurements by SQUID magnetometry revealed an intrinsic coercivity of 10–15 mT, indicative of magnetically soft magnetic particles in a single domain SPM state (Hsu et al., 2007). Furthermore, studying thermal decay of remanence, Desoil et al. (2005) conclude that honey bees possess SD magnetic particles (size: > 30 nm) in their abdomens, and SPM particles in their heads and thoraces. Magnetic coercivity in bees was also reported by Ferreira et al. (2005) who conducted measurements with various social insects.

Temperature-dependent (5 K to 310 K) hysteresis measurements, with SQUID fields applied parallel or perpendicular to bee abdomens, revealed that the energetically favorable direction (“easy axis”) of spontaneous magnetization of magnetic material is parallel to the bees’ body axis (Esquivel et al., 2002). The magnetic material analyzed in this study was inferred to be magnetite, as the “Verway transition” (the temperature above which SPM particles act like permanent magnets) was determined to be 120 K (Desoil et al., 2005).

Wajnberg et al. (2001) report induced remanent magnetizations of honey bee abdomens, with SPM particles in abdomens measuring < 30 nm in size. In previous reports, magnetic moments of nanoparticles vary between test samples, and are affected by e. g., the location of the bee hive from which the sample(s) originated, the

water content of samples, and the sample storage solution (El-Jaick et al., 2001; Chambarelli et al., 2008).

1.5. Overview of research chapters

My thesis consists of six chapters. Chapter 1 (this chapter) outlines my study area and describes the most pertinent background information for my study. Chapters 2-6 are research chapters that I present in article format. Chapter 2 (PLoS ONE), Chapter 3 (Proceedings of the Royal Society B) Chapter 4 (Journal of Comparative Physiology) and Chapter 5 (Review of Scientific Instruments) have already been published and Chapter 6 constitutes a concluding summary of my major findings. All research chapters are presented according to the format requirements of the target journal; they include an abstract, an introduction, methods, results, a discussion and a list of references. Below, I will outline the content of each chapter.

In Chapter 2, I study a potential function of the honey bees' magnetoreceptor in the context of their waggle dance, a unique in-hive communication system of bees. A forager bee returning to the hive performs a waggle dance on a vertical comb to inform her hive mates about a potent food source she has just found and to recruit them to its location. She dances a repeated figure-8 pattern interspersed by a short straight segment, the "waggle run". Directional information pointing to the food source relative to the sun's azimuth is encoded in the angle between the waggle run line and a reference line, which is generally thought to be established by gravity. Yet, there is tantalizing evidence that the local (ambient) geomagnetic field (LGMF) could play a role. I tested the effect of the LGMF on the recruitment success of forager bees by placing observation hives inside large Helmholtz coils, and then either reducing the LGMF to 2% or shifting its apparent declination. My data show that neither of these two treatments reduced the number of nest mates that waggle dancing forager bees recruited to an experimental feeding station located 200 m north of the hive. My results support the conclusion that the LGMF does not act as the reference for the alignment of waggle-dancing bees and that the magnetic sense of bees must play a role in a context other than information conveyance during the waggle dance.

In Chapter 3, I investigate the magnetic sense of honey bees. Previous studies of magnetoreception in honey bees have aimed at identifying the magnetic material, the

location of the magnetoreceptor, and potential underlying sensory mechanisms. A major contribution of my work in Chapter 3 is that I directly link the bees' magnetic material to a magnetoreceptive function. In laboratory and field experiments, I show that ferromagnetic material consistent with magnetite plays an integral role in the bees' magnetoreceptor. I lyophilized and pelletized bee tagmata and subjected tagma samples to analyses by a Superconducting Quantum Interference Device (SQUID). These analyses revealed a distinct hysteresis loop for the abdomen but not for the thorax or the head of bees, indicating the presence of ferromagnetic material in the bee abdomen. Moreover, I could show that magnetic remanence of abdomen pellets produced from bees that I did, or did not, expose to a 2.2 kOe field of a magnet while alive differed, indicating that magnet-exposure altered the magnetization of this magnetite in live bees. In behavioral two-choice field experiments, I then demonstrate that the bees, which I had briefly exposed to the same magnet, but not the sham-treated control bees, failed to sense a custom-generated magnetic anomaly. These results indicate that magnet-exposure had rendered the bees' magnetoreceptor dysfunctional. My data support the conclusion that honey bees possess a ferromagnetite-based magnetoreceptor located in the abdomen.

In Chapter 4, I study whether honey bees sense the inclination of magnetic field lines or the polarity of magnetic fields. I built on the results of Chapter 3 that honey bees possess magnetite in their abdomen and have a magnetite-based magnetoreceptor. In general, this type of receptor is thought to be sensitive to magnetic field polarity. Accordingly, I tested the hypothesis that honey bees sense the polarity of magnetic fields. With support from Dr. Michael Hayden, I designed a magnetic anomaly that crudely resembles the dipole field in the vicinity of one end of a bar magnet, analogous to the geomagnetic field near one of the poles. In behavioral field studies, I trained bees to associate this anomaly with a sugar water reward. I then subjected trained bees in random order to a control or a treatment experiment. In each experiment, I presented a sugar water reward in two separate watch glasses, placing one reward in the center of the anomaly that I either kept the same as during bee training (control experiment) or that I altered by reversing its polarity (treatment experiment). My prediction was that bees would fail to respond to the altered anomaly, if they were sensitive to the polarity of the magnetic field. I found that bees continued to respond to the magnetic anomaly when its polarity was kept unaltered, but failed to respond to the anomaly when its

polarity was reversed. These results support the hypothesis that honey bees possess a polarity-sensitive magnetoreceptor.

In Chapter 5 (with strong support by Dr. Michael Hayden and Surath Gomis), I describe and evaluate a new method for extracting magnetic moments from SQUID magnetometry data that is particularly well suited to experiments in which one is searching for very weak magnetic signatures. Typically, one fits a model function of the SQUID response as a function of sample position to the acquired data. The conventional fitting function works well for samples ≤ 0.5 cm in length, but fails for longer samples. Since biological samples such as bee tagmata often contain little magnetite with small magnetic moments, it is advantageous to employ as much analyte in each sample as possible to increase the amplitude of the detected signal. Therefore, we derived and applied a fitting function that explicitly accounts for sample size and can be applied to elongated samples of arbitrary length. This methodology was employed in the work described in Chapter 2, but it is much more generally applicable to problems in magnetometry as long as sufficient quantities of analyte are available.

In Chapter 6, I summarize my most important findings and comment on their implications.

1.6. References

- Binhi, V. N. (2006). Stochastic dynamics of magnetosomes and a mechanism of biological orientation in the geomagnetic field. *Bioelectromagnetics*, 27, 58–63.
- Blakemore, R. (1975). Magnetotactic bacteria. *Science*, 190(4212), 377–379.
- Burda, H., Marhold, S., Westenberger, T., Wiltshko, R., & Wiltshko, W. (1990). Magnetic compass orientation in the subterranean rodent *Cryptomys hottentotus* (Bathergidae). *Experientia*, 46, 528–530.
- Chambarelli, L. L., Pinho, M. a., Abraçado, L. G., Esquivel, D. M. S., & Wajnberg, E. (2008). Temporal and preparation effects in the magnetic nanoparticles of *Apis mellifera* body parts. *Journal of Magnetism and Magnetic Materials*, 320, 207–210.
- Chapman, S. (1918). An outline of a theory of magnetic storms. *Royal Society of London*, 95(666), 61–83.

- De Jong, D. (1982). Orientation of comb building by honeybees. *Journal of Comparative Physiology A*, 147, 495–501.
- Desoil, M., Gillis, P., Gossuin, Y., Pankhurst, Q., & Hautot, D. (2005). Definitive identification of magnetite nanoparticles in the abdomen of the honeybee *Apis mellifera*. *Journal of Physics: Conference Series*, 17, 45–49.
- Diebel, C. E., Proksch, R., Green, C. R., Neilson, P., & Walker, M. M. (2000). Magnetite defines a vertebrate magnetoreceptor. *Nature*, 406(6793), 299–302.
- El-Jaick, L. J., Acosta-Avalos, D., De Souza Esquivel, D. M., Wajnberg, E., & Linhares, M. P. (2001). Electron paramagnetic resonance study of honeybee *Apis mellifera* abdomens. *European Biophysics Journal*, 29, 579–586.
- Esquivel, D. M. S., Wajnberg, E., Cernicchiaro, G. R., Acosta-Avalos, D., & Garcia, B. (2002). Magnetic material arrangement in *Apis mellifera* abdomens. *Materials Research Society Symposium Proceedings*, 724, 3–6.
- Ferreira, J., Cernicchiaro, G., Winklhofer, M., Dutra, H., De Oliveira, P. S., Esquivel, D. M. S., & Wajnberg, E. (2005). Comparative magnetic measurements on social insects. *Journal of Magnetism and Magnetic Materials*, 289, 442–444.
- Frankel, R. B., Blakemore, R. P., & Wolfe, R. S. (1979). Magnetite in freshwater magnetotactic bacteria. *Advancement Of Science*, 203(4387), 1355–1356.
- Frier, H., Edwards, E., Smith, C., Neale, S., & Collett, T. (1996). Magnetic compass cues and visual pattern learning in honeybees. *The Journal of Experimental Biology*, 199, 1353–61.
- Gould, J. L., Kirschvink, J. L., & Deffeyes, K. S. (1978). Bees have magnetic remanence. *Science (New York, N.Y.)*, 201(4360), 1026–1028.
- Gould, J. L., Kirschvink, J. L., & Deffeyes, K. S. (1980). Orientation of demagnetized bees. *The Journal of Experimental Biology*, 86, 1–8.
- Gould, J. L. (1984). Magnetic sensitivity in animals. *Annual Review Physiology*, 46, 585–98.
- Guerra, P. A., Gegear, R. J., & Reppert, S. M. (2014). A magnetic compass aids monarch butterfly migration. *Nature Communications*, 5(May), 4164.
- Hautot, D., Pankhurst, Q. A., & Dobson, J. (2005). Superconducting quantum interference device measurements of dilute magnetic materials in biological samples. *Review of Scientific Instruments*, 76, 1–5.
- Hsu, C., & Li, C. (1993). The ultrastructure and formation of iron granules in the honeybee (*Apis mellifera*). *Journal of Experimental Biology*, 180, 1–13.

- Hsu, C.-Y., Ko, F.-Y., Li, C.-W., Fann, K., & Lue, J.-T. (2007). Magnetoreception system in honeybees (*Apis mellifera*). *PloS One*, 2(4), e395.
- Johnsen, S., & Lohmann, K. J. (2008). Magnetoreception in animals. *Physics Today*, 61(3), 29–35.
- Kalmijn AD (1982) Electric and magnetic field detection in Elasmobranch fishes. *Science* 218(4575) 916-918
- Kilbert, K. (1979). Geräuschanalyse der Tanzlaute der Honigbiene (*Apis mellifica*) in unterschiedlichen magnetischen Feldsituationen. *Journal of Comparative Physiology A*, 132, 11–25.
- Kirschvink, J. L. (1981). The horizontal magnetic dance of the honeybee is compatible with a single-domain ferromagnetic magnetoreceptor. *BioSystems*, 14, 193–203.
- Kirschvink, J. L. (1981). Biogenic magneite as a basis for magnetic field detection in animals. *BioSystems*, 13(3), 181-201
- Kirschvink, J. L., & Kobayashi-Kirschvink, A. (1991). Is geomagnetic sensitivity real? Replication of the Walker-Bitterman magnetic conditioning experiment in honey bees. *Integrative and Comparative Biology*, 31, 169–186.
- Kirschvink, J. L., Kuwajima, T., Ueno, S., Kirschvink, S. J., Diaz-Ricci, J., Morales, A., Barwig S., Quinn, K. J. (1992). Discrimination of low-frequency magnetic fields by honeybees: biophysics and experimental tests. In *Society of General Physiologists series* 47, 225–240.
- Kirschvink, J., Padmanabha, S., Boyce, C., & Oglesby, J. (1997). Measurement of the threshold sensitivity of honeybees to weak, extremely low-frequency magnetic fields. *The Journal of Experimental Biology*, 200, 1363–8.
- Kirschvink JL, Dizon AE, Westphal JA (1986) Evidence from strandings for geomagnetic sensitivity in cetaceans. *Journal of Experimental Biology* 120, 1-24
- Klinowska M (1985) Cetacean live stranding sites relate to geomagnetic topography. *Aquatic Mammals* 1, 27-32
- Klinowska M (1988) Cetacean 'navigation' and the geomagnetic field. *Journal of Navigation* 41(1), 52-71
- Kuterbach, D. A, & Walcott, B. (1986). Iron-containing cells in the honey bee (*Apis mellifera*) I. Adult Morphology and Physiology. *Journal of Experimental Biology*, 126, 375–387.
- Lambinet V, Hayden ME, Reigel K, Gomis S, Gries G. (2017) Linking magnetite in the abdomen of honey bees to a magnetoreceptive function. *Proc R Soc B* 284: 20162873.

- Lambinet V, Hayden ME, Reid C, Gries G. (2017) Honey bees possess a polarity-sensitive magnetoreceptor. *J Comp Physiol A*
- Liang, C.-H., Chuang, C.-L., Jiang, J.-A., & Yang, E.-C. (2016). Magnetic sensing through the abdomen of the honey bee. *Scientific Reports*, 6, 1–7.
- Liboff, A. R. (2013). Why are living things sensitive to weak magnetic fields? *Electromagnetic Biology and Medicine*, 8378, 1–5.
- Lindauer, M., & Martin, H. (1968). Die Schwereorientierung der Bienen unter dem Einfluss des Erdmagnetfeldes. *Zeitschrift für Vergleichende Physiologie*, 60, 219–243.
- Lindauer, M., (1976). Recent advances in the orientation and learning of honey bees. *Proceedings of the 25th International Congress of Entomology* 25, 450-60
- Lohmann, K. J., & Willows, A. O. D. (1987). Lunar-modulated geomagnetic orientation by a marine mollusk. *Science* AAAs, 235(4786), 331–334.
- Lohmann, K. J. (1991). Magnetic orientation by hatchling loggerhead sea turtles (*Caretta caretta*). *The Journal of Experimental Biology*, 155, 37–49.
- Lohmann, K., & Lohmann, C. (1993). A light-independent magnetic compass in the leatherback sea turtle. *The Biological Bulletin*, 185(5), 149–151.
- Lohmann, K. J., & Lohmann, C. M. F. (1994). Detection of magnetic inclination angle by sea turtles: A possible mechanism for determining latitude. *The Journal of Experimental Biology*, 194, 23–32.
- Lohmann, K., Pentcheff, N., Nevitt, G., Stetten, G., Zimmer-Faust, R., Jarrard, H., & Boles, L. (1995). Magnetic orientation of spiny lobsters in the ocean: experiments with undersea coil systems. *The Journal of Experimental Biology*, 198(Pt 10), 2041–2048.
- Lohmann, K. J., & Lohmann, C. M. F. (1996). Detection of magnetic field intensity by sea turtles. *Nature*, 380(6569), 59–61.
- Lohmann, K. J., Putman, N. F., & Lohmann, C. M. F. (2008). Geomagnetic imprinting: A unifying hypothesis of long-distance natal homing in salmon and sea turtles. *PNAS*, 105, 19096–19101.
- Lohmann, K. J., Putman, N. F., & Lohmann, C. M. F. (2012). The magnetic map of hatchling loggerhead sea turtles. *Current Opinion in Neurobiology*, 22(2), 336–342.
- Marhold, S., Wiltschko, W., & Burda, H. (1997). A magnetic polarity compass for direction finding in a subterranean mammal. *Naturwissenschaften*, 84, 421–423.

- Martin, H., & Lindauer, M. (1977). Der Einfluss des Erdmagnetfeldes auf die Schwereorientierung der Honigbiene (*Apis mellifera*). *Journal of Comparative Physiology A*, 122, 145–187.
- Phillips, J. B. (1977). Use of the earth's magnetic field by orienting cave salamanders (*Eurycea lucifuga*). *Journal of Comparative Physiology A*, 121, 273–288.
- Phillips, J. B. (1986). Magnetic compass orientation in the Eastern red-spotted newt (*Notophthalmus viridescens*). *Journal of Comparative Physiology A*, 158, 103–109.
- Phillips, J. B., & Borland, S. C. (1992). Behavioural evidence for use of a light-dependent magnetoreception mechanism by a vertebrate. *Nature*, 359, 142–144.
- Phillips, J. B., Freake, M. J., Fischer, J. H., & Borland, S. C. (2002). Behavioral titration of a magnetic map coordinate. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology*, 188, 157–160.
- Quinn, T. P. (1980). Evidence for celestial and magnetic compass orientation in lake migrating sockeye salmon fry. *Journal of Comparative Physiology A*, 137, 243–248.
- Quinn, T. P., Merrill, R. T., & Brannon, E. L. (1981). Magnetic field detection in sockeye salmon. *Journal of Experimental Zoology*, 217, 137–142.
- Ritz, T., Adem, S., & Schulten, K. (2000). A model for photoreceptor-based magnetoreception in birds. *Biophysical Journal*, 78, 707–718.
- Ritz, T., Ahmad, M., Mouritsen, H., Wiltschko, R., & Wiltschko, W. (2010). Photoreceptor-based magnetoreception: optimal design of receptor molecules, cells, and neuronal processing. *Journal of the Royal Society, Interface*, 7, S135–46.
- Sandoval, E. L., Wajnberg, E., Esquivel, D. M. S., de Barros, H. L., & Acosta-Avalos, D. (2012). Magnetic orientation in *solenopsis* sp. ants. *Journal of Insect Behavior*, 25(6), 612–619.
- Schiff, H., & Canal, G. (1993). The magnetic and electric fields induced by superparamagnetic magnetite in honeybees Magnetoperception: an associative learning? *Biological Cybernetics*, 69, 7–17.
- Schulten, K. (1978) A mechanism for the light-driven proton pump of *Halobacterium halobium*. *Nature* 272(2), 85–86
- Souza, J. J., Poluhowich, J. J., & Guerra, R. J. (1988). Orientation responses of American eels, *Anguilla rostrata*, to varying magnetic fields. *Comparative Biochemistry and Physiology. A, Comparative Physiology*, 90A, 57–61.

- Thalau, P., Ritz, T., Stapput, K., Wiltschko, R., & Wiltschko, W. (2005). Magnetic compass orientation of migratory birds in the presence of a 1.315 MHz oscillating field. *Naturwissenschaften*, 92(2), 86–90.
- Thalau, P., Holtkamp-Rötzler, E., Fleissner, G., & Wiltschko, W. (2007). Homing pigeons (*Columba livia f. domestica*) can use magnetic cues for locating food. *Naturwissenschaften*, 94(10), 813–819.
- Von Frisch, K. (1948). Gelöste und ungelöste Rätsel der Bienensprache. *Die Naturwissenschaften*, Heft 2, 38–43.
- Wajnberg, E., Cernicchiaro, G., Acosta-Avalos, D., El-Jaick, L. ., & Esquivel, D. M. (2001). Induced remanent magnetization of social insects. *Journal of Magnetism and Magnetic Materials*, 226–230, 2040–2041.
- Wajnberg, E., Acosta-Avalos, D., Alves, O. C., de Oliveira, J. F., Srygley, R. B., & Esquivel, D. M. S. (2010). Magnetoreception in eusocial insects: an update. *Journal of the Royal Society, Interface*, 7(January), S207–S225.
- Walcott, C., & Green, R. P. (1974). Orientation of homing pigeons altered by a change in the direction of an applied magnetic field. *Science (New York, N.Y.)*, 184(4133), 180–182.
- Walker, M. M., & Bitterman, M. E. (1985). Conditioned responding to magnetic-fields by honeybees. *Journal of Comparative Physiology A-Sensory Neural and Behavioral Physiology*, 157, 67–71.
- Walker, M. M., Baird D. L., & Bitterman M. E., (1989). Failure of stationary but not of flying honeybees to respond to magnetic field stimuli. *Journal of Comparative Physiology*, 103, 62–69.
- Walker, M., & Bitterman, M. (1989a). Attached magnets impair magnetic field discrimination by honeybees. *Journal of Experimental Biology*, 141, 447–451.
- Walker, M., & Bitterman, M. (1989b). Honeybees can be trained to respond to very small changes in geomagnetic field intensity. *Journal of Experimental Biology*, 145(1989), 489–494.
- Walker, M. M., Diebel, C. E., Haugh, C. V, Pankhurst, P. M., Montgomery, J. C., & Green, C. R. (1997). Structure and function of the vertebrate magnetic sense. *Nature*, 390(6658), 371–376.
- Walker, M. M. (2008). A model for encoding of magnetic field intensity by magnetite-based magnetoreceptor cells. *Journal of Theoretical Biology*, 250, 85–91.
- Wang, Y., Pan, Y., Parsons, S., Walker, M., & Zhang, S. (2007). Bats respond to polarity of a magnetic field. *Proceedings of the Royal Society B: Biological Sciences*, 274, 2901–2905.

- Wiltschko, W., & Wiltschko, R. (1972). Magnetic compass of european robins. *Science, New Series*, 176(4030), 62–64.
- Wiltschko, W. (1980). The earth's magnetic field and bird orientation. *TINS*, 140–144.
- Wiltschko, W., Munro, U., Ford, H., & Wiltschko, R. (1993). Red light disrupts magnetic orientation of migratory birds. *Nature*, 364, 525–527.
- Wiltschko R., Wiltschko W., (1995). Magnetic orientation in animals. Springer-Verlag Berlin Heidelberg New York. ISBN 3-540-59257-1.
- Wiltschko, W., Gesson, M., & Wiltschko, R. (2001). Magnetic compass orientation of European robins under 565 nm green light. *Naturwissenschaften*, 88, 387–390.
- Wiltschko, W., & Wiltschko, R. (2002). Magnetic compass orientation in birds and its physiological basis. *Naturwissenschaften*, 89(10), 445–452.
- Wiltschko, R., & Wiltschko, W. (2003). Avian navigation: from historical to modern concepts. *Animal Behaviour*, 65, 257–272.
- Wiltschko, R., & Wiltschko, W. (2006). Magnetoreception. *BioEssays*, 28, 157–168.
- Wiltschko, W., Freire, R., Munro, U., Wiltschko, Ritz T., Rogers I., Thalau P., Wiltschko R., (2007). The magnetic compass of domestic chickens. *The Journal of Experimental Biology*, 210, 2300–2310.
- Winklhofer, M. (2010). Magnetoreception. *Journal of The Royal Society Interface*, 7, S131–S134.
- Winklhofer, M., & Kirschvink, J. L. (2010). A quantitative assessment of torque-transducer models for magnetoreception. *Journal of the Royal Society, Interface / the Royal Society*, 7(January), S273–S289.
- Yoshii, T., Ahmad, M., & Helfrich-Förster, C. (2009). Cryptochrome mediates light-dependent magnetosensitivity of *Drosophila*'s circadian clock. *PLoS Biology*, 7(4)

1.7. Figures

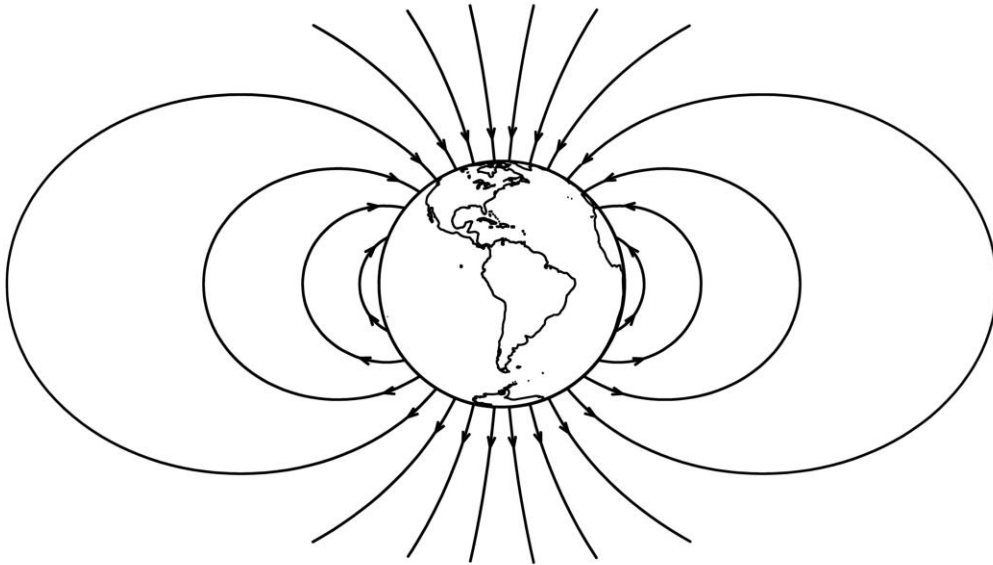


Figure 1.1. Approximate variation of the orientation of the Earth's field (black arrows) as a function of location.

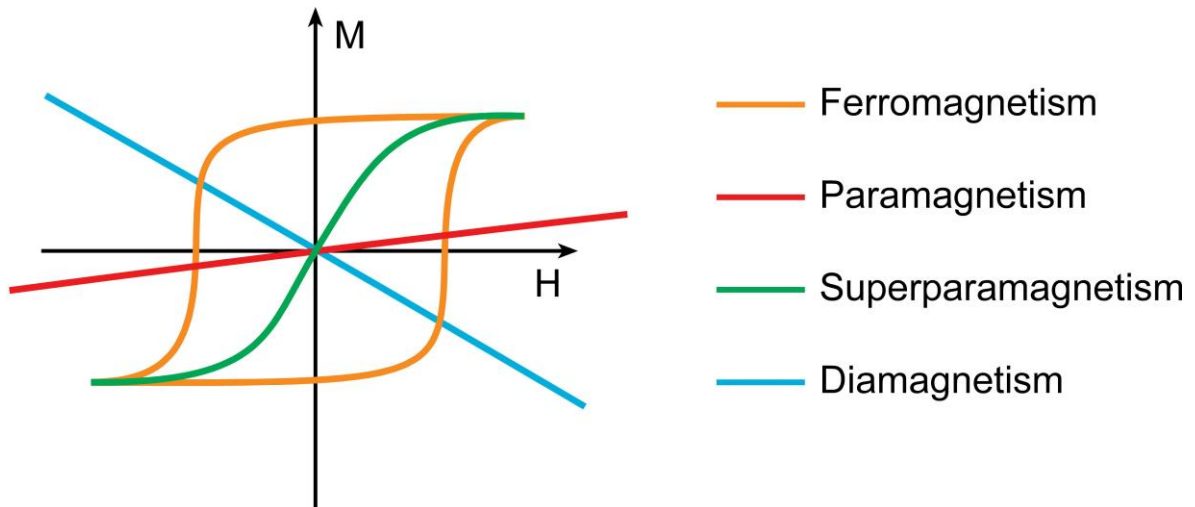


Figure 1.2. Characteristic hysteresis for single-domain ferromagnetic particles (orange curve), paramagnetic particles (red curve), superparamagnetic particles (green curve), and diamagnetism (blue line). H is the applied magnetic field in Oe (Oersted), whereas M is the sample moment (emu).



Figure 1.3. Triaxial Helmholtz coil system capable of cancelling or arbitrarily modifying the local geomagnetic field. Note the honey bee observation hive in the center of the three pairs of octagonal Helmholtz coils.

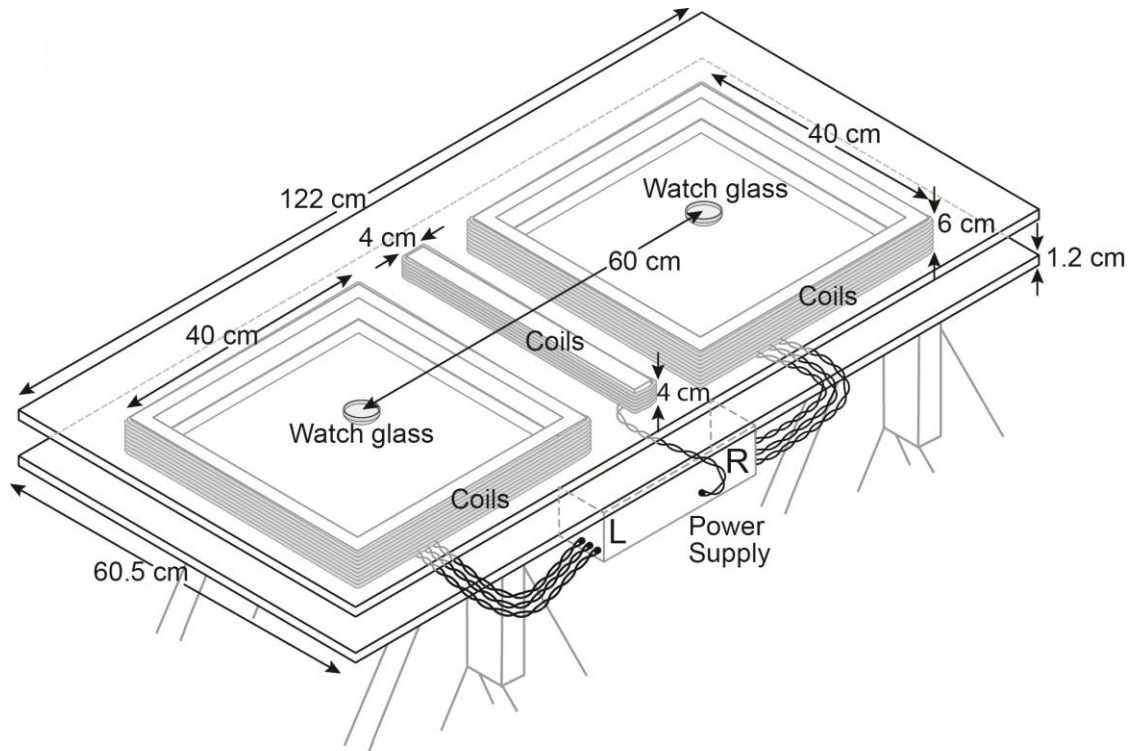


Figure 1.4. Two-choice bioassay table equipped with custom-built coils (sandwiched between plywood sheets) that were used to generate magnetic field anomalies in the vicinity of one watch glass or the other. Visits of number-tagged bees to watch glasses were scored as evidence for their (in)ability to sense magnetic anomalies.

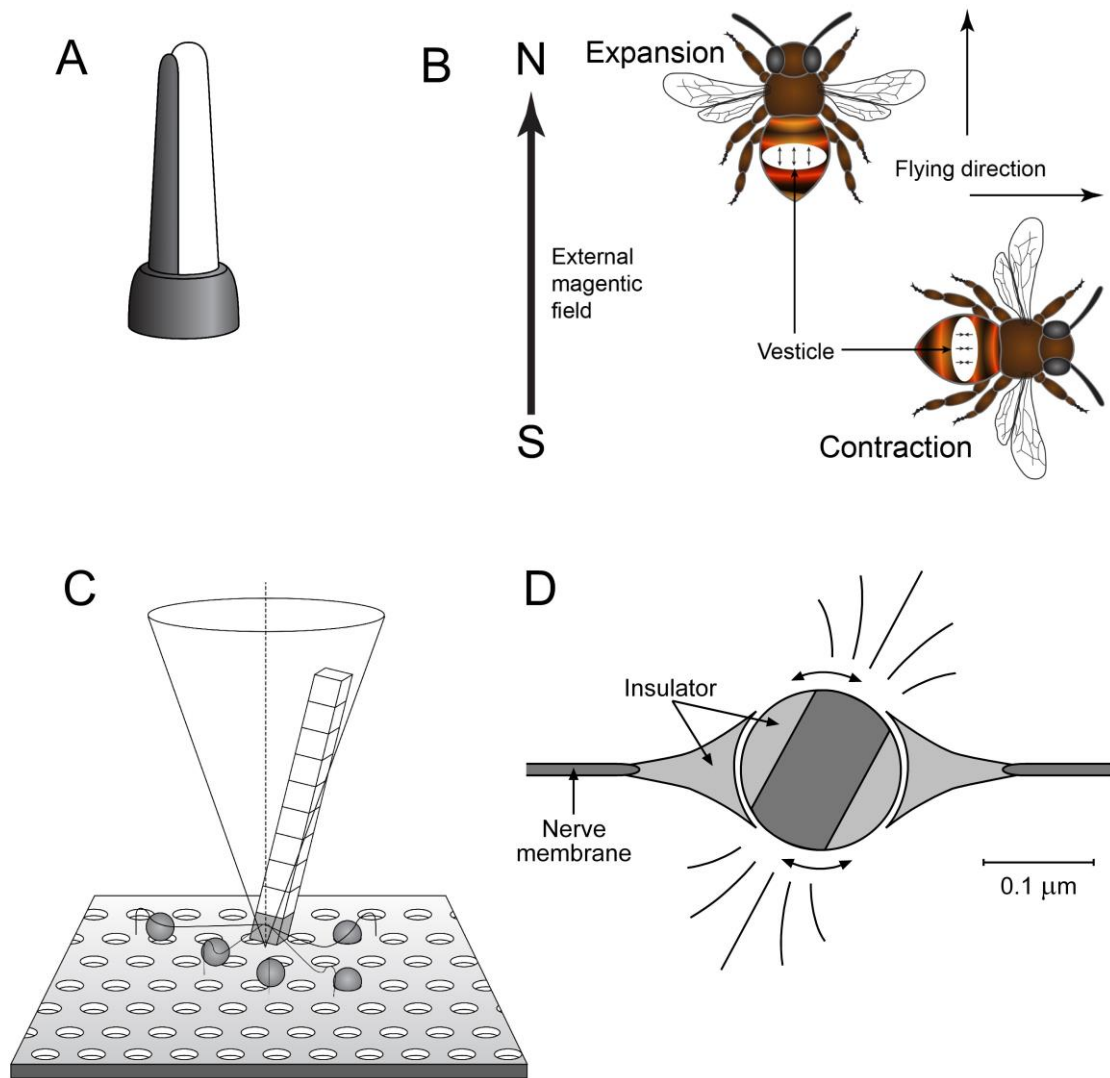


Figure 1.5. Graphical illustrations of proposed magnetoreceptor models: (A) superparamagnetic (SPM) particles arranged in a rod and ring structure (adapted from Schiff and Canal, 1993): Vertical and horizontal gradients of the Earth Magnetic Field (EMF) are tracked through electromagnetic induction, while bees cross magnetic flux lines during their flight. These SPM particles induce very small electric fields in dendrites of the second abdominal ganglion, which then mediate the opening of ion channels, thus causing a depolarization of the nervous system; (B) SPM iron granules in trophocytes (adapted from Kirschvink and Gould, 1981 Hsu and Li, 2007): As SPM particles align themselves with the EMF, the direction of the EMF to the iron granules causes an expansion or a contraction of iron granules. The cytoskeleton associated with the trophocytes expands or contracts with the iron granules, acting as a signal, magnified by gap junctions that trigger the release of synaptic vesicles, thus initiating neural responses; (C) Ferromagnetite-based magnetoreception model (adapted from Walker 2008): SD ferromagnetic particles are arranged in a chain that is embedded in a cellular membrane and rotates freely within the cell. Cytoskeleton filaments are attached to the chain and coupled to ion channels in the cell membrane. Once the chain aligns itself with an external field, the resulting torque is transmitted to the filaments, ion channels in the cell membrane open, and the cell membrane depolarizes, thus causing signal transduction; (D) slightly elongated SD ferromagnetic particles (adapted from Kirschvink, 1981): The particles are wrapped with an organic insulator such as myelin, and rotate freely in the thickened membrane of a nerve cell. While an SD particle aligns itself with an external magnetic field, the membrane resistance changes as a function of the angle between a particle and the membrane, thereby “passively” depolarizing the membrane. This model is indifferent to the polarity of the EMF, and is not sensitive to alternating magnetic fields.

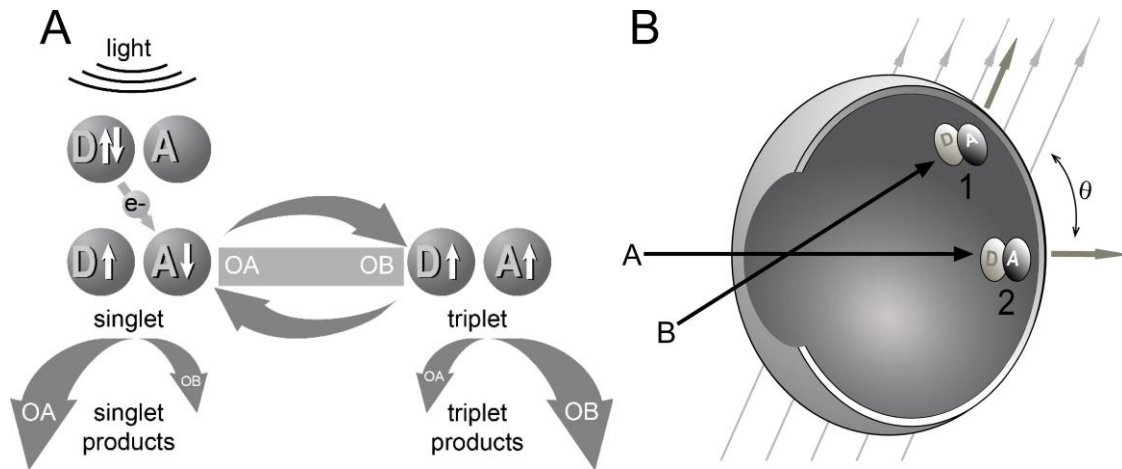


Figure 1.6. The chemical magnetoreception model (adapted from Ritz et al., 2000): Photosensitive flavoproteins such as cryptochrome (CRY) are believed to absorb photons of specific wavelength, and to function as e⁻ transfer agents. The e⁻ spin of a radical pair has a small magnetic moment that is affected by the Earth Magnetic Field (EMF) and that creates two radical states, the singlet state and the triplet state. The EMF alters the rate and the proportion of these two biochemically distinct states depending on the orientation of the radical molecule within the EMF. The proportion of singlet and triplet states is dependent on the radical pairs' alignment to the EMF, rendering the receptor sensitive to the inclination of the EMF but insensitive to the polarity of the EMF. With CRY residing in the eyes of birds, the birds are thought "to see" the EMF.

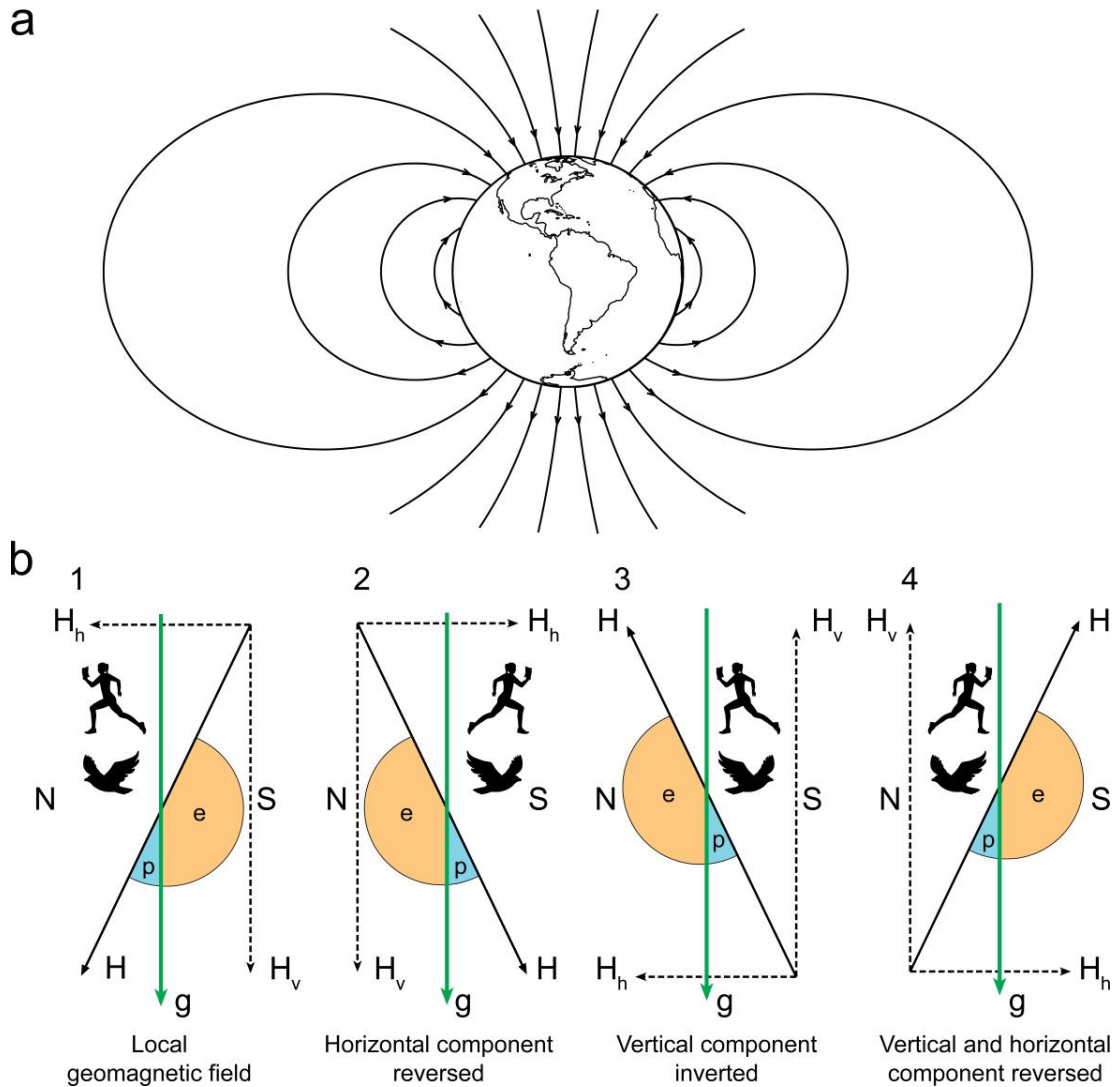


Figure 1.7. Gross features of the geomagnetic field are dipolar, and resemble the field of a (fictitious) centrally-located bar magnet, with field lines emerging from the Earth's surface in the southern hemisphere and re-entering in the northern hemisphere; (b) Graphical comparison of behavioral responses governed by an inclination compass and a polarity compass (adapted from Wiltschko and Wiltschko, 2005). The local magnetic field \vec{H} is decomposed into vertical (H_v) and horizontal (H_h) components, and the sense of the gravitational field is denoted by g . N and S indicate geographic North and South, while p and e denote poleward and equatorward, the readings of the inclination compass. The migration direction of the bird is poleward, which in this example corresponds to North when the field is unaltered. The direction chosen by the orienteer using a polarity compass (an ordinary magnetic compass) is the apparent magnetic north.

Chapter 2.

Does the Earth's Magnetic Field Serve as a Reference for Alignment of the Honeybee Waggle Dance?

A very similar version of this chapter has been published: Veronika Lambinet, Michael E. Hayden, Marco Bieri, Gerhard Gries. (2014) PLoS ONE 9(12): e115665.

2.1. Abstract

The honeybee (*Apis mellifera*) waggle dance, which is performed inside the hive by forager bees, informs hive mates about a potent food source, and recruits them to its location. It consists of a repeated figure-8 pattern: two oppositely directed turns interspersed by a short straight segment, the “waggle run”. The waggle run consists of a single stride emphasized by lateral waggling motions of the abdomen. Directional information pointing to a food source relative to the sun’s azimuth is encoded in the angle between the waggle run line and a reference line, which is generally thought to be established by gravity. Yet, there is tantalizing evidence that the local (ambient) geomagnetic field (LGMF) could play a role. We tested the effect of the LGMF on the recruitment success of forager bees by placing observation hives inside large Helmholtz coils, and then either reducing the LGMF to 2% or shifting its apparent declination. Neither of these treatments reduced the number of nest mates that waggle dancing forager bees recruited to a feeding station located 200 m north of the hive. These results indicate that the LGMF does not act as the reference for the alignment of waggle-dancing bees.

2.2. Introduction

The waggle dance of the honeybee, *Apis mellifera*, is performed by a forager bee inside the hive and informs nest mates about the existence and location of a rich food source (von Frisch, 1968). It is undoubtedly one of the most sophisticated means of information transfer amongst insects and probably the entire animal kingdom.

The waggle dancing bee describes a figure 8 on a vertical comb in the hive (Figure 2.1A) (von Frisch, 1968). The straight portion of her dance, known as the “waggle-run”, consists of a single stride (von Frisch, 1948) emphasized by lateral wagging motions of the abdomen at 12-15 Hz. The angle at which the waggle run is performed on the vertical comb correlates with the angle between the target food source and the azimuth of the sun (angular direction) or that of or sun-linked patterns of polarized skylight (Figure 2.1A) (von Frisch, 1948, 1949, 1968). The distance to the food source is encoded in the number of sound pulses generated during the waggle run (Wenner, 1962), with more pulses conveying a more distant food source. Following each waggle run, the bee alternately turns left or right and returns to her starting point, describing a figure 8 in the process. She may repeat this procedure many times. The hypothesis that the waggle dancing bee recruits hive mates to a food source has been confirmed by radar-tracking the flight of bees that attended a waggle dance (Riley et al., 2005).

The directional information pointing to the location of a food source is encoded in the angle between two lines, the waggle run line and a reference line. Gravity was implicated as the natural basis for this reference line following the discovery of a gravity-sensing organ, consisting of paired hairs at the joints between the head and thorax, and the thorax and abdomen (Lindauer and Nedel, 1959). This hypothesis is supported by findings that bees with experimentally impaired gravity receptors failed to perform meaningful waggle dances. It is conceivable however, that the impairment procedure inhibited the bees’ ability to waggle dance properly, and that instead the local (ambient) geomagnetic field (LGMF) serves as the reference line. Like gravity, the LGMF is uniform and stable over long distances.

The ability of bees to detect the LGMF has been reported many times; a few examples are highlighted here. Both the orientation of a vertical comb with respect to the LGMF and the intensity of the LGMF affect the ability of bees to describe food locations during their waggle dances, and alter the bees’ dance behavior (Lindauer and Martin, 1968). When bees are forced to waggle dance on a horizontal comb, they align their dance primarily N-S or E-W irrespective of the location of the food source (Martin and Lindauer, 1977), particularly when the intensity of the LGMF is experimentally increased by a factor of 10. On the other hand, bees, dancing on horizontal combs fail to align their

dance N-S or E-W when the intensity of the LGMF is reduced by 96% (Martin and Lindauer, 1977).

Studies of the apparent magnetoreceptivity of bees revealed their sensitivity to changes in the magnetic field (Walker and Bitterman, 1989), their sensitivity to the direction of the magnetic field (Kirschvink and Kobayashi-Kirschvink, 1991), and their insensitivity to alternating magnetic fields (Kirschvink et al., 1997). Nevertheless, the nature of the receptor that is involved, the manner in which it functions, and its location are unknown or controversial (Desoil et al., 2005; Gould et al., 1978; Hsu et al., 2007; Hsu and Li, 1994; Kuterbach and Walcott, 1986; Válková and Vácha, 2012; Walker, 2008).

In light of the paramount importance of honeybees as crop pollinators (Breeze et al., 2011; Klein et al., 2014), it is essential that we understand their in-hive communication system, which ultimately recruits nest mates to crops. We need to demonstrate unequivocally whether the directions established by the Earth's gravitational or magnetic fields serve as a reference line for waggle dancing bees. Because honeybees are sensitive to the LGMF (Walker and Bitterman, 1989) and may sense residual fields, rigorous LGMF manipulations should include both a reduction in field strength and a shift of its declination around the hive. This has not yet been done. Moreover, previous studies that altered the strength of the LGMF to test its effect on in-hive communication of bees report misdirections (“Missweisungen” (Lindauer and Martin, 1968; Martin and Lindauer, 1977) during waggle dances as the experimental response criterion rather than the all important success of a dancing bee to recruit nest mates to a food source.

Given the current state of knowledge, the directions established by both the Earth's gravitational and magnetic fields are plausible reference lines for the bees' waggle dance. Experimental tests of either field as the basis for a reference line require the ability to manipulate or eliminate the relevant field strength and/or direction. As it is difficult to modify gravity without affecting bee behavior, we focused our studies on modulating the LGMF in the vicinity of hives. We carried out experiments in which we suppressed (reduced to 2%), or rotated the declination of the magnetic field around a hive, and assessed the subsequent success of waggle dancing bees to recruit nest mates to a feeding station.

2.3. Results

A set of six large current-carrying coils arranged to form three orthogonal Helmholtz pairs (Figure 2.1B) allowed us to arbitrarily control the ambient magnetic field around a single-frame observation hive containing up to 2000 individually marked bees. During 2-h treatment sessions, but not 2-h control sessions, we reduced the measured intensity of the ambient magnetic field in the hive to $< 2\%$ of the LGMF (residual fields: $0.8 \mu\text{T} \pm 0.5 \mu\text{T}$; stability limited by the influence of environmental temperature changes on the magnetometer and current sources).

In experiment 1 (East–West alignment of hive) and experiment 2 (North–South alignment of hive), the number of nest mates that forager bees recruited to a feeding station located 200 m north of the hive did not differ between treatment and control sessions [experiment 1: $F_{1,10} = 0.9548$, $p = 0.35$ (Figure 2.2A); experiment 2: $F_{1,7} = 0.9660$, $p = 0.36$ (Figure 2B)], indicating that the LGMF had no effect on the ability of forager bees to recruit nest mates to a food source.

In experiment 3, we deployed a single-frame observation hive with orange- or green-coded bees in each of two separate Helmholtz coil systems; during 2-h treatment sessions, but not concurrently-run 2-h control sessions, we rotated the declination of the ambient magnetic field toward the East (Figure 2.1E), on average by $84^\circ \pm 4^\circ$, while maintaining its intensity constant within $< 3\%$ ($\pm 1.4 \mu\text{T}$). This manipulation essentially achieves a rotation of the horizontal component of the magnetic field. In experiment 3, forager bees recruited similar numbers of nest mates to the feeding station during treatment and control sessions [$F_{1,14} = 0.0274$, $p = 0.87$ (Figure 2.3)], again indicating that the direction of the LGMF field had no effect on the ability of forager bees to recruit nest mates to a food source.

2.4. Discussion

Our data show that suppressing the LGMF to 2% of its nominal value has no effect on the number of nest mates that forager bees recruit to a feeding station, irrespective of hive alignment North–South or East–West. Furthermore, our data show that rotating the declination of the field from North to East had no effect on the number of recruits to a feeding station. Combined, these results support the conclusion that the

LGMF is not likely being used by honeybees as a reference line during the waggle dance. With the LGMF experimentally eliminated as the possible reference, the direction of the Earth's gravitational field is the obvious alternative reference line.

The gravitational field always points to the center of the Earth and would make a highly reliable reference line for the waggle run. It was already implicated as a potential reference for the waggle-run alignment when Lindauer and Nedel (1959) discovered a gravity receptor in bees, and discussed its potential role in the context of their waggle dance. The perfectly vertical honeycombs that bees build in their nest may facilitate optimal detection and reading of this reference during information conveyance in the waggle dance.

The suppression of the LGMF by >98% in experiments 1 and 2 of our study was likely sufficient to reveal any potential effect of the LGMF on the recruitment success of waggle-dancing bees. Even a 96-% suppression of the LGFM was previously shown to have a distinct effect on the waggle dance behavior of bees (Lindauer and Martin, 1968). In a 96-% suppressed LGMF bees exhibit smaller communication inaccuracies ("Missweissungen") on vertical combs, and align their dance randomly (instead of N-S or E-W) on horizontal combs (Martin and Lindauer, 1977). Even if bees sensed the residual magnetic field in experiments 1 and 2 (Walker and Bitterman, 1989) and thus prevented us from detecting an LGMF-treatment effect, the results of experiment 3 indicate that the LGMF is not essential for communication conveyance of waggle dancing bees. In experiment 3, we shifted the declination of LGMF without affecting the number of nest mates that waggle dancing bees recruited to a feeding station (Figure 2.3). These results provide strong evidence that the LGMF is not the reference line for the waggle dance alignment of bees.

The ability of bees to sense magnetic fields (Kirschvink and Kobayashi-Kirschvink, 1991; Lindauer and Martin, 1968; Martin and Lindauer, 1977; Walker and Bitterman, 1989; Walker et al., 1989) is well accepted but the biological significance of this ability is poorly understood. Its single known function is in the context of comb alignment. Bees building combs in a new hive orient them according to the comb alignment in their parent hive by making reference to the LGMF (De Jong, 1982). Another factor that is poorly understood is the biological significance of the sensitivity of the bees' magnetoreceptor, which can sense changes as small as 0.6% of the LGMF

(Walker and Bitterman, 1989). If the magnetoreceptor plays a significant role in the bees' everyday life, the ever increasing magnetic noise pollution of the industrial world could interfere with its optimal functioning and could contribute to the bees colony collapse disorder (Válková and Vácha, 2012).

In conclusion, we have tested a potential role of the LGMF in the waggle dance language of honeybees. Neither cancelling the ambient magnetic field nor shifting its declination from North to East had any measurable effect on the recruitment success of waggle-dancing forager bees. These results likely eliminate the LGMF as a reference for the waggle-run alignment of a dancing bee that attempts to inform her hive mates about a food source. These results also implicate, yet again (Lindauer and Nedel, 1959; Lindauer and Martin, 1968; Martin and Lindauer, 1977; Walker and Bitterman, 1989a, 1989b), gravity as the more plausible reference line, which together with the waggle-run line of the dancing bee appears to form the angle that encodes the direction to a food source. Considerable speculation has been devoted to the manner in which hive mates read the waggle-run line of the dancing bee (Dreller and Kirchner, 1993; Michelsen, 1993; Michelsen and Andersen, 1992; Nieh and Tautz, 2000; Rohrseitz and Tautz, 1999; Tanner and Visscher, 2008, 2009; Thom et al., 2007; Tsujiuchi et al., 2007) but the definitive answer has yet to be found.

2.5. Material and Methods

2.5.1. Experimental location

We ran all experiments on a property near Sicamous, British Colombia, Canada (50°52N, 118°56W) where no power lines or roads interfered with the measured LGMF. The local magnetic field intensity was $55 \mu\text{T} \pm 1.3 \mu\text{T}$ which is consistent with expectations for this location (intensity: $56 \mu\text{T}$; inclination: 72° ; declination: 17° East [33]).

2.5.2. Manipulation of ambient magnetic fields

We custom-built two triaxial Helmholtz coil systems (Figure 2.1B) to control the ambient magnetic field around a single-frame observation hive (51 cm wide \times 29 cm high \times 13 cm deep). We assembled each of the six (regular) octagonal coils from wood studs

(3.8 × 8.9 cm), with a groove (2 cm wide × 1.5 cm deep) cut into one surface to accommodate 21 turns of insulated solid 14 AWG wire. With respect to the hive orientation, the coils producing the vertical-, perpendicular-, and horizontal in-plane components of the applied magnetic field had minimum outer diameters of 1.70 m, 1.85 m, and 2.10 m, respectively. Under typical operating conditions the fields produced by these coils are uniform over the hive volume at the level of 1% or better.

We altered the ambient magnetic field around the observation hive by independently controlling the current in each of the three pairs of coils using Hewlett Packard 6002A power supplies (Hewlett-Packard Company, Palo Alto CA, USA). We deployed a Honeywell model HMC 2003 three-axis magnetic sensor (Honeywell International Inc., Plymouth, MN, USA) to monitor the magnetic field at the hive; we acquired data using a DATAQ® Instruments model DI-149 data acquisition system and processed it using WinDaq®/XL software (DATAQ® Instruments, Akron, Ohio, USA).

2.5.3. Preparation of the observation hive

We placed a single-frame Plexiglas observation hive containing up to 2000 individually marked bees in the center of the Helmholtz coil system. Marking bees allowed us to recognize them as recruits in experiments and to distinguish them from other bees on the property. To be able to mark young bees (<24-h-old) that were not yet able to fly and sting, we kept a frame with bee larvae and pupae in the observation hive for 19 days and then transferred it to an incubator (Narco, Model 310, San Diego, CA, USA). We checked the incubator every 24 h and collected emergent bees. In experiments 1 and 2 (2011), we marked bees individually with colored-number tags (Wienold Imkereibedarf, Lauterbach, Germany). In experiment 3 (2012; North-South alignment of the hive) during which we concurrently deployed two observation hives, we again marked bees individually but coded them with an orange or green dot (Opaque Paint Markers; (Elmer's Products, Inc. Toronto, ON, Canada) as an indicator of the hive from which they originated.

During experiments, we excluded visual cues inside the hive by (*i*) placing thick pieces of Styrofoam over the transparent walls of the hive, (*ii*) using duct tape to seal all gaps between the Styrofoam and the wood frame of the hive, and (*iii*) taping paper tubes over the hive entrance to block direct sunlight. This was done because inhomogeneous

comb illuminations can have an adverse effect on the waggle dance (von Frisch 1965). Thus, instead of using illuminated hives and recording the accuracy of waggle dance alignments (or the degree of misdirections (Martin and Lindauer 1977)) as the experimental treatment effect, we measured the success of waggle dancing bees to recruit nest mates to a feeding station 200 m away from the hive.

2.5.4. Training of bees to visit a feeding station

We trained bees to visit a foraging station consisting of a table (45 cm × 45 cm × 60 cm high) with a yellow plastic cover as a distinct visual cue. At the foraging station we offered bees a watch glass (2.5 cm diameter) filled with anise-scented sugar water or an empty honeycomb (3 cm × 6 cm) filled with diluted honey. Once bees had learned to locate and revisit the foraging station near the observation hive, we moved the station in multiple steps to its final destination 200 m north of the observation hive. We trained three bees from each hive to forage at the station (henceforth “foragers”) and allowed them to recruit other nest mates (henceforth “recruits”) to the station.

2.5.5. Experiments 1 and 2 (August to September, 2011): Effect of suppressed ambient magnetic field on the recruitment success of bees.

We suppressed the LGMF at the hive to by applying opposing fields directed along the vertical, horizontal, and perpendicular directions (with respect to the hive) as shown in Figure 2.1C2. In experiment 1 (3-29 August 2011), we aligned the plane of the observation hive perpendicular (East–West) to the horizontal component of the LGMF; in experiment 2 (14-28 September 2011), we aligned the plane of the observation hive parallel (North–South) to the horizontal component of the LGMF. We tested the same hive in both experiments, but separated the experiments by two weeks so that the bees could adapt to the new magnetic field alignment. Prior to the first experiment, the plane of the observation hive had been in East–West alignment for 2.5 weeks.

Each replicate of the experiment involved a treatment and a control session. During the 2-h treatment session, but not the 2-h control session, we cancelled the ambient magnetic field around the colony. We alternated the order of treatment and control sessions daily. Each replicate commenced after one of the three foragers arrived

at the feeding station. We photographed every bee at the station to document the time and number of visits for each bee. We allowed foragers to return to the colony, but aspirated recruits that landed on the station in a glass jar so that they could not recruit other nest mates.

2.5.6. Experiment 3 (September 2012): Effect of shifting the magnetic declination on the recruitment success of bees

We shifted the declination of the magnetic field at the location of the hive from North to East, by applying currents to the Helmholtz coils (Figure 2.1 C3). The change was made so as to maintain the intensity and the inclination of the field constant. The experimental design for testing the recruitment success of bees consisted of a single-frame, magnetic North–South-aligned observation hive, in each of two separate concurrently run Helmholtz coil systems. Each experimental replicate had a 2-h treatment and a 2-h control session. During the treatment session, but not the control session, we shifted the ambient magnetic declination in the vicinity of the hive toward the East (on average, by $84^{\circ} \pm 4^{\circ}$). During each of two experimental replicates per day, we assigned one hive to a treatment session and the other to a control session, alternating the sequence of assignments of treatment or control session for each hive between days. We took day- and time-of-day-stamped photographs during sessions to document recruits at the feeding station, and to compare the number of recruits during treatment and control sessions. These photographs also enabled us to determine the exact time the first forager bee arrived at the station, at which time we started the experiment.

2.5.7. Data analyses

We analyzed data from experiments 1 and 2 using a linear mixed effect model of the form: $\text{Log (Recruits)} = \text{Treatment} + \text{Date}(R)$. The *Treatment* term is a fixed effect representing the reduction in the number of recruited nest mates for treated hives, while *Date(R)* is a random effect representing the day-to-day variability in the number of recruits. We used a log transformation of the number of recruits to normalize the data, and to be able to report the *Treatment* effect as a direct estimate of the Log (Recruits) ratio, where the biological meaning of a recruitment ratio is easy to interpret.

We analyzed data from experiment 3 using a similar linear mixed effect model, but included additional random effects to account for modifications of the experimental design: $\text{Log}(\text{Recruits}) = \text{Treatment} + \text{Date}(R) + \text{Hive}(R) + \text{Date} * \text{Hive}(R) + \text{Date} * \text{Time of Day}(R)$. Here $\text{Hive}(R)$ accounts for differences between hives, $\text{Date} * \text{Hive}(R)$ accounts for differences between hives on a given day, and $\text{Date} * \text{Time of Day}(R)$ accounts for potential differences in the number of recruits between morning and afternoon sessions. By accounting for these additional sources of variability we reduced the residual error by more than a factor of two, thus increasing the statistical power to detect the main *Treatment* effect (reduction in recruited nest mates). All analyses were executed using JMP 9.0.2 (JMP®, Version 9.0.2. SAS Institute Inc., Cary, NC, 1989-2007).

2.6. Acknowledgments

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2.7. References

- Breeze TD, Bailey AP, Balcombe KG, Potts SG (2011) Pollination services in the UK: How important are honeybees? *Agric Ecosyst environ* 142: 137–143.
- De Jong D (1982) Orientation of comb building by honeybees. *J Comp Physiol A* 147: 495–501.
- Desoil M, Gillis P, Gossuin Y, Pankhurst QA, Hautot D (2005) Definitive identification of magnetite nanoparticles in the abdomen of the honeybee *Apis mellifera*. *J Phys Conf Ser* 17: 45–49.
- Dreller C, Kirchner WH (1993) How honeybees perceive the information of the dance language. *Naturwissenschaften* 80: 319–321.
- Finlay CC, Maus S, Beggan CD, Bondar TN, Chambodut A, et al. (2010) International Geomagnetic Reference Field: the eleventh generation. *Geophysical Journal International* 183: 1216–1230.
- Gould JL, Kirschvink JL, Deffeyes KS (1978) Bees Have Magnetic Remanence. *Science* 201: 1026–1028.

- Hsu C, Li C (1994) Magnetoreception in honeybees. *Science* 265: 95–97.
- Hsu C, Ko F, Li C, Fann K, Lue J (2007) Magnetoreception system in honeybees (*Apis mellifera*). *PloS ONE* 2: e395.
- Kirschvink JL, Kobayashi-Kirschvink A (1991) Is Geomagnetic Sensitivity Real? Replication of the Walker-Bitterman Magnetic Conditioning Experiment in Honey Bees. *Amer Zool* 31: 169–185.
- Kirschvink JL, Padmanabha S, Boyce C, Oglesby J (1997) Measurement of the threshold sensitivity of honeybees to weak, extremely low-frequency magnetic fields. *J Exp Biol* 200: 1363–1368.
- Kuterbach DA, Walcott B (1986) Iron-containing cells in the honey bee (*Apis mellifera*) I. Adult morphology and physiology. *J Exp Biol* 126: 375–387.
- Klein AM, Vaissière BE, Cane JH, Steffan-Dewenter I, Cunningham SA, et al. (2014) Importance of pollinators in changing landscapes for world crops. *Proc R Soc B* 274: 303–313. *Proc R Soc B* 274: 303–313.
- Lindauer M, Nedel O (1959) Ein Schweresinneorgan der Honigbiene. *Z vergl Physiol* 42: 334–364.
- Lindauer M, Martin H (1968) Die Schwereorientierung der Bienen unter dem Einfluß des Erdmagnetfeldes. *Z vergl Physiol* 60: 219–243.
- Martin H, Lindauer M (1977) Der Einfluß des Erdmagnetfeldes auf die Schwereorientierung der Honigbiene (*Apis mellifica*). *J Comp Physiol A* 122: 145–187.
- Michelsen A (1993) The transfer of information in the dance language of honeybees: progress and problems. *J Comp Physiol A* 173: 135–141.
- Michelsen A, Andersen B (1992) How honeybees perceive communication dances, studied by means of a mechanical model. *Behav Ecol Sociobiol* 30: 143–150.
- Nieh JC, Tautz J (2000) Behaviour-locked signal analysis reveals weak 200-300 Hz comb vibrations during the honeybee waggle dance. *J Exp Biol* 203: 1573–1579.
- Riley JR, Greggers U, Smith a D, Reynolds DR, Menzel R (2005) The flight paths of honeybees recruited by the waggle dance. *Nature* 435: 205–207.
- Rohrseitz K, Tautz J (1999) Honey bee dance communication: waggle run direction coded in antennal contacts? *J Comp Physiol A*: 184: 463–470.
- Tanner DA, Visscher PK (2008) Do honey bees average directions in the waggle dance to determine a flight direction? *Behav Ecol Sociobiol* 62: 1891–1898.

- Tanner DA, Visscher K (2009) Does the body orientation of waggle dance followers affect the accuracy of recruitment? *Apidologie* 40: 55–62. doi:10.1051/apido.
- Thom C, Gilley DC, Hooper J, Esch HE (2007) The scent of the waggle dance. *PLoS Biol* 5: e228.
- Tsujiuchi S, Sivan-Loukianova E, Eberl DF, Kitagawa Y, Kadowaki T (2007) Dynamic range compression in the honey bee auditory system toward waggle dance sounds. *PLoS ONE* 2: e234.
- Válková T, Vácha M (2012) How do honeybees use their magnetic compass? Can they see the North? *Bull Entomol Res* 102: 461–467.
- Von Frisch K (1948) Gelöste und ungelöste Rätsel der Bienensprache. *Naturwissenschaften* 35: 38–43.
- Von Frisch K (1949) Die Polarisation des Himmelslichtes als orientierender Faktor bei den Tänzen der Bienen. *Experientia* 5: 142–148.
- Von Frisch K (1968) The dance language and orientation of bees. Harvard University Press.
- Walker MM, Baird DL, Bitterman ME (1989) Failure of stationary but not of flying honeybees (*Apis mellifera*) to respond to magnetic field stimuli. *J Comp Psychol* 103: 62–69.
- Walker M, Bitterman M (1989)a Attached magnets impair magnetic field discrimination by honeybees. *J Exp Biol* 141: 447–451.
- Walker M, Bitterman M (1989)b Honeybees can be trained to respond to very small changes in geomagnetic field intensity. *J Exp Biol* 149: 489–494.
- Walker MM (2008) A model for encoding of magnetic field intensity by magnetite-based magnetoreceptor cells. *J Theor Biol* 250: 85–91.
- Wenner A (1962) Sound production during the waggle dance of the honey bee. *Anim Behav* 10: 79–95.

2.8. Figures

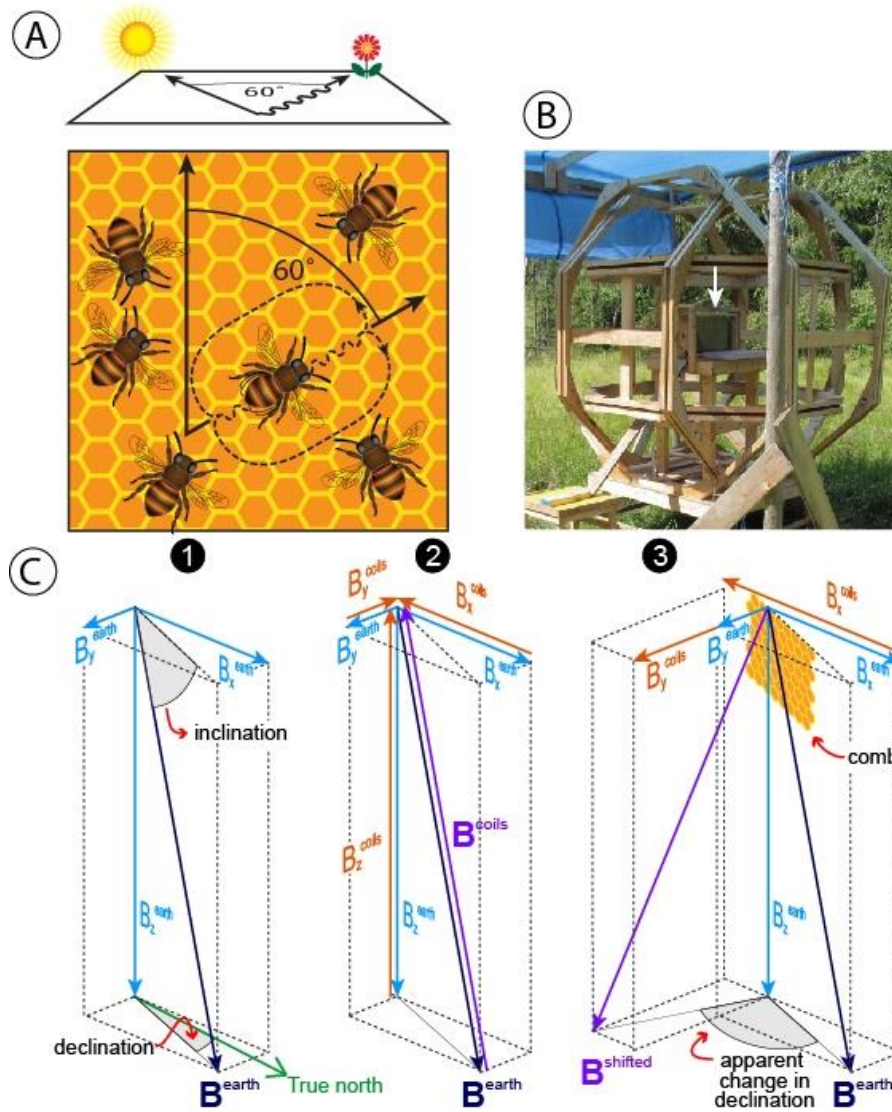


Figure 2.1. Experimental design to test the effect of the Earth's magnetic field on the recruitment success of waggle-dancing honeybees. (A) bottom: waggle dancing honeybee describing a figure 8 on the vertical comb in a hive; top: the angle of the waggle run relative to vertical correlates with the angle between the target food source and the azimuth of the sun (angular direction); (B) observation hive (see arrow) inside a triaxial Helmholtz coil system capable of cancelling or arbitrarily modifying the local geomagnetic field; (C1) components (blue vectors) of the local (unmodified) geomagnetic field (B_{earth}); (C2 & C3) manipulation of field components (purple vectors) to cancel the local geomagnetic field (C2) or to rotate its declination to the East (C3).

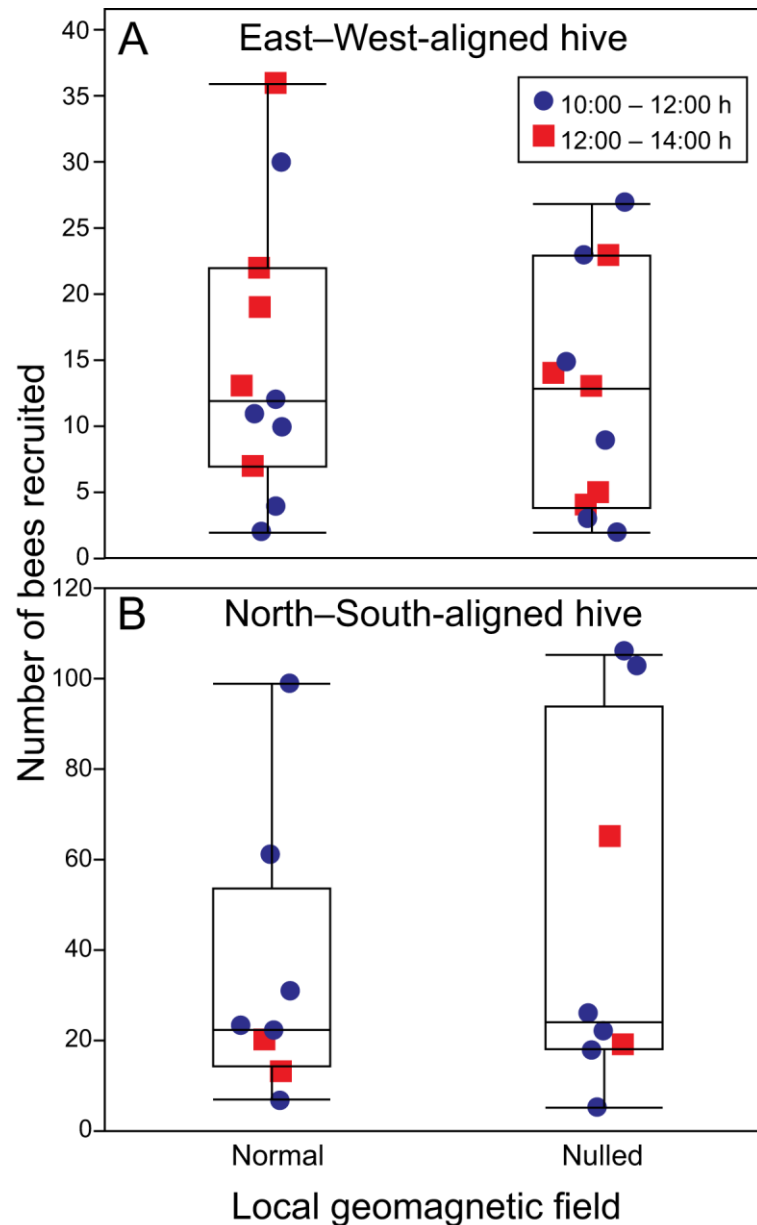


Figure 2.2. Effect of suppressing the ambient magnetic field on the recruitment success of waggle-dancing bees. Boxplots show the mean, median lower and upper quartiles, and \pm whiskers (minimum/maximum data points) of the number of honeybee nest mates recruited in morning and afternoon sessions to a feeding station in the presence (control session) or absence (treatment session) of the ambient magnetic field (see Figures 1B, C2). The presence or absence of the ambient magnetic field had no effect on the number of recruits irrespective of the alignment of the observation hive (East-West: linear mixed effect model analysis; $F_{1,10} = 0.9548$, $p = 0.35$; North-South: linear mixed effect model analysis; $F_{1,7} = 0.9660$, $p = 0.3$).

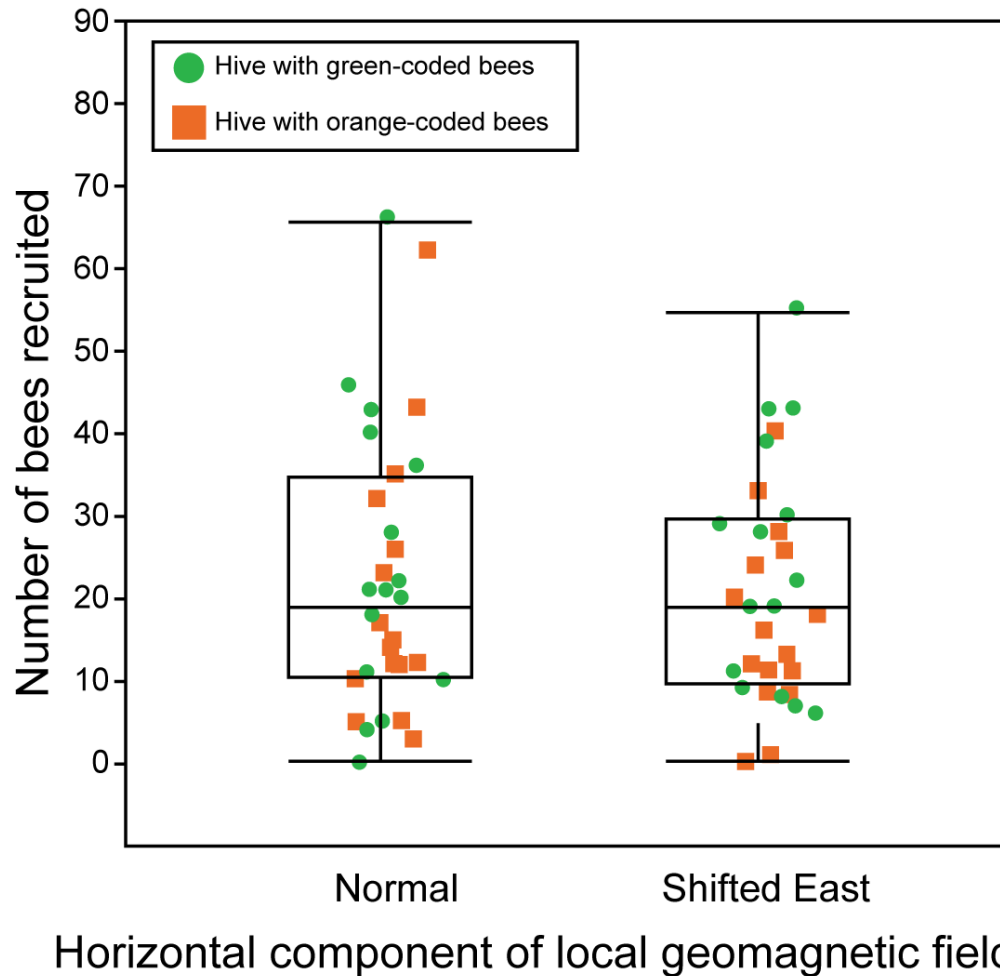


Figure 2.3. Effect of shifting the declination (83°) of the ambient magnetic field to the East on the recruitment success of waggle-dancing bees. Boxplots show the mean, median, lower and upper quartiles, and \pm whiskers (minimum/maximum data points) of the number of honeybee nest mates recruited to a feeding station when the hive was exposed to either the ambient magnetic field (control session) or to the magnetic field with its declination shifted East (treatment session) (see Figure 1, C3). In each replicate ($N = 15$), treatment and control sessions were run concurrently using a single-frame observation hive with green- or orange-coded bees in each of two separate but identical Helmholtz coil systems (one of which is shown in Figure 1B). The number of nest mates recruited from hives in treatment or control sessions did not differ (linear mixed effect model analysis; $F_{1,14} = 0.0274$, $p = 0.87$).

Chapter 3.

Linking magnetite in the abdomen of honey bees to a magnetoreceptive function

A very similar version of this chapter has been published: Veronika Lambinet, Michael E. Hayden, Katharina Reigel, Surath Gomis, Gerhard Gries (2017) Proceedings of the Royal Society B 284:20162873

3.1. Abstract

Previous studies of magnetoreception in honey bees, *Apis mellifera*, focused on the identification of magnetic material, its formation, the location of the receptor, and potential underlying sensory mechanisms, but never directly linked magnetic material to a magnetoreceptive function. In our study, we demonstrate that ferromagnetic material consistent with magnetite plays an integral role in the bees' magnetoreceptor.

Subjecting lyophilized and pelletized bee tagmata to analyses by a Superconducting Quantum Interference Device (SQUID) generated a distinct hysteresis loop for the abdomen but not for the thorax or the head of bees, indicating the presence of ferromagnetic material in the bee abdomen. Magnetic remanence of abdomen pellets produced from bees that were, or were not, exposed to the 2.2-kOe field of a magnet while alive differed, indicating that magnet-exposure altered the magnetization of this magnetite in live bees. In behavioral two-choice field experiments, bees briefly exposed to the same magnet, but not sham-treated control bees, failed to sense a custom-generated magnetic anomaly, indicating that magnet-exposure had rendered the bees' magnetoreceptor dysfunctional. Our data support the conclusion that honey bees possess a magnetite-based magnetoreceptor located in the abdomen.

3.2. Introduction

Magnetoreception (the sensory modality that enables organisms to detect magnetic fields) is widespread among animals, including vertebrates (mammals (Burda et al., 1990; Marhold et al., 1997, Klinowska 1985), birds (Wiltschko and Wiltschko, 2003; Wiltschko and Wiltschko, 1972), fish (Putman et al., 2013; Quinn, 1980; Taylor, 1986), reptiles (Lohmann, 1991; Lohmann and Lohmann, 1993)), insects (e.g., monarch butterflies (Etheredge et al., 1999), beetles (Vácha and Soukopova, 2004), bees (Lindauer and Martin, 1968; Martin and Lindauer, 1977; Walker and Bitterman, 1985, Kirschvink et al., 1997)), and even microorganisms (Blakemore, 1975). Sensing the geomagnetic field aids orientation and navigation behaviour (Wiltschko and Wiltschko, 2005), and enables the exploitation of regional variations in magnetic fields (magnetic “maps”) (Lohmann et al., 2001). Migratory animals relate to the geomagnetic field as a source of directional information (Wiltschko and Wiltschko 1995). For example, birds migrating north or south over thousands of kilometers relate to the geomagnetic field as a substitute cue for the position of the sun, polarized light or star patterns (Wiltschko and Wiltschko, 2003). Similarly, loggerhead sea turtles “read” local magnetic fields for positional information to stay on their migratory course in the North Atlantic (Lohmann et al., 2001).

The underlying mechanisms of magnetoreception and sensory transduction pathways are generally not well understood but three major models have been proposed (see (Johnsen and Lohmann, 2008; Block, 1991) or reviews): chemical magnetoreception, magnetite-based magnetoreception, and electromagnetic induction. According to the chemical magnetoreception model, magnetoreception is light-dependent. This model proposes that exposure of a photoreceptor (e.g., cryptochrome) to ultraviolet/blue light induces “magnetically sensitive photochemical reactions with radical pairs as fleeting intermediates” (Ritz et al., 2000; Ritz et al., 2010), ultimately allowing an animal to “see” the geomagnetic field. Light-dependent magnetoreception has been demonstrated for migratory birds (Ritz et al., 2010; Ritz et al., 2002; Thalau et al., 2005), vinegar flies (Gegear, 2008), American cockroaches (Vácha, 2006; Vácha et al., 2009), and monarch butterflies (Reppert et al. 2010).

The magnetite-based magnetoreception model was likely inspired by the magnetite in teeth of polyplacophora molluscs and the discovery of magnetite in

magnetotactic bacteria that can orient in magnetic fields (Lowenstam, 1962; Blakemore, 1975; Frankel et al., 1979). This model proposes that animals sense the geomagnetic field through magnetite (Fe_3O_4) crystals present in their bodies (Kirschvink and Gould, 1981). When these crystals track the direction of the geomagnetic field, their mechanical orientation changes, thus affecting ion channels in cellular membranes and enabling signal transduction (Winklhofer and Kirschvink, 2010). Magnetite-based magnetoreception has been shown in eukaryotic protists (Bazylinski et al., 2000), ants (de Oliveira et al., 2010), sockeye salmon (Sakaki et al. 1990), and several species of migratory or homing birds (Walcott et al., 2016, Beason and Semm, 1987; Fleissner et al., 2003), bats (Holland et al., 2006, 2008), as well as honey bees (Kirschvink et al., 1997).

The electromagnetic induction model applies only to sharks, stingrays and fish; it proposes that the electroreceptive organ of these marine vertebrates is capable not only of detecting electric fields of potential predators or prey but also of sensing magnetic fields (see (Wiltschko and Wiltschko, 1995) for a review).

Locating magnetoreceptors is challenging and has been compared to searching for “a needle in a haystack” (Kirschvink, 1981). Unlike other sensory receptors such as ears or eyes, magnetoreceptors are potentially exceedingly small and diffuse (spread over a large volume of body tissue) (Kirschvink, 1982). Indeed, if the signal transduction process occurs as a sequence of chemical reactions, as proposed in the model for light-dependent magnetoreception in birds (Ritz et al., 2000), then there may not even be any obvious sensory organ devoted to magnetoreception (Wiltschko and Wiltschko, 1995).

Honey bees, *Apis mellifera*, are good model organisms for locating and characterizing a magnetoreceptor because they (i) use the geomagnetic field for orientation during foraging (Walker and Bitterman, 1985; Frier et al., 1996) and for alignment of their combs (De Jong, 1982), (ii) detect small magnetic anomalies relative to the geomagnetic background (Walker and Bitterman, 1989a), (iii) distinguish between magnetic anomalies in behavioral experiments (Walker and Bitterman, 1985), and (iv) can readily be obtained from large hives for laboratory analyses and field testing.

In an early quest to locate the honey bee magnetoreceptor, Gould et al. (1978) subjected honey bees to analyses by a superconducting quantum interference device

(SQUID) and concluded that the posterior abdomen contains ferromagnetic material. However, the study does not address the question of whether the magnetic material in the abdomen of honey bees is indeed part of their magnetoreceptor. To address this question, one could attempt to impair or modify the magnetoreceptor by applying sufficiently intense magnetic fields, and then bioassaying the subsequent behavior of treated honey bees. This principle was elegantly demonstrated by Wiltschko et al. (1998; 2002) who re-magnetized ferromagnetic material in the beaks of pigeons and Australian silvereyes and thereby temporarily altered the migratory direction of such treated birds. In contrast, Gould et al. (1980) could not de-magnetize the magnetic material of honey bees, and concluded that it was in the form of superparamagnetic crystals, a conclusion that was later questioned (Kirschvink et al., 1997).

In follow-up experiments, Walker and Bitterman (1989b) demonstrated that honey bees distinguish between the presence and absence of magnetic anomalies but fail to do so when a magnetic wire is attached to the anterodorsal surface of their abdomen. The data (Walker and Bitterman, 1989b) demonstrate that the magnetoreceptor is likely located in the abdomen but do not reveal any characteristics of the magnetic particles involved. Kirschvink et al. (1997) tested the response of honey bees to magnetic fields of varying intensity and frequency. Their findings that honey bees readily distinguish between alternating fields when the frequency is kept below 10 Hz, but require stronger fields when the frequency is raised, support the hypothesis of a magnetite-based magnetoreceptor in honey bees, although neural filtering as the root cause of the frequency dependence cannot be excluded.

Also searching for the magnetoreceptor of honey bees, Liang et al. (2016) took a two-pronged approach, running proboscis extension reflex (PER) bioassays with immobilized honey bees and recording electrophysiological signals from the bees' ventral nerve cord in response to magnetic field exposure. When they severed the ventral nervous cord between the abdomen and thorax, honey bees failed to extend their proboscis in response to magnetic fields in PER bioassays. Importantly, these honey bees still demonstrated PER in response to odour stimuli, indicating that the sensory impairment induced by the neural micro-surgery was selective in nature. Collectively, these observations effectively localize the magnetoreceptor to the abdomen of honey bees. In our parallel search for the honey bee magnetoreceptor, we coupled SQUID experiments with behavioral field experiments. In SQUID experiments, we show that the

abdomen but not the head or the thorax of honey bees contains ferromagnetic material consistent with being magnetite, and for which remanent magnetization can be demonstrated. Moreover, in behavioral bioassays, we demonstrate that magnetized honey bees do not respond to magnetic anomalies, whereas control honey bees consistently do. Collectively, our data bridge a critical gap in the data record and demonstrate that honey bees have a ferromagnetic magnetoreceptor located in the abdomen, complementing and enhancing the finding by Liang et al. (2016).

3.3. Methods

3.3.1. Preparation of samples for SQUID analyses

To reduce systematic diamagnetic effects in test samples, we developed a procedure to increase analyte and reduce water content, as follows: we collected bees from a hive in a jar, cold-euthanized them, washed them twice in 70% ethanol [prepared with deionized water from 95% ethanol (Commercial Alcohols, Brampton, ON, Canada)] and – after drying at room temperature – stored them temporarily at -70°C (Panasonic® Ultra low Temperature Freezer; MDF-U76VC, Wood Dale, IL, USA). While each bee was still frozen, we placed her in liquid nitrogen, then retrieved her and while still frozen severed her tagmata [head (typically including antennae and mouthparts), thorax (without wings and legs), and abdomen] using Teflon-coated forceps. We then air-dried and subsequently lyophilized (VirTis Freeze mobile Freeze dryer, 25 EL Sentry 2.0; SP Scientific; Warminster, PA, USA) tagmata for 10 to 14 days. We pressed an average of 13 abdomens, 17 thoraces or 45 heads into pellets (on average 0.4 cm dia. \times 1.1 cm long) (Figure 3.1a), using a pellet press (Figure 3.1d) with its body (3.8 cm dia. \times 7.5 cm long) manufactured from acrylic. We placed bee material into the central cylindrical bore (0.41 cm dia. \times 2.8 cm long) and compressed it by counter-rotating two close-fitting (0.4 cm dia.) Nylon pistons on threaded shafts (Figure 3.1d). We then removed the pistons, pushed the pellet out of the press body, wrapped it in a thin sheet of low-density polyethylene plastic (2 cm long \times 1.5 cm wide; Saran™ Premium wrap; S.C. Johnson & Son, Racine, WI, USA), and inserted the wrapped pellet into a translucent plastic drinking straw (0.5 cm dia. \times 19.4 cm long; Dixie Foodservice model JW74, Georgia-Pacific, Atlanta, GA, USA) (figure 1a) for SQUID analyses as the sample holder.

3.3.2. Potential hysteretic effects associated with bee heads, thoraces and abdomens

We used a SQUID (Superconducting Quantum Interference Device) based magnetometer (MPMS-XL-7 Quantum Design Inc., San Diego, CA, USA) to generate magnetization curves for bee head, thorax and abdomen pellets ($N = 3$, 3 and 4, respectively) at a temperature of 295 K and magnetic fields spanning the range ± 2 kOe.

3.3.3. Remanent magnetization of bee abdomen pellets

We produced abdomen pellets as described above but worked with two groups of live bees: one that we did magnetize (mag-bees), and the other that we did not (non-mag-bees). We magnetized mag-bees by exposing them for 5 s to a 2.2 ± 0.2 kOe magnetic field in the vicinity of a grade 50 neodymium iron boron (NdFeB) disc magnet (5.1 cm dia. \times 1.2 cm thick; Applied Magnets, Plano, TX, USA) holding its broad face parallel to, and 0.5 - 1.0 cm from, the body axis of a live bee (Figure 3.1*b*). We sham-treated control bees (non-mag-bees) by exposing them for 5 s to an aluminum object of similar circumference as the NdFeB disc magnet.

For each pellet from either group, we measured its axial magnetic moment at 295 K and 0 Oe, following a standardized procedure. First, we degaussed the external permalloy shield of the SQUID magnetometer and reset the magnet (raised its temperature above the superconducting transition) 12 h prior to performing any measurements to ensure a stable, uniform, near-zero field. We then measured the magnetic moment of a pellet twice, removing and re-inserting the sample holder from the magnetometer between measurements to reveal any effect on data caused simply by handling the sample holder.

3.3.4. Behavioral responses of bees to magnetic anomalies in two-choice experiments

We tested behavioral responses of mag-bees and non-mag-bees to magnetic anomalies in a backyard in East Vancouver (British Columbia, Canada), with beehives 20 m away from the test location.

3.3.5. Experimental design for two-choice behavioral bioassays

We produced magnetic field anomalies in the vicinity of one watch glass or the other with custom-built coils wound using insulated stranded 16 AWG wire and supported beneath a yellow painted plywood surface (Figure 3.1c). The two lateral coils produce the anomalies. They each comprise 149 turns of wire wound on a square (40 cm × 40 cm) former, but in detail they are subdivided into three co-wound coils (73 turns, 73 turns, and 3 turns, respectively) that can be accessed individually. The central coil shunts magnetic flux from the anomaly near one watch glass away from the other watch glass, and thus acts as a screen. It comprises 100 turns of wire wound on a rectangular (40 cm × 4 cm) former. All seven coils are connected in series and are powered by a single current source (Hewlett Packard model 6002A power supply; Hewlett-Packard Company, Palo Alto, CA, USA). A remote switchbox is then employed to alternate between two configurations. In one configuration, the current in all 149 turns of one lateral coil flows in the same sense (sense A; i.e., CW), producing the desired magnetic field anomaly, whereas the current in the central coil flows in the opposite sense (sense B; i.e., CCW). In our experiments, we always chose sense A to enhance rather than oppose the vertical component of the earth's field. Meanwhile, the windings of the other lateral coil are interconnected so that current flows in sense A through 76 turns of wire (i.e., CW) and in sense B through 73 turns of wire (i.e., CCW). This yields a near cancelation of the magnetic fields produced by the current in this coil, and ensures that any thermal or other systematic effects associated with the flow of current through the lateral coils are constrained to be identical. The net field that is produced by this lateral coil acts in concert with the stray fields from the other coils to minimize perturbations of the magnetic field above the second watch glass when the anomaly is generated. In the other configuration, the roles of the two lateral coils (and hence the location of the anomaly) are interchanged. Representative magnetic field lines in the region of space above the table surface are shown in Figure 3.1e, and the average magnetic fields within specified volumes are listed in Table 3.2. Note that the leads of all coils are individually twisted in pairs so that they make no contribution to the field. Note also that the entire apparatus (table top, coil formers, and support structure) was constructed from wood and (non-magnetic) brass fasteners.

3.3.6. Training of bees

The objective was to let bees learn to associate a sugar reward with a 15-Oe magnetic anomaly that we regularly monitored with a Hall probe (F.W. Bell Model 6010 Gauss/Teslameter equipped with a Model HAD61-2508-15 axial probe; Bell Technologies, Sipris, ON, Canada). To this end, we placed watch glasses (2.5 cm dia.) on either side of the table (Figure 3.1c). The watch glass associated with the magnetic anomaly contained sugar water, and the other contained salt water. We marked bees visiting the watch glass containing sugar water with a queen number tag (Imkershoperzgebirge.de; Schönbrunn; Germany) so that we could distinguish them from other foraging bees. Between visits of bees to watch glasses, we pseudo randomly switched the position of the sugar reward and its corresponding magnetic anomaly to the left or right side of the table. Once a bee had re - “located” the food reward guided by the anomaly in 10 consecutive visits, we considered her to be “trained”.

3.3.7. Testing of bees

We assigned trained bees to a treatment group or a control group. While bees were lapping up sugar water from a watch glass, we magnetized treatment bees (mag-bees) by a 5-s exposure to the NdFeB disc magnet (detail described above, Figure 3.1b), and sham-treated control bees (non-mag-bees) by a 5-s exposure to a (non-magnetic) cylindrical aluminum object of similar circumference as the magnet. Following treatment, we randomly assigned the magnetic anomaly to one lateral coil or the other, replaced both watch glasses with new ones containing plain water, and recorded the responses of bees, predicting that mag-bees would no longer exhibit a preference for watch glasses associated with the magnetic anomaly. After completing the tests, we removed all test bees from the experimental site.

3.3.8. Analyses of data

We acquired all SQUID data as a function of sample position, at 295 K and fixed magnetic field. We then determined magnetic moments from nonlinear least squares fits of the anticipated response function to these data, accounting for the known (i.e., measured) extent of each sample [MEH, SG, VL, GG; unpubl. data]. We repeated each such scan and subsequent fit a minimum of six times. We obtained magnetization

curves, comprising an initial magnetization curve from 0 Oe to 2 kOe followed by one complete cycle of the main loop (2 kOe to - 2 kOe and back again), by repeating this sequence following stepwise increments of the applied magnetic field with no overshoot. We then fit a heuristic analytic model comprising the sum of a single linear (diamagnetic) term and a Langevin function to the mean magnetic moment data for the demagnetizing curve (Downscan) and the remagnetizing curve (2nd Upscan), with the constraint that the saturation magnetizations of the anhysteretic terms are equal. We subtracted the linear contribution to the magnetic moment so determined from the data to yield hysteresis curves.

We performed statistical analyses using Maple 17 (Maplesoft, Waterloo, ON, Canada). In the case of remnant magnetic moments, we conducted Shapiro-Wilk tests for normality on all samples; we then compared variances using conventional F-tests for equality of variances, or Brown-Forsythe tests when there was reason to question normality; we compared means using either Welch's t-test (for unequal variances) or Student's t-test (for equal variances), as appropriate. For two-choice bioassays, we used a Pearson's χ^2 test to evaluate the significance of deviations from a discrete random equal probability distribution.

3.4. Results

3.4.1. Magnetization of bee heads, thoraces and abdomens

We observed qualitatively different magnetization curves (magnetic moment m versus magnetic field H) when we studied pellets of bee heads, thoraces and abdomens using a SQUID magnetometer (Figure 3.1a, Figure 3.2; see Methods for details). Distinct hysteresis loops, indicating the presence of ferromagnetic material, are evident in data acquired from bee abdomens, but not from heads or thoraces (Figure 3.2; electronic supplementary materials 1-3). Instead, the latter reveal contributions from weak anhysteretic (s-shaped) components, consistent with superparamagnetism or trace quantities of ferromagnetic material. All three responses are well fit by Langevin functions of the form $\coth[(H - \Delta H)/H_0] - H_0/(H - \Delta H)$, with relative amplitudes in the ratio 70: 4: 9 (abdomen: head: thorax) for the example shown in Figure 3.2b. Meanwhile, the initial magnetic moments of four abdomen pellets were all zero to within 1.09×10^{-7} emu/bee (Figure 3.2c, 1st Upscan). The mean remanent magnetic moments and

coercive fields of the subsequent hysteresis loops were of order 3×10^{-7} emu/bee and 1×10^2 Oe, respectively (Figure 3.2b). And, the ratios of remanent to saturation magnetic moments were typically of order 0.2. The localized scatter of data evident in Figure 3.2 is an instrumental effect, and is not associated with the sample or sample holder, as verified in ancillary control experiments with paramagnetic samples mounted in quartz sample holders.

3.4.2. Remanent magnetization of the bee abdomen

With distinct evidence for hysteretic behavior in the abdomen but not in the thorax or the head of bees (see Figure 3.2), we focused further studies on remanent magnetic moments. We report data separately for bees that we magnetized while they were alive (mag-bees) (Figure 3.1b) and for those that we did not magnetize (non-mag-bees) (see Methods for detail). We prepared abdomen pellets from lyophilized mag-bees, and from lyophilized non-mag-bees, and first subjected all pellets to two consecutive control measurements (M_1 , M_2) in which we attempted to detect a residual (i.e., remanent) magnetic moment with the applied SQUID field set to 0 Oe. In both groups (mag- and non-mag-bees), we observed a high degree of reproducibility between paired control measurements M_1 and M_2 . We thus analyzed and present data in terms of the equivalent measurements $M_{\overline{12}}$ and $M_{\Delta 12}$, comprising the means and half-differences between paired observations of sample magnetic moments [i.e., $(m_2 + m_1)/2$ and $(m_2 - m_1)/2$, respectively].

For non-mag-bee pellets, the control ($M_{\overline{12}}$) data are tightly clustered around a mean value of zero (Figure 3.3; electronic supplementary material 4), consistent with our earlier observation of zero initial magnetization in bee abdomen pellets. The control data for mag-bee pellets are also consistent with a mean value of zero, but their distribution is significantly broader than it is for non-mag-bee pellets. This variation reflects the effect of the magnetization treatment to which this group of bees was subjected while still alive (Table 3.1).

3.4.3. Behavioral experiments

Once bees had learned to associate a sugar reward (presented in a watch glass) with a 15-Oe magnetic anomaly produced by one of two lateral coils mounted beneath a

plywood table top (Figure 3.1c), we exposed them to the field of a NdFeB magnet (figure 1b) (mag-bees) or kept them as sham-treated controls (non-mag-bees). We then presented mag-bees and non-mag-bees with the opportunity of choosing between the presence or absence of the 15-Oe magnetic anomaly randomly assigned to the left or right side of the table but always presented in combination with a watch glass now containing plain water.

In these two-choice experiments, non-mag-bees selected the watch glass associated with the magnetic anomaly significantly more often than could be expected by chance (50%) [χ^2 (1, N = 21) = 5.8, p = 0.016], whereas mag-bees did not [χ^2 (1, N = 29) = 0.035, p = 0.85] (Figure 3.4; electronic supplementary material 5). We infer that the magnetoreceptors of mag-bees were temporarily or permanently rendered dysfunctional as a result of the prior magnet exposure.

3.5. Discussion

Previous studies of magnetoreception in honey bees have addressed the location of the magnetoreceptor (Gould et al., 1978; Hsu et al., 2007; Hsu and Li, 1993; Hsu and Li, 1994; Kuterbach and Walcott, 1986), its formation (Hsu and Li, 1993; Hsu and Li, 1994), and potential mechanisms for encoding the direction and intensity of the (geo)magnetic field (Winklhofer and Kirschvink, 2010; Hsu et al., 2007; Hsu and Li, 1994; Walker 2008). While some of these studies provide evidence for the presence of magnetite in honey bees (reviewed in (Wajnberg et al. 2010)), no studies have directly linked magnetite to a magnetoreceptive function.

In a recent study, Liang et al. (2016) not only link proboscis extension of honey bees to their sensing of magnetic stimuli, they also trace the origin of the neuronal signal triggering the proboscis reflex to the abdomen. Their data convincingly demonstrate that the magnetoreceptor of honey bees is located in the abdomen, but they do not reveal its material characteristics. In our study, we demonstrate that honey bees possess ferromagnetic material in their abdomen that is consistent with magnetite or a magnetite-like substance, and that this ferromagnetic material is indeed an integral part of the bees' magnetoreceptor. Our conclusion is based on experiments that integrate data from SQUID analyses of pelletized bee tagmata for the presence of magnetizable magnetite,

and data from field studies with live bees that characterize behavior subsequent to magnetization.

Our SQUID studies of bee tagma pellets reveal magnetic hysteresis in abdomen pellets, but not thorax or head pellets (Figure 3.2). This hysteresis, which is characteristic of ferromagnetic materials such as magnetite, indicates that materials within the abdomen can be permanently magnetized through application of a sufficiently strong magnetic field. This was further evident in remanent magnetization studies wherein live bees were magnetized, cold-euthanized, and pelletized prior to being subjected to SQUID analysis. Abdomen pellets prepared in this manner retained a clear magnetic signature that distinguished them from similar pellets prepared from the abdomens of bees that were only sham-treated prior to cold-euthanization (Figure 3.3 and Table 3.1). Indications that the ferromagnetic material in these samples is magnetite is provided by experiments in which we monitored the magnetic moment of magnetized bee tagma pellets (in zero applied field) as the temperature of the sample was cycled from 295 K to 10 K and back again. Features analogous to those reported by Desoil et al. (2005), and consistent with the Verwey transition in magnetite, are observed at temperatures in the range 110 to 130 K. And, the coercive fields and ratios of remanent to saturation magnetic moments we extract from hysteresis loops (Figure 3.2) are similarly consistent with expectations for magnetite particles (Banerjee et al., 1985).

The magnetoreceptive function of the magnetite in the abdomen of honey bees became particularly obvious in two-choice field experiments. Following exposure of live bees to the same NdFeB magnet employed in laboratory studies, these magnetized bees, unlike sham-treated control bees, failed to sense, or respond to, the presence of a magnetic anomaly. This demonstrates a functional connection between magnetite in the abdomen and the magnetoreceptor, and temporary or permanent disablement of the receptor through magnet-exposure.

From the outset, we anticipated that signatures of ferromagnetism would be weak and readily obscured by the diamagnetic response of biological tissues to applied magnetic fields (Hautot et al., 2005). We minimized the diamagnetic component of the signal by lyophilizing severed bee tagmata to reduce water content. We then maximized the signal amplitude by compressing lyophilized bee body tissue into pellets (Figure 3.1a,d). Often, when commercially available SQUID magnetometer systems are

employed, sample dimensions are chosen to be of order a few millimeters or less so that the sample can be treated as a point source. To further increase signal strength, we intentionally produced large cylindrical pellets (0.4 cm dia. × 0.6 to 1.3 cm long) and then explicitly accounted for sample dimensions in our data analyses.

Given the universal challenge of magnetite crystals in any type of magnetoreceptor to interact sufficiently with the geomagnetic field to overcome thermal buffeting (Kirschvink et al., 2001), it is plausible that some elements of “receptor design” are conserved across taxa. For rainbow trout, *Oncorhynchus mykiss*, there exists one of the most detailed descriptions of a magnetite-based magnetoreceptor (Diebel et al., 2000). Using an array of analytical techniques including confocal and atomic-force microscopy imaging, Diebel et al. (2000) report that iron-rich crystals in olfactory lamellae of rainbow trout are single-domain magnetite particles that are arranged in a 1- μm long braided chain enclosed within a single receptor cell. The magnetic moments of individual crystals in this chain sum linearly, thereby improving the magnetic to thermal energy ratio of the receptor and rendering it capable of aligning with an external magnetic field. If a chain of single-domain ferromagnetic magnetite crystals were to be part of the honey bees’ magnetoreceptor, and if it was mechanically constrained to preclude realignment, that chain could potentially crumble, buckle, detach from the cell membrane, or rupture constraining tethers upon exposure to a magnetic field substantially larger than its coercive field. The 2.2-kOe field in the vicinity of the NdFeB disc magnet to which we exposed bees (Figure 3.1b) is well in excess of the coercive fields of bulk and particulate magnetite (a few hundred Oe; (Goya et al., 2003)) and of the ferromagnetic magnetite we report in bee abdomens (Figure 3.2; of order one hundred Oe), and thus could presumably impair bee magnetoreceptors. This scenario is consistent with our observation that magnet-exposed bees, unlike control bees, failed to detect magnetic anomalies in two-choice experiments (Figure 3.4).

Studies addressing whether exposure to intense magnetic fields renders the magnetoreceptor of honey bees temporarily or permanently dysfunctional would provide insight into the microstructure of the receptor and its ability, or not, to effect repairs. Some insight along these lines can be gleaned from our SQUID-based measurements of remnant magnetic moments performed on abdomen pellets of bees that were, or were not, exposed to the fields of a magnet while they were still alive (mag-bees vs. non-mag-bees). The data from these experiments (Figure 3.3) are consistent with a scenario in

which the applied fields or their gradients reconfigure envisaged chains of ferromagnetic magnetite particles associated with a magnetoreceptor.

A signature stemming from magnetizing live honey bees is retained when mag-bees are lyophilized and pelletized (variance of M_{12}^{mag} relative to $M_{12}^{\text{non-mag}}$), suggesting that the magnetite in the abdomen of these bees is not naturally oriented with respect to the body axis; or, if it is oriented, the degree of orientation is much less than that which is induced by a 2.2-kOe field. Our results differ from previous reports of magnetic alignment in bees (Gould et al. 1978; Esquivel et al., 2002). Irrespectively, our laboratory-based magnetization studies and complementary behavioral response bioassays convincingly demonstrate that exposure of live bees to an intense magnetic field alters the magnetization of ferromagnetic magnetite in their abdomens, and renders their magnetoreceptors dysfunctional.

While we can posit a microstructure for the honey bees' magnetoreceptor, rigorous experimentation is needed to assign a definitive structure. These experiments might include dynamic SQUID analyses of pelletized bee abdomens or various types of microscopy imaging (e.g., TEM) of crystals in thin sections of otherwise untreated abdominal bee tissue. Moreover, there are models (e.g., Walker, 2008) but only few experimental data as to how the direction and the intensity of an external magnetic field are encoded by magnetite-based magnetoreceptor cells. Studying the process by which a chain of crystals, or any other crystal formation, transduces a magnetic field into an electrical signal in the nervous system will be particularly challenging but essential to gain a complete understanding of the magnetite-based magnetic sense.

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3.7. References

- Banerjee, S.K. Moskowitz, B.M. in: Kirschvink JL, Jones DS MB. 1985 Magnetite biomineralization and magnetoreception in organisms: a new biomagnetism. New York: Plenum Press.
- Bazylinski D.A., Schlezinger D.R., Howes B.H., Frankel R.B., Epstein S.S. 2000 Occurrence and distribution of diverse populations of magnetic protists in a chemically stratified coastal pond. *Chemical Geology* 169(3-4), 319-328
- Beason RC, Semm P. 1987 Magnetic responses of the trigeminal nerve system of the bobolink (*Dolichonyx oryzivorus*). *Neurosci Lett.* 80, 229–234.
- Blakemore R. 1975 Magnetotactic bacteria. *Science* 190, 377–379.
- Block SM. 1991 Biophysical Principles of Sensory Transduction. In *Sensory Transduction* (eds DP Corey, SD Roper), pp 1-17. The Rockefeller Press, New York.
- Burda H, Marhold S, Westenberger T, Wiltshko R, Wiltshko W. 1990 Magnetic compass orientation in the subterranean rodent *Cryptomys hottentotus* (Bathyergidae). *Experientia* 46, 528–530.
- De Jong D. 1982 Orientation of comb building by honeybees. *J Comp Physiol A* **147**, 495–501.
- Desoil M, Gillis P, Gossuin Y, Pankhurst Q, Hautot D. 2005 Definitive identification of magnetite nanoparticles in the abdomen of the honeybee *Apis mellifera*. *J Phys Conf Ser.* 17, 45–49.
- Diebel CE, Proksch R, Green CR, Neilson P, Walker MM. 2000 Magnetite defines a vertebrate magnetoreceptor. *Nature* 406, 299–302.
- de Oliveira JF, Wajnberg E, Esquivel DMDS, Weinkauff S, Winklhofer M, Hanzlik M. 2010 Ant antennae: are they sites for magnetoreception? *J R Soc Interface* 7, 143–152.
- Esquivel DMS, Wajnberg E, Cernicchiaro GR, Acosta-Avalos D, Garcia B. 2002 Magnetic material arrangement in *Apis mellifera* abdomens. *Mater Res Soc Symp Proc.* 724, 3–6.
- Etheredge JA, Perez SM, Taylor OR, Jander R. 1999 Monarch butterflies (*Danaus plexippus* L.) use a magnetic compass for navigation. *PNAS* 96, 13845–13846.
- Fleissner G, Holtkamp-Rötzler E, Hanzlik M, Winklhofer M, Fleissner G, Petersen N, Wiltshko W. 2003 Ultrastructural analysis of a putative magnetoreceptor in the beak of homing pigeons. *J Comp Neurol.* 458, 350–360.

- Frankel RB, Blakemore RP, Wolfe RS. 1979 Magnetite in freshwater magnetotactic bacteria. *Adv Sci.* 203, 1355–1356.
- Frier H, Edwards E, Smith C, Neale S, Collett T. 1996 Magnetic compass cues and visual pattern learning in honeybees. *J Exp Biol.* 199, 1353–1361.
- Gegear RJ, Casselman A, Waddell S, Reppert SM. 2008 Cryptochrome mediates light-dependent magnetosensitivity in *Drosophila*. *Nature* 454, 1014–1018.
- Gould JL, Kirschvink JL, Deffeyes KS. 1978 Bees have magnetic remanence. *Science* 201, 1026–1028.
- Gould JL, Kirschvink JL, Deffeyes KS, Brines ML. 1980 Orientation of demagnetized bees. *J Exp Biol.* 86, 1–8
- Goya GF, Berquo TS, Fonseca FC, Morales MP. 2003 Static and dynamic magnetic properties of spherical magnetite nanoparticles. *J Appl Phys.* 94, 3520–3528.
- Hautot D, Pankhurst Q A., Dobson J. 2005 Superconducting quantum interference device measurements of dilute magnetic materials in biological samples. *Rev Sci Instrum.* 76, 1–5.
- Holland R.A., Thorup K., Vonhof M.J., Cochran W.W., Wikelski M. 2006 Navigation: Bat orientation using Earth's magnetic field. *Nature* 444, 702.
- Holland R.A., Kirschvink J.L. Doak T., Wikelski M. 2008 Bats use magnetite to detect the Earth's magnetic field. *PLoS One* 3:e1676
- Hsu C, Li C. 1993 The ultrastructure and formation of iron granules in the honeybee (*Apis mellifera*). *J Exp Biol.* 180, 1–13.
- Hsu CY, Li CW. 1994 Magnetoreception in honeybees. *Science* 265, 95–97.
- Hsu C-Y, Ko F-Y, Li C-W, Fann K, Lue J-T. 2007 Magnetoreception system in honeybees (*Apis mellifera*). *PLoS One* 2, e395.
- Johnsen S, Lohmann KJ. 2008 Magnetoreception in animals. *Phys. Today* 61, 29–35.
- Kirschvink JL. 1981 The horizontal magnetic dance of the honeybee is compatible with a single-domain ferromagnetic magnetoreceptor. *BioSystems* 14,193–203.
- Kirschvink JL, Gould JL. 1981 Biogenic magnetite as a basis for magnetic field detection in animals. *Biosystems* 13,181-201.
- Kirschvink J. 1982 Birds, bees and magnetism: A new look at the old problem of magnetoreception. *Trends in Neurosciences* 5, 160–167.

- Kirschvink JL, Walker MM, Diebel CE. 2001 Magnetite-based magnetoreception. *Curr Opin Neurobiol.* 11, 462–468.
- Kirschvink J, Padmanabha S, Boyce C, Oglesby J. 1997 Measurement of the threshold sensitivity of honeybees to weak, extremely low-frequency magnetic fields. *J Exp Biol.* 200, 1363–1368.
- Klinowska M 1985 Cetacean live stranding sites relate to geomagnetic topography. *Aquatic Mammals* 1, 27-32.
- Kuterbach D A, Walcott B. 1986 Iron-containing cells in the honey bee (*Apis mellifera*) I. Adult morphology and physiology. *J Exp Biol.* 126, 375–387.
- Kuterbach D A, Walcott B. 1986 Iron-containing cells in the honey-bee (*Apis mellifera*) II. Accumulation during development. *J Exp Biol.* 126, 389–401.
- Liang C-H, Chuang C-L, Jiang J-A, Yang E-C. 2016 Magnetic sensing through the abdomen of the honey bee. *Sci. Rep.* 6, 1–7.
- Lindauer M, Martin H. 1968 Die Schwereorientierung der Bienen unter dem Einfluss des Erdmagnetfeldes. *Z. Vergl. Physiol.* 60, 219–243.
- Lohmann KJ. 1991 Magnetic orientation by hatchling loggerhead sea turtles (*Caretta caretta*). *J Exp Biol.* 155, 37–49.
- Lohmann K, Lohmann C. 1993 A light-independent magnetic compass in the leatherback sea turtle. *Biol Bull.* 185, 149–151.
- Lohmann KJ, Cain SD, Dodge SA, Lohmann CMF. 2001 Regional magnetic fields as navigational markers for sea turtles. *Science* 294, 364–366.
- Lowenstam HA (1962) Magnetite in denticle capping in recent chitons (Polyplacophora). *GSA Bulletin* 73(4), 435-438
- Marhold S, Wiltschko W, Burda H. 1997 A magnetic polarity compass for direction finding in a subterranean mammal. *Naturwissenschaften* 84, 421–423.
- Martin H, Lindauer M. 1977 Der Einfluss des Erdmagnetfeldes auf die Schwereorientierung der Honigbiene (*Apis mellifica*). *J Comp Physiol A* 122, 145–187.
- Putman NF, Lohmann KJ, Putman EM, Quinn TP, Klimley AP, Noakes DLG. 2013 Evidence for geomagnetic imprinting as a homing mechanism in pacific salmon. *Curr Biol.* 23, 312–316.
- Quinn TP. 1980 Evidence for celestial and magnetic compass orientation in lake migrating sockeye salmon fry. *J Comp Physiol A* 137, 243–248.

- Reppert SM, Geegear RJ, Merlin C. 2010 Navigational mechanisms of migrating monarch butterflies. *Trends Neurosci.* 33, 399–406.
- Ritz T, Adem S, Schulten K. 2000 A model for photoreceptor-based magnetoreception in birds. *Biophys J.* 78, 707–718.
- Ritz T, Dommer DH, Phillips JB. 2002 Shedding light on vertebrate magnetoreception. *Neuron* 34, 503–506.
- Ritz T, Ahmad M, Mouritsen H, Wiltschko R, Wiltschko W. 2010 Photoreceptor-based magnetoreception: optimal design of receptor molecules, cells and neuronal processing. *J R Soc Interface* 7, 135–46.
- Sakaki Y, Motomiya T, Kato M, Ogura M. 1990 Possible mechanism of biomagnetic sense organ extracted from sockeye salmon. *IEEE Trans Magn.* 26, 1554–1556.
- Taylor PB. 1986 Experimental evidence for geomagnetic orientation in juvenile salmon, *Oncorhynchus tshawytscha* Walbaum. *J Fish Biol.* 28, 607–623.
- Thalau P, Ritz T, Stapput K, Wiltschko R, Wiltschko W. 2005 Magnetic compass orientation of migratory birds in the presence of a 1.315 MHz oscillating field. *Naturwissenschaften* 92, 86–90.
- Vácha M, Soukopova H. 2004 Magnetic orientation in the mealworm beetle *Tenebrio* and the effect of light. *J Exp Biol.* 207, 1241–1248.
- Vácha M. 2006 Laboratory behavioural assay of insect magnetoreception: magnetosensitivity of *Periplaneta americana*. *J Exp Biol.* 209, 3882–6.
- Vácha M, Puzová T, Kvícalová M. 2009 Radio frequency magnetic fields disrupt magnetoreception in American cockroach. *J Exp Biol.* 212, 3473–3477.
- Wajnberg E, Acosta-Avalos D, Alves OC, de Oliveira JF, Srygley RB, Esquivel DMS. 2010 Magnetoreception in eusocial insects: an update. *J R Soc Interface* 7, 207–225.
- Walker MM, Bitterman ME. 1985 Conditioned responding to magnetic fields by honeybees. *J Comp Physiol A* 157, 67–71.
- Walker M, Bitterman M. 1989a Short Communication: Honeybees can be trained to respond to very small changes in geomagnetic field intensity. *J Exp Biol.* 145, 489–494.
- Walker M, Bitterman M. 1989b Short communication: Attached magnets impair magnetic field discrimination by honeybees. *J Exp Biol.* 141, 447–451.
- Walker MM. 2008 A model for encoding of magnetic field intensity by magnetite-based magnetoreceptor cells. *J Theor Biol.* 250, 85–91.

- Walcott C, Gould JL, Kirschvink JL. 2016 Pigeons have magnets. *Am Assoc Adv Sci.* 205, 1027–1029.
- Wiltschko W, Wiltschko R. 1972 Magnetic compass of European Robins. *Sci New Ser.* 176, 62–64.
- Wiltschko R, Wiltschko W. 1995 Magnetic orientation in animals. *Zoophysiology*, Berlin, Heidelberg, New York: Springer-Verlag.
- Wiltschko W, Munro U, Ford H, Wiltschko R. 1998 Effect of a magnetic pulse on the orientation of silvereyes, *Zosterops l. lateralis*, during spring migration. *J Exp Biol.* 201, 3257–3261.
- Wiltschko W, Munro U, Wiltschko R, Kirschvink JL. 2002 Magnetite-based magnetoreception in birds: the effect of a biasing field and a pulse on migratory behavior. *J Exp Biol.* 205, 3031–3037.
- Wiltschko R, Wiltschko W. 2003 Avian navigation: from historical to modern concepts. *Anim Behav.* 65, 257–272.
- Wiltschko W, Wiltschko R. 2005 Magnetic orientation and magnetoreception in birds and other animals. *J Comp Physiol A* 191, 675–693.
- Winklhofer M, Kirschvink JL. 2010 A quantitative assessment of torque-transducer models for magnetoreception. *J R Soc Interface* 7, 273–289.

3.8. Figures

Table 3.1. Summary of test statistics for magnetometry data: Tests for (a) equality of variances (left; Brown-Forsythe W50 or F-test) and (b) equality of means (right; two-sample t-tests for equal or unequal variances).

Measurements		Comparison of Variances		Comparison of Means	
		W_{50} -/F-statistic	p -value	t-statistic	p -value
$M_1^{\text{non-mag}}$	$M_2^{\text{non-mag}}$	$W_{50:14,14} = 2.2$	$p = 0.2$	$t_{\text{eq}:28} = 0.5$	$p = 0.6$
M_1^{mag}	M_2^{mag}	$F_{10,10} = 1.08$	$p = 0.90$	$t_{\text{eq}:20} = 0.12$	$p = 0.90$
$M_{12}^{\text{non-mag}}$	M_{12}^{mag}	$W_{50:10,14} = 14$	$p < 10^{-4}$	$t_{\text{un}:24} = 1.3$	$p = 0.22$
$M_{\Delta 12}^{\text{non-mag}}$	$M_{\Delta 12}^{\text{mag}}$	$W_{50:10,14} = 1.8$	$p = 0.4$	$t_{\text{eq}:24} = 1$	$p = 0.3$

Table 3.2. Root-mean-square (RMS) magnetic field computed in right cylindrical volumes of height h aligned with the axes of the lateral coils and located with their bases on the surface of the two-choice bioassay table (figure 1 a,e). The background field in the absence of applied currents is 0.540 Oe.

h (cm)	RMS Magnetic Field (Oe)	
	Above anomaly	Opposite anomaly
5	14.7	0.554
10	14.1	0.545
20	13.2	0.532
30	12.4	0.521

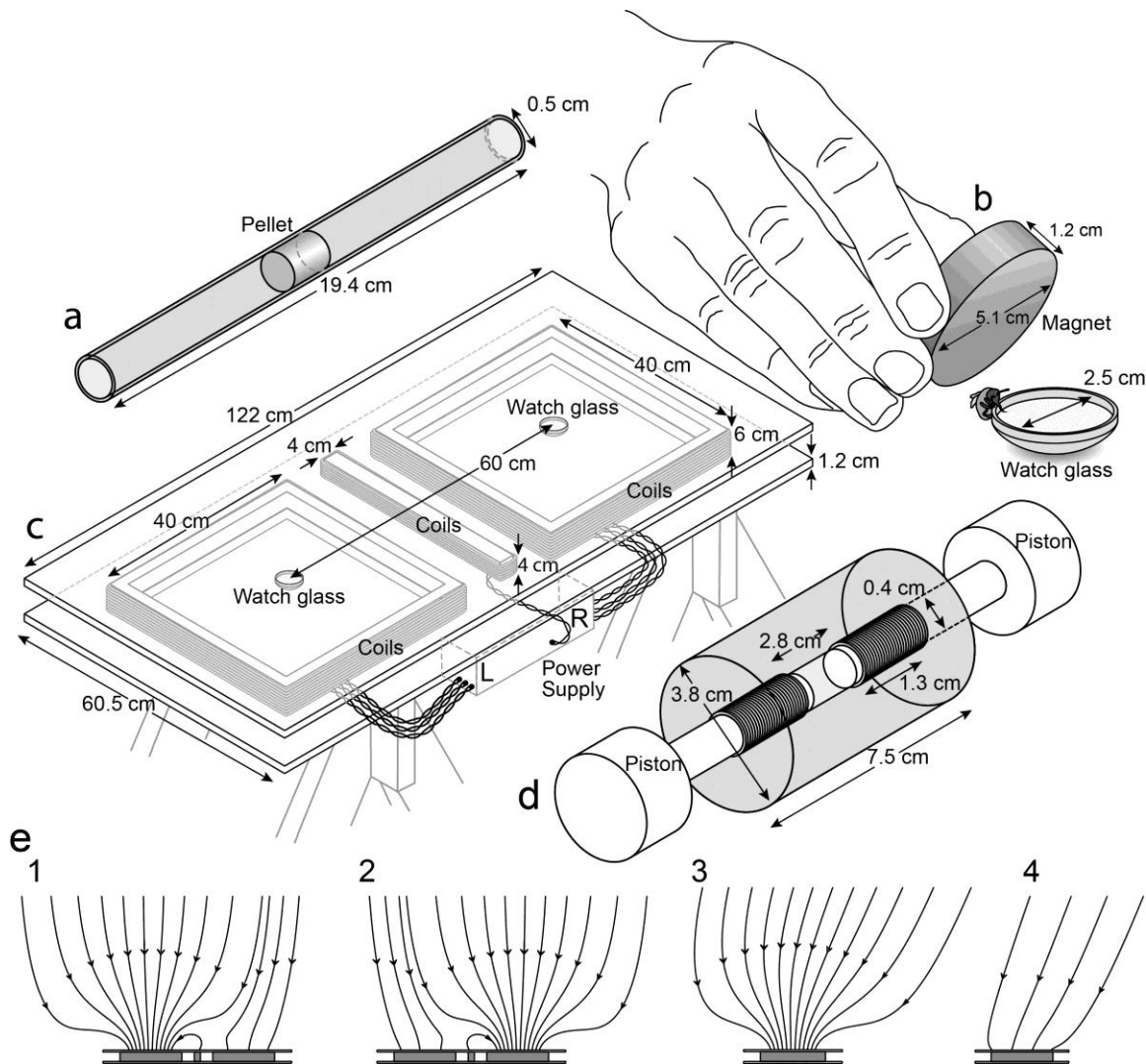


Figure 3.1. Schematic drawings of various experimental elements. (a) Pellet of lyophilized and compressed honey bee abdomens in the center of a straw to be subjected to analysis by a Superconducting Quantum Interference Device (SQUID) magnetometer; (b) Exposure of a NdFeB disc magnet while visiting a watch glass filled with sugar water; (c) Two-choice bioassay table equipped with custom-built coils (sandwiched between plywood sheets) that are used to generate magnetic field anomalies in the vicinity of one watch glass or the other; (d) Custom-built “pellet press” capable of compacting tagma tissue of honey bees for SQUID analysis; (e) Projections of magnetic field lines passing through coil midplanes at the surface of the bioassay table. The anomaly is shown alternately positioned above the (1) left- and (2) right-hand coils. Also shown are side projections of (different) lines above the coil (3) with and (4) without the anomaly. Note that the various sets of magnetic field lines shown are in general not coplanar. For simplicity, the short axis of the bioassay table is shown aligned with the horizontal component of the geomagnetic field.

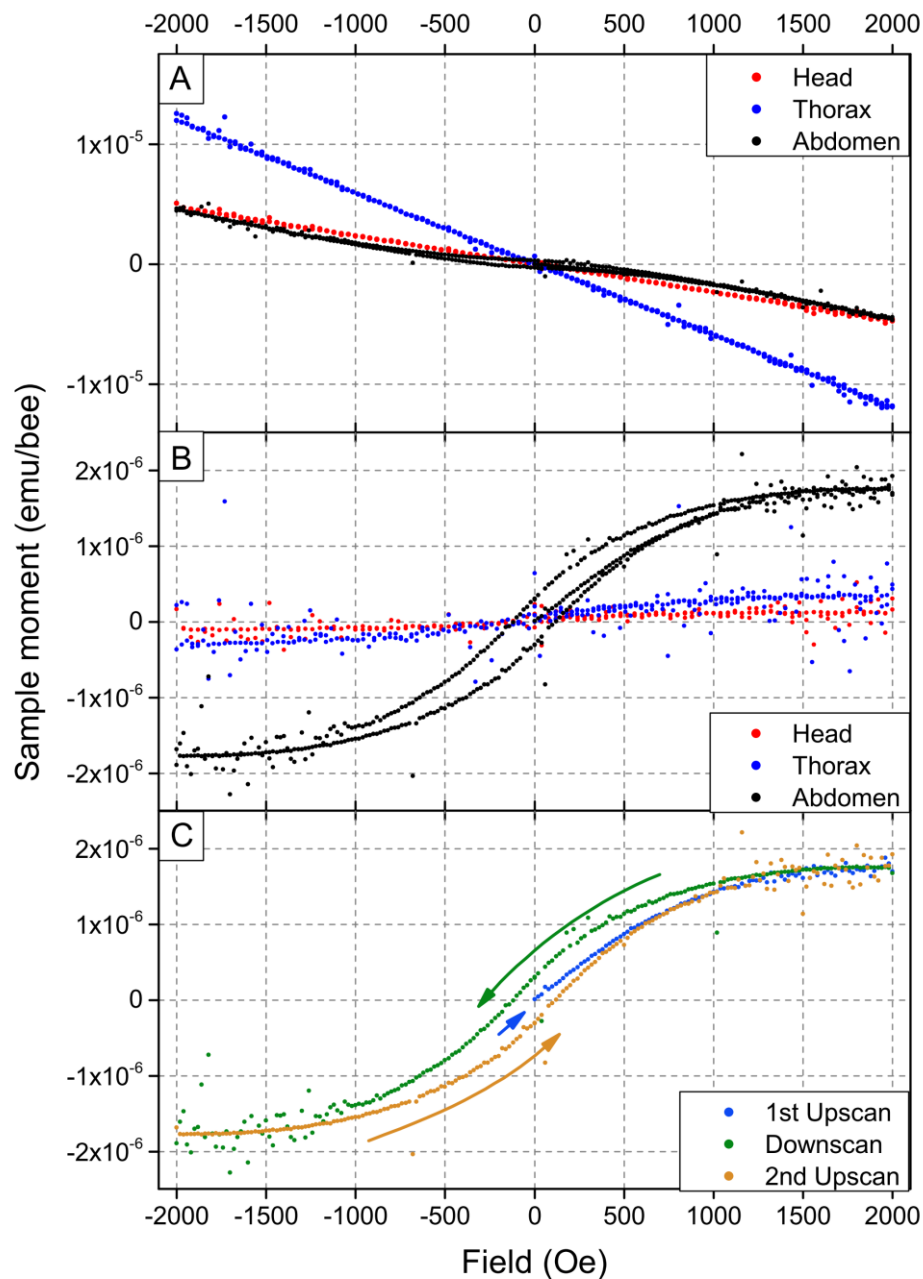


Figure 3.2. SQUID-detected magnetization curves for honey bee head, thorax and abdomen pellets. (a) Magnetic moment (in emu/bee) of honey bee abdomen, thorax and head pellets as a function of applied magnetic field H (in Oe); (b) The same data after subtracting linear diamagnetic terms (note the distinct hysteresis loop only for the abdomen pellet); (c) Magnetic moment of the abdomen pellet, illustrating the sequence of data acquisition for each sample (1st Upscan, Downscan, 2nd Upscan).

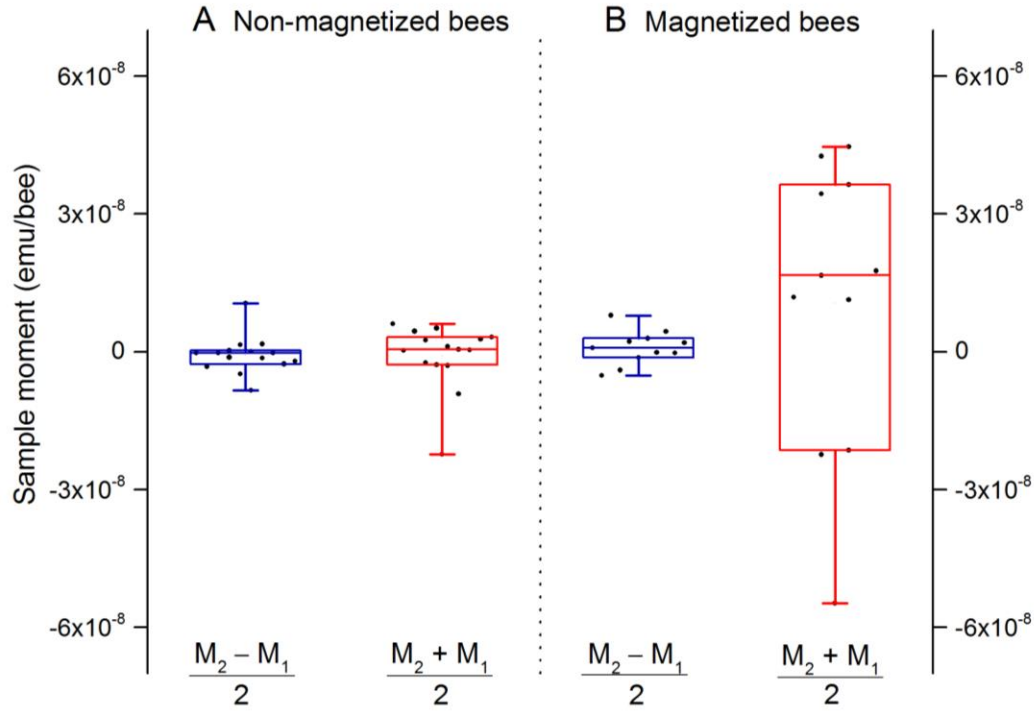


Figure 3.3. SQUID analyses for remanent magnetization in honey bee abdomen pellets; (a,b) Remanent magnetic moments of honey bee abdomen pellets prepared from live bees that were (a), or were not (b), exposed to the field of a NdFeB disc magnet (figure 1b); note: (1) boxplots show the mean, median lower and upper quartiles, and \pm whiskers (minimum/maximum data points) of remanent magnetic moments; (2) there is a significant difference in the variance of the average of the data for abdomen pellets prepared from live bees that were (a), or were not (b), exposed to the field of a NdFeB disc magnet (see table 1 for detailed statistical analyses).

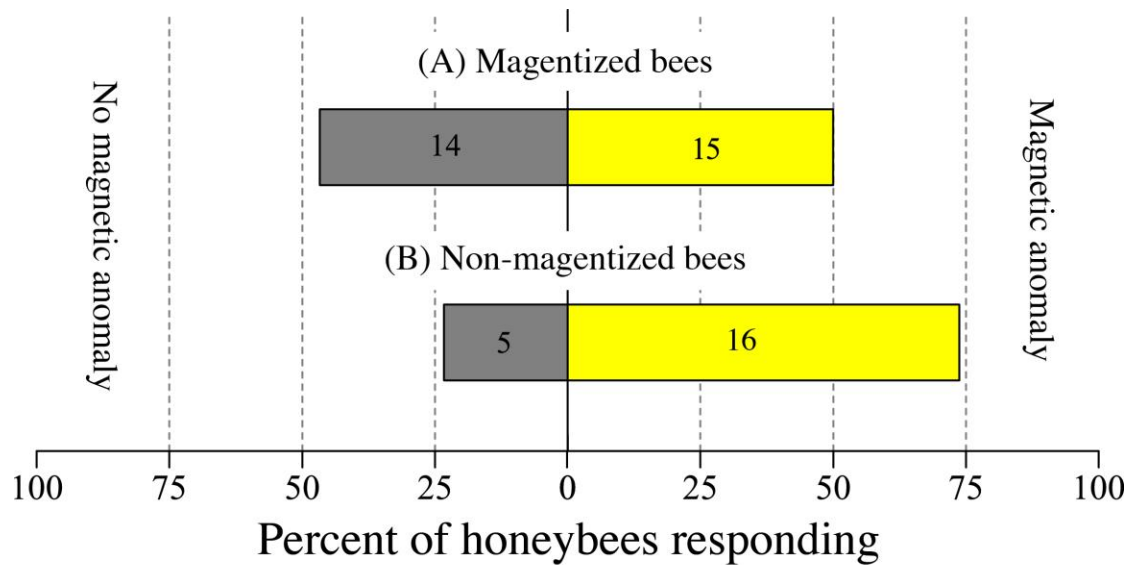


Figure 3.4. Effect of magnet-exposure of honey bees on their ability to detect a magnetic field anomaly. Response of honey bees that had previously learned to associate a sugar reward with a 15-Oersted (Oe) magnetic field anomaly (produced by a custom-built set of coils; figure 1c) following exposure (a), or not (b), to the 2.2-kOe field of a NdFeB disc magnet (figure 1b). Non-magnet-exposed bees detected the magnetic anomaly significantly more often than could be expected by chance (50%) [χ^2 test, $p = 0.0164$], whereas magnet-exposed (magnetized) bees did not [χ^2 test, $p = 0.8527$], indicating that the magnetoreceptor of magnetized bees was rendered dysfunctional.

Chapter 4.

Honey bees possess a polarity-sensitive magnetoreceptor

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4.1. Abstract

Honey bees, *Apis mellifera*, exploit the geomagnetic field for orientation during foraging and for alignment of their combs within hives. We tested the hypothesis that honey bees sense the polarity of magnetic fields. We created an engineered magnetic anomaly in which the magnetic field generally either converged toward a sugar reward in a watch glass, or away from it. After bees in behavioral field studies had learned to associate this anomaly with a sugar water reward, we subjected them to two experiments performed in random order. In both experiments, we presented bees with two identical sugar water rewards, one of which was randomly marked by a magnetic field anomaly. During the control experiment the polarity of the magnetic field anomaly was maintained the same as it was during the training session. During the treatment experiment it was reversed. We predicted that bees would not respond to the altered anomaly if they were sensitive to the polarity of the magnetic field. Our findings that bees continued to respond to the magnetic anomaly when its polarity was in its unaltered state, but did not respond to it when its polarity was reversed, support the hypothesis that honey bees possess a polarity-sensitive magnetoreceptor.

4.2. Introduction

The magnetic field of the Earth (geomagnetic field hereafter) is a rich source of information for any animal capable of sensing it (Wiltschko and Wiltschko 2005). The general form of the geomagnetic field is dipolar, as if it originated from a powerful bar magnet located near the center of the Earth (Figure 4.1a). Field lines emerge from the Earth's surface in the southern hemisphere and re-enter in the northern hemisphere. The intensity and the inclination of this field with respect to the Earth's surface vary with latitude. The geomagnetic field is most intense near the poles ($> 60 \mu\text{T}$) and least intense near the equator ($< 35 \mu\text{T}$). Its inclination ranges from $\pm 90^\circ$ (vertical) at the magnetic poles to 0° (horizontal) at the magnetic equator.

There are at least two types of information that magnetoreceptive migrating animals can glean from the geomagnetic field. First, the alignment of the field provides directional (compass) information, enabling an animal to maintain a consistent heading in a particular direction, such as north or south. Orientation based on magnetic compass mechanisms is known to occur in diverse taxa including vertebrates [fish (Quinn, 1980; Mann et al., 1988; Putman et al., 2013), amphibians (Phillips, 1977), reptiles (Lohmann, 1991; Fuxjager et al., 2011), birds (Wiltschko and Wiltschko, 2003), mammals (Marhold et al., 1997; Wang et al., 2007)], mollusks (Lohmann and Willows, 1987), crustaceans (Lohmann et al., 1995), and insects (Wajnberg et al., 2010). Second, aspects of the field such as its local intensity and inclination with respect to the Earth's surface provide indications of "magnetic latitude" (Wiltschko and Wiltschko, 2003), from which magnetoreceptive migrating animals are able to gauge their current position relative to their final destination (Lohmann et al., 2008). For example, animals migrating north from the magnetic equator to the magnetic pole encounter progressively steeper inclination angles and progressively stronger magnetic fields, either or both of which could be used to derive some kind of positional information (Lohmann and Lohmann, 1994, 1996; Phillips et al., 2002). More generally, the ability of some animals including European robins, *Erithacus rubecula* (Wiltschko and Wiltschko, 1972), African mole rats (Marhold et al., 1997), and honey bees, *Apis mellifera* (Walker and Bitterman, 1989a) to sense subtle deviations or abnormalities in the local geomagnetic field enables them to learn the "magnetic topography" of their home range, which may then facilitate orientation towards specific resources such as nesting or foraging sites (Thalau et al., 2007;

Lohmann et al., 2012). Given the wealth of directional and positional information that might be derived from the geomagnetic field, and the possibility that this information might overload the capacity of a single receptor system, it has been suggested that some migrating animals such as birds might rely on two separate receptors that are based on different sensory mechanisms (Wiltschko and Wiltschko, 2006).

The question of whether migrating animals sense the inclination of the magnetic field (inclination compass) or the polarity of the horizontal component of the field (polarity compass) was first and most rigorously studied with birds (e.g., Wiltschko and Wiltschko, 1972, 2002). Evidence that birds sense the inclination of the magnetic field, rather than its polarity, was generated by testing migratory birds in experimental settings where the horizontal and vertical components of the magnetic field could be independently manipulated, typically using Helmholtz coil systems (Firester, 1966; Crosser et al., 2010, and references cited therein). Inverting the horizontal component of the field (which reverses its polarity relative to geographic coordinates) or the vertical component of the field (which inverts its inclination with respect to the Earth's surface), both caused the same re-direction of the birds' directional preference (Figure 4.1). These observations indicate that the avian magnetic compass does not sense the polarity of the geomagnetic field (magnetic north or south). Instead, it recognizes "polewards" as the horizontal component of the direction in which local magnetic field lines appear to intersect the Earth's surface, and "equatorwards" as the horizontal component of the direction in which magnetic field lines appear to "intersect" the sky (Wiltschko, 1980; Figure 4.1b).

Ever since these pioneering experiments were performed, inverting the vertical component of the geomagnetic field (which alters the axial course of the field lines but not polarity) (Figure 4.1b) has become a standard diagnostic test to determine the type of magnetoreceptor (inclination vs. polarity compass) that magnetically sensitive animals possess. Inverting the vertical component of the field alters the migratory direction or orientation behavior of animals that possess an inclination compass. Those that do not re-orient their behavior in response to this type of manipulation likely possess a polarity compass. Supporting evidence for polarity sensitivity can then be obtained by shifting the horizontal component of the field, and observing a corresponding shift in orientation behavior. On the basis of these types of experimental manipulations it has been demonstrated that sockeye salmon, *Oncorhynchus nerka* (Quinn, 1980), African mole

rats (Marhold et al., 1997), and spiny lobsters (Lohmann et al., 1995) possess a polarity compass.

Honey bees exploit the geomagnetic field for orientation during foraging (Walker and Bitterman, 1985; Frier et al., 1996) and for alignment of their combs within a hive or nest cavity (De Jong, 1982). They are capable of detecting natural and engineered magnetic anomalies relative to the background geomagnetic field (Walker and Bitterman, 1989a; Lambinet et al., 2017). However, the underlying mechanism of the magnetoreceptor in honey bees is still unknown.

The primary mechanisms that have been proposed for magnetoreception rely on electromagnetic induction, magnetic field-mediated biochemical reactions (plausibly initiated by photoexcitation), or forces acting on magnetic (chiefly biogenic magnetite) particles (Johnsen and Lohmann, 2008). Based on current understanding, (marine) animals in the “electromagnetic induction” group and (terrestrial) animals in the “particle-based” group sense the polarity of the geomagnetic field, whereas animals in the “biochemical reaction” group do not (Ritz et al., 2000; Davila et al. 2003; Winklhofer and Kirschvink, 2010). Because honey bees possess ferromagnetic particles (magnetite) in their abdomen (Gould et al., 1978; Hsu and Li, 1994; Desoil et al., 2005) that are integral to their magnetoreceptor (Lambinet et al., 2017), we hypothesized that honey bees belong to the “particle-based” group and thus sense the polarity of a magnetic field.

Our experimental design to test this hypothesis took into account the fact that honey bees are not long-distance migrants that use the geomagnetic field for long-distance compass orientation but that they sense magnetic anomalies in their home range during foraging. Drawing on previous studies (Walker and Bitterman, 1989a; Kirschvink and Kobayashi-Kirschvink, 1991), we performed two-choice experiments in which we presented honey bees with an engineered and controllable magnetic anomaly. Once bees had learned to associate a sugar reward with the anomaly, we reversed its polarity (but not its inclination), predicting that bees with a polarity-sensitive magnetoreceptor would not respond to the inverted anomaly.

4.3. Material and Methods

4.3.1. Experimental design

Bees were always presented with two watch glasses, one on either side of a featureless plywood surface painted yellow (Figure 4.2). We produced engineered magnetic field anomalies in the vicinity of one watch glass or the other using custom-built coils that have been described, characterized, and employed previously (Lambinet et al., 2017). They are wound using insulated stranded 16 AWG wire and supported beneath the table. The two lateral coils produce the anomalies. They each comprise 149 turns of wire wound on a square (40 cm x 40 cm) former, but in detail they are subdivided into three co-wound coils (73 turns, 73 turns, and 3 turns, respectively) that can be accessed individually. The central coil shunts magnetic flux from the anomaly near one watch glass away from the other watch glass, and thus acts as a screen. It comprises 100 turns of wire wound on a rectangular (40 cm x 4 cm) former. All seven coils are connected in series and are powered by a single current source (Hewlett Packard model 6002A power supply; Hewlett-Packard Company, Palo Alto, CA 94304-1126, USA). A switchbox is then employed to alternate between two basic configurations. In one configuration, the current in all 149 turns of one lateral coil flows in the same sense (sense A; e.g. CW), producing the desired magnetic field anomaly, while the current in the central coil flows in the opposite sense (sense B; e.g. CCW). Meanwhile, the windings of the other lateral coil are interconnected so that current flows in sense A through 76 turns of wire and in sense B through 73 turns of wire. This yields a near (but intentionally imperfect) cancellation of the magnetic fields produced by the current in this coil, and ensures that any thermal or other systematic effects associated with the flow of current through the lateral coils are constrained to be identical. The net field that is produced by the second lateral coil acts in concert with the stray fields from the other coils to minimize perturbations of the magnetic field above the corresponding watch glass when the anomaly is generated (see Table 3.1 and Figure 3.1e in Lambinet et al. 2017). In the other configuration, the roles of the two lateral coils (and hence the location of the anomaly) are interchanged. Note that the leads of all coils are individually twisted in pairs so that they make no contribution to the field. Note also that the entire apparatus (table top, coil formers, and support structure) was constructed from wood and (non-magnetic) brass.

The full three-dimensional magnetic field produced by this system of coils, and the vector superposition of this field with the local geomagnetic field, are readily calculated from first-principles (e.g., Griffiths, 2013) or using magnetic field modeling packages (e.g., BiotSavart, Ripplon Software Inc., New Westminster BC, Canada). We have done both, and have compared these design calculations with experimentally-determined magnetic field maps generated using a fluxgate magnetometer (Fluxmaster, Stefan Mayer Instruments, Dinslaken, Germany). We find that, as constructed, the system of coils employed in our study produces magnetic fields that agree with expectations at the level of a few percent.

During bee-training sessions described below, the vertical component of the total magnetic field at the location of the watch glass on the side of the table where the anomaly was generated was set to 1.5 Gauss, directed downwards (Figure 4.2b). In this configuration the horizontal component of the field associated with the anomaly is generally directed toward the associated watch glass. During subsequent treatment experiments, but not control experiments, we inverted the magnetic anomaly (by reversing the sense of current flow through all coils) and adjusted the intensity of the vertical component of the total magnetic field at the location of the watch glass to 1.5 Gauss, directed upwards (Figure 4.2c). In this treatment configuration the horizontal component of the field associated with the anomaly is generally directed away from the associated watch glass.

4.3.2. Training of bees

We worked with honey bees from an experimental hive that we placed near Texas Creek Road in Lillooet (British Columbia). The local geomagnetic field intensity and inclination were 0.5 Gauss and 72 degrees, respectively. The two-choice table (Figure 4.2a) was positioned 20 m south of this hive, with its long axis aligned with the magnetic meridian. To initiate training, we set a watch glass (2.5 cm dia.) on both sides of the table. We filled the watch glass associated with the magnetic anomaly with concentrated sugar water, and filled the other with diluted sugar water. To distinguish trainee bees from other non-foraging bees, we marked trainees with a queen number tag (supplier: Imkershoperzgebirge.de; Schönbrunn; Germany). We allowed only one bee at any time to participate in the experiment, aspirating recruits from the hive and bees that coincidentally alighted on the experimental table into a jar. Between visits of number-

tagged bees to a watch glass, we pseudo-randomly switched the position of the magnetic anomaly and the associated sugar reward from one side of the table to the other. Furthermore, we replaced watch glasses contacted by bees with clean watch glasses and filled them with a new sugar solution. Once a bee correctly identified the location of the randomly positioned magnetic anomaly and its associated sugar reward on six consecutive visits (1 in 64 chance of being a random sequence of events), we considered her to be trained.

4.3.3. Testing of bees

We subjected each trained bee to two experiments, pseudo-randomly assigning the order in which they were performed. In both cases, we filled both watch glasses with a rich sugar reward. In the control experiment, we employed the same randomly positioned magnetic anomaly used during the training session. In the treatment experiment, we inverted the randomly positioned magnetic anomaly by running the current through the coils in the opposite sense. When a bee had completed the first experiment (control or treatment), we returned her to the training sequence described above. Once she again had successfully tracked the position of the magnetic anomaly and its associated sugar reward for six consecutive visits, we subjected her to the second experiment.

4.3.4. Data analyses

We analyzed data from the control experiment and the treatment experiment using chi-squared (χ^2) tests, testing the null hypothesis that the watch glasses containing identical sugar rewards were chosen at random, with equal probability. We also employed Barnard's exact test, testing the null hypothesis that the likelihood of choosing the sugar reward marked by the altered magnetic anomaly was not reduced in the treatment experiment, relative to the control experiment.

4.4. Results

During the control experiment, bees visited the watch glass associated with the magnetic anomaly far more often than could be expected by chance (50%) [$df = 1$, $N = 30$, $\chi^2 = 8.53$, $p = 0.0035$] (Figure 4.3a), thus showing that they had indeed learned to

associate the magnetic anomaly with a food reward. During the treatment experiment, on the other hand, bees visited the two watch glasses equally often [$df = 1$, $N = 30$, $\chi^2 = 0.13$, $p = 0.72$] (Figure 4.3b), indicating that they did not respond to the magnetic anomaly when its polarity was reversed. These observations are reinforced by Barnard's exact test, which indicates significantly fewer behavioral responses to the magnetic anomaly when its polarity was reversed [Wald statistic = 1.895, 1-tailed, $p = 0.045$].

4.5. Discussion

In order to test the hypothesis that honey bees can sense the polarity of magnetic fields we designed a magnetic anomaly in which magnetic field lines could be made to generally converge toward (Figure 4.2b), or diverge away from (Figure 4.2c), either one of two watch glasses placed 60 cm apart from one another, on an otherwise featureless table (Figure 4.2a). We hypothesized that bees would not respond to an altered anomaly if they were sensitive to the polarity of the magnetic field. We found that bees responded to the magnetic anomaly in its normal state (the polarity used during training sessions; Figure 4.3a), but did not respond to the anomaly when its polarity was reversed (Figure 4.3b). These observations support the hypothesis that honey bees have a polarity-sensitive magnetoreceptor rather than an inclination-sensitive magnetoreceptor.

Previous studies of polarity-sensitive animals have shown that their orientation behavior is not affected when Helmholtz coil systems are used to invert the vertical component of the magnetic field (and hence its inclination), but that it is influenced when the horizontal component of the field is reoriented (Quinn, 1980; Lohmann et al., 1995; Marhold et al., 1997). Here it is worth emphasizing that the second test alone is not sufficient if the goal is to probe sensitivity to polarity (cf. Figure 4.1b subpanels 1 and 2); reorienting the horizontal component of the magnetic field necessarily changes the direction indicated by both inclination and polarity compasses. In our experiments, we simultaneously inverted the local sense of all components of the magnetic field in the vicinity of the sugar reward (Figure 4.2b, c). This has the effect of inverting the polarity of the (horizontal component of the) magnetic field, but not its local inclination (cf. Figure 4.1b subpanels 1 and 4). In this sense, our single test is in principle a more direct probe of polarity sensitivity than the sequence of experiments that is usually employed.

A characteristic of the magnetite-based polarity compass is that it can detect minute changes in the intensity and polarity of a magnetic field (Johnsen and Lohmann, 2005) and that it is functional in both well-lit and entirely dark conditions. Light-independent functioning of polarity-sensitive magnetoreceptors has been shown in experiments with mole rats (Marhold et al., 1997), spiny lobsters (Lohmann et al., 1995), sockeye salmon (Quinn, 1980), and bats (Wang et al., 2007). In complete darkness, subterranean mole rats, e.g., adjust their nest-building activity in accordance with experimentally-shifted magnetic north, and visually occluded spiny lobsters change the course of their orientation in response to experimental manipulations of the field polarity.

Honey bees respond to the geomagnetic field in the darkness of their hive (De Jong, 1982) and during foraging in bright daylight (Walker and Bitterman, 1989a). They also detect natural and engineered magnetic anomalies under both dark and well-lit experimental conditions (Walker and Bitterman 1985, 1989a,b; Kirschvink and Kobayashi-Kirschvink, 1991; Liang et al., 2016). While it is conceivable that honey bees possess both light-independent and light-dependent magnetoreceptors, it is more plausible that their magnetic sense relies on only one type of receptor, which is fully functional under both photopic and scotopic conditions. Moreover, light-dependent chemical magnetoreception by bees is not supported by studies that investigated the formation and ultrastructure of iron granules in honey bees (Hsu and Li, 1993; Hsu and Chan, 2011). Instead, there is a mounting base of evidence in support of a magnetite-based magnetoreceptor located in the abdomen of bees (Válková and Vácha, 2012; Liang et al., 2016; Lambinet et al., 2017).

Compared to vertebrates, little is known about the magnetic sense in invertebrates. Work on insects started in the mid-1970s, when Arendse and Vrinis (1975) and Arendse (1978) studied the magnetic sense of the mealworm beetle *Tenebrio molitor*. At the time, the authors concluded that mealworm beetles employ a light-independent polarity compass. However, subsequent studies (Vácha and Soukopova, 2004; Vácha et al., 2008) questioned this conclusion and proposed instead that *Tenebrio* beetles use a light-dependent inclination compass. While there is increasing evidence that insects can perceive the geomagnetic field or magnetic anomalies (for a review see Wajnberg et al., 2010), only a few studies have definitively determined the type of compass (inclination or polarity) that the study insects use. For example, *Drosophila* vinegar flies and monarch butterflies, *Danaus plexippus*, have a magnetoreceptor that is

not only light-dependent but also frequency-specific, requiring wavelengths of 420 nm or longer (Yoshii et al., 2009).

The magnetite-based, polarity-sensitive magnetoreceptor model is thought to apply to eusocial insects like honey bees (Wajnberg et al., 2010), and data on the geomagnetic sensitivity of honey bees (Kirschvink and Kobayashi-Kirschvink, 1991) were later interpreted (Winklhofer and Kirschvink, 2010) as evidence that honey bees may possess a polarity compass. In our study, we explicitly tested this hypothesis, and report strong experimental evidence that honey bees indeed possess a polarity-sensitive magnetoreceptor.

4.6. Acknowledgements

We thank Brian Andrade for assistance in the construction of the two-choice table; the owners of the Spray Creek ranch and the Texas Creek ranch for permitting experimentation on their land; Hannah Crisp for assisting in data collection; Tristan Banwell for various hands-on work and help; Stephen DeMuth and Stephen Takács for graphical illustrations; Gitta Lambinet and Fritz Lambinet for supporting VL's education and providing financial assistance; and two anonymous reviewers for constructive comments. The research was supported by Graduate Fellowships from Simon Fraser University to VL, NSERC Discovery Grants to MEH and GG, and by an NSERC - Industrial Research Chair to GG, with Scotts Canada Ltd. as the industrial sponsor.

4.7. References

- Arendse MC, Vries JCM (1975) Magnetic orientation and its relation to photic orientation in *T. molitor*. Netherlands J Zool 25:407–437.
- Arendse MC (1978) Magnetic field detection is distinct from light detection in the invertebrates *Tenebrio* and *Talitrus*. Nature 274:358–362.
- Crosser MS, Scott S, Clark A, Wilt PM (2010) On the magnetic field near the center of Helmholtz coils. Rev Sci Instrum 81:084701.
- Block SM (1991) Biophysical principles of sensory transduction. In: Corey DP, Roper SD (eds) Sensory transduction, The Rockefeller Press, New York, pp 1-17.

- Davila AF, Fleissner G, Winklhofer M, Petersen N (2003) A new model for a magnetoreceptor in homing pigeons based on interacting clusters of superparamagnetic magnetite. *Phys Chem Earth* 28:647–652.
- De Jong D (1982) Orientation of comb building by honeybees. *J Comp Physiol A* 147:495–501.
- Desoil M, Gillis P, Gossuin Y, Pankhurst QA, Hautot D (2005) Definite identification of magnetite nanoparticles in the abdomen of honeybees *Apis mellifera*. *J Phys Conf Ser* 17:45-49.
- Firester AH (1966) Design of square Helmholtz coil systems. *Rev Sci Instrum* 37:1264.
- Frier H, Edwards E, Smith C, Neale S, Collett TS (1996) Magnetic compass cues and visual pattern learning in honeybees. *J Exp Biol* 199:1353–1361.
- Fuxjager MJ, Eastwood BS, Lohmann KJ (2011) Orientation of hatchling loggerhead sea turtles to regional magnetic fields along a transoceanic migratory pathway. *J Exp Biol* 214:2504–2508.
- Gould JL, Kirschvink JL, Deffeyes KS (1978) Bees have magnetic remanence. *Science* 201:1026–1028.
- Griffiths DJ (2013). Introduction to electrodynamics. 4th edition, Prentice-Hall, Upper Saddle, New Jersey.
- Hsu CY, Li CW (1993) The ultrastructure and formation of iron granules in the honeybee (*Apis mellifera*). *J Exp Biol* 180:1–13.
- Hsu CY, Li CW (1994) Magnetoreception in honeybees. *Science* 265:95-97.
- Hsu CY, Chan YP (2011) Identification and localization of proteins associated with biomineralization in the iron deposition vesicles of honeybees (*Apis mellifera*). *PLoS One*.
- Johnsen S, Lohmann KJ (2005) The physics and neurobiology of magnetoreception. *Nat Rev Neurosci* 6:703–712.
- Johnsen S, Lohmann KJ (2008) Magnetoreception in animals. *Phys Today* 61:29–35.
- Kirschvink JL, Kobayashi-Kirschvink A (1991) Is geomagnetic sensitivity real? Replication of the Walker-Bitterman magnetic conditioning experiment in honey bees. *Integr Comp Biol* 31:169–186.
- Lambinet V, Hayden ME, Reigel K, Gomis S, Gries G. Linking magnetite in the abdomen of honey bees to a magnetoreceptive function. *Proc R Soc B* 284: 20162873.

- Liang C-H, Chuang C-L, Jiang J-A, Yang E-C (2016) Magnetic sensing through the abdomen of the honey bee. *Sci Rep* 6:1–7.
- Lohmann K, Pentcheff N, Nevitt G, Stetten G, Zimmer-Faust R, Jarrard H, Boles L (1995) Magnetic orientation of spiny lobsters in the ocean: experiments with undersea coil systems. *J Exp Biol* 198:2041–2048.
- Lohmann KJ (1991) Magnetic orientation by hatchling loggerhead sea turtles (*Caretta caretta*). *J Exp Biol* 155:37–49.
- Lohmann KJ, Lohmann CMF (1996) Detection of magnetic field intensity by sea turtles. *Nature* 380:59–61.
- Lohmann KJ, Lohmann CMF (1994) Detection of magnetic inclination angle by sea turtles: a possible mechanism for determining latitude. *J Exp Biol* 194:23–32.
- Lohmann KJ, Putman NF, Lohmann CMF (2012) The magnetic map of hatchling loggerhead sea turtles. *Curr Opin Neurobiol* 22:336–342.
- Lohmann KJ, Putman NF, Lohmann CMF (2008) Geomagnetic imprinting: a unifying hypothesis of long-distance natal homing in salmon and sea turtles. *PNAS* 105:19096–19101.
- Lohmann KJ, Willows AOD (1987) Lunar-modulated geomagnetic orientation by a marine mollusk. *Sci AAAs* 235:331–334.
- Mann S, Sparks NH, Walker MM, Kirschvink JL (1988) Ultrastructure, morphology and organization of biogenic magnetite from sockeye salmon, *Oncorhynchus nerka*: implications for magnetoreception. *J Exp Biol* 140:35–49.
- Marhold S, Wiltshko W, Burda H (1997) A magnetic polarity compass for direction finding in a subterranean mammal. *Naturwissenschaften* 84:421–423.
- Phillips JB (1977) Use of the earth's magnetic field by orienting cave salamanders (*Eurycea lucifuga*). *J Comp Physiol A* 121:273–288.
- Phillips JB, Freake MJ, Fischer JH, Borland SC (2002) Behavioral titration of a magnetic map coordinate. *J Comp Physiol A* 188:157–160.
- Putman NF, Lohmann KJ, Putman EM, Quinn TP, Klimley AP, Noakes DLG (2013) Evidence for geomagnetic imprinting as a homing mechanism in pacific salmon. *Curr Biol* 23:312–316.
- Quinn TP (1980) Evidence for celestial and magnetic compass orientation in lake migrating sockeye salmon fry. *J Comp Physiol A* 137:243–248.
- Ritz T, Adem S, Schulten K (2000) A model for photoreceptor-based magnetoreception in birds. *Biophys J* 78:707–718.

- Thalau P, Holtkamp-Rötzler E, Fleissner G, Wiltschko W (2007) Homing pigeons (*Columba livia f. domestica*) can use magnetic cues for locating food. *Naturwissenschaften* 94:813–819.
- Vácha M, Drštková D, Půžová T (2008) Tenebrio beetles use magnetic inclination compass. *Naturwissenschaften* 95:761–765.
- Vácha M, Soukopova H (2004) Magnetic orientation in the mealworm beetle *Tenebrio* and the effect of light. *J Exp Biol* 207:1241–1248.
- Válková T, Vácha M (2012) How do honeybees use their magnetic compass? Can they see the North? *Bull Entomol Res* 102:461–467.
- Wajnberg E, Acosta-Avalos D, Alves OC, de Oliveira JF, Srygley RB, Esquivel DMS (2010) Magnetoreception in eusocial insects: an update. *J R Soc Interface* 7:S207–S225.
- Walker M, Bitterman M (1989a) Honeybees can be trained to respond to very small changes in geomagnetic field intensity. *J Exp Biol* 145:489–494.
- Walker M, Bitterman M (1989b) Attached magnets impair magnetic field discrimination by honeybees. *J Exp Biol* 141:447–451.
- Walker MM, Bitterman ME (1985) Conditioned responding to magnetic fields by honeybees. *J Comp Physiol* 157:67–71.
- Walker MM, Baird DL, Bitterman ME (1989) Failure of stationary but not of flying honeybees to respond to magnetic field stimuli. *J Comp Physiol* 103:62–69.
- Wang Y, Pan Y, Parsons S, Walker M, Zhang S. (2007) Bats respond to polarity of a magnetic field. *Proc R Soc B* 274 (1627), 2901–2905.
- Wiltschko R, Wiltschko W (2003) Avian navigation: from historical to modern concepts. *Anim Behav* 65:257–272.
- Wiltschko R, Wiltschko W (2006) Magnetoreception. *BioEssays* 28:157–168.
- Wiltschko W (1980) The earth's magnetic field and bird orientation. *TINS* 140–144.
- Wiltschko W, Wiltschko R (2005) Magnetic orientation and magnetoreception in birds and other animals. *J Comp Physiol A* 191:675–693.
- Wiltschko W, Wiltschko R (1972) Magnetic compass of European robins. *Sci New Ser* 176:62–64.
- Wiltschko W, Wiltschko R (2002) Magnetic compass orientation in birds and its physiological basis. *Naturwissenschaften* 89:445–452.

Winklhofer M, Kirschvink JL (2010) A quantitative assessment of torque-transducer models for magnetoreception. *J R Soc Interface* 7:S273–S289.

Yoshii T, Ahmad M, Helfrich-Förster C (2009) Cryptochrome mediates light-dependent magnetosensitivity of *Drosophila*'s circadian clock. *PloS Biol* 7:e1000086.

4.8. Figures

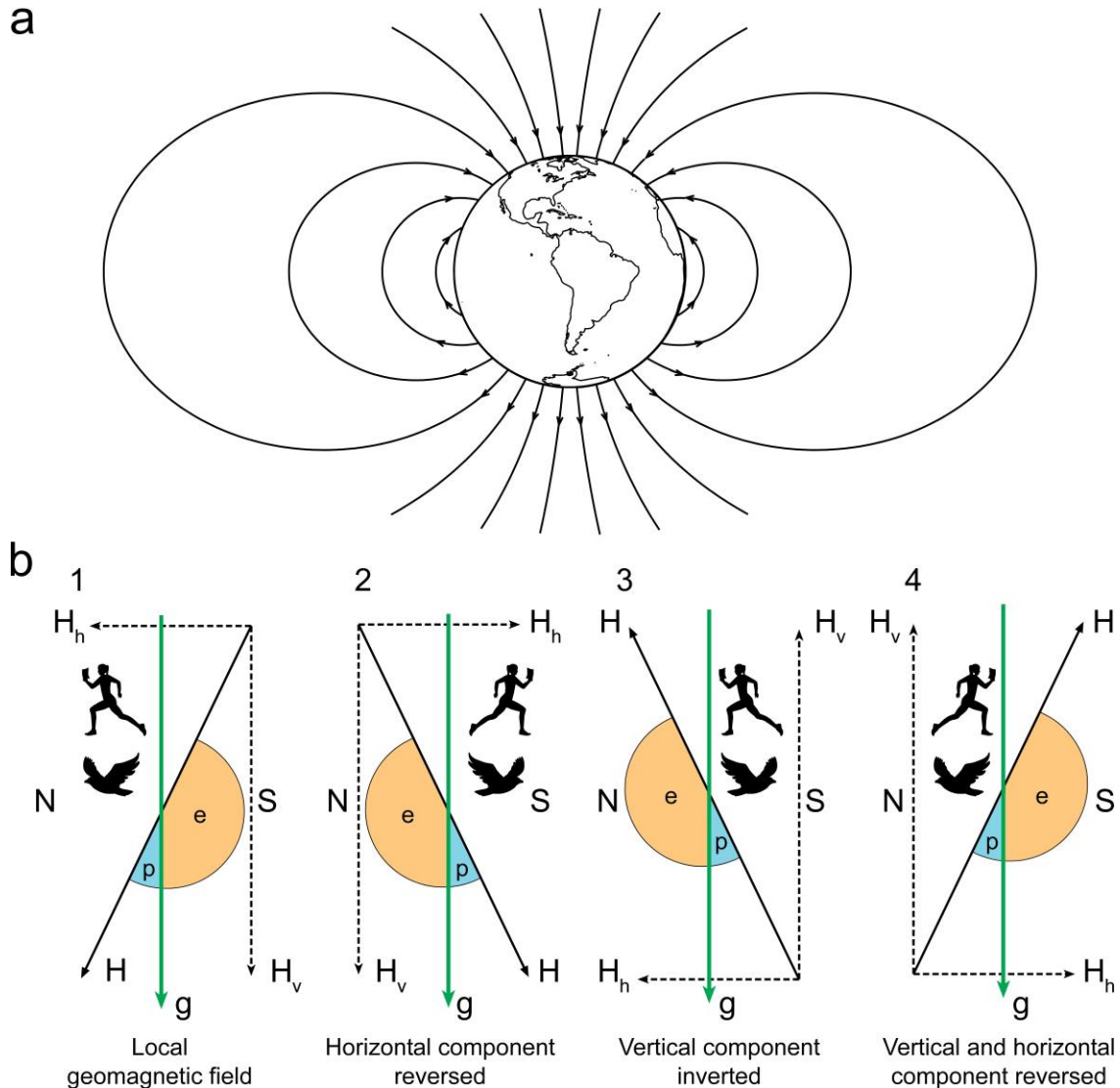


Figure 4.1. (a) Gross features of the geomagnetic field are dipolar, and resemble the field of a (fictitious) centrally-located bar magnet, with field lines emerging from the Earth's surface in the southern hemisphere and re-entering in the northern hemisphere; (b) Graphical comparison of behavioral responses governed by an inclination compass and a polarity compass (adapted from Wiltschko and Wiltschko, 2005). The local magnetic field \vec{H} is decomposed into vertical (H_v) and horizontal (H_h) components, and the sense of the gravitational field is denoted by g . N and S indicate geographic North and South, while p and e denote poleward and equatorward, the readings of the inclination compass. The migration direction of the bird is poleward, which in this example corresponds to North when the field is unaltered. The direction chosen by the orienteer using a polarity compass (an ordinary magnetic compass) is the apparent magnetic north.

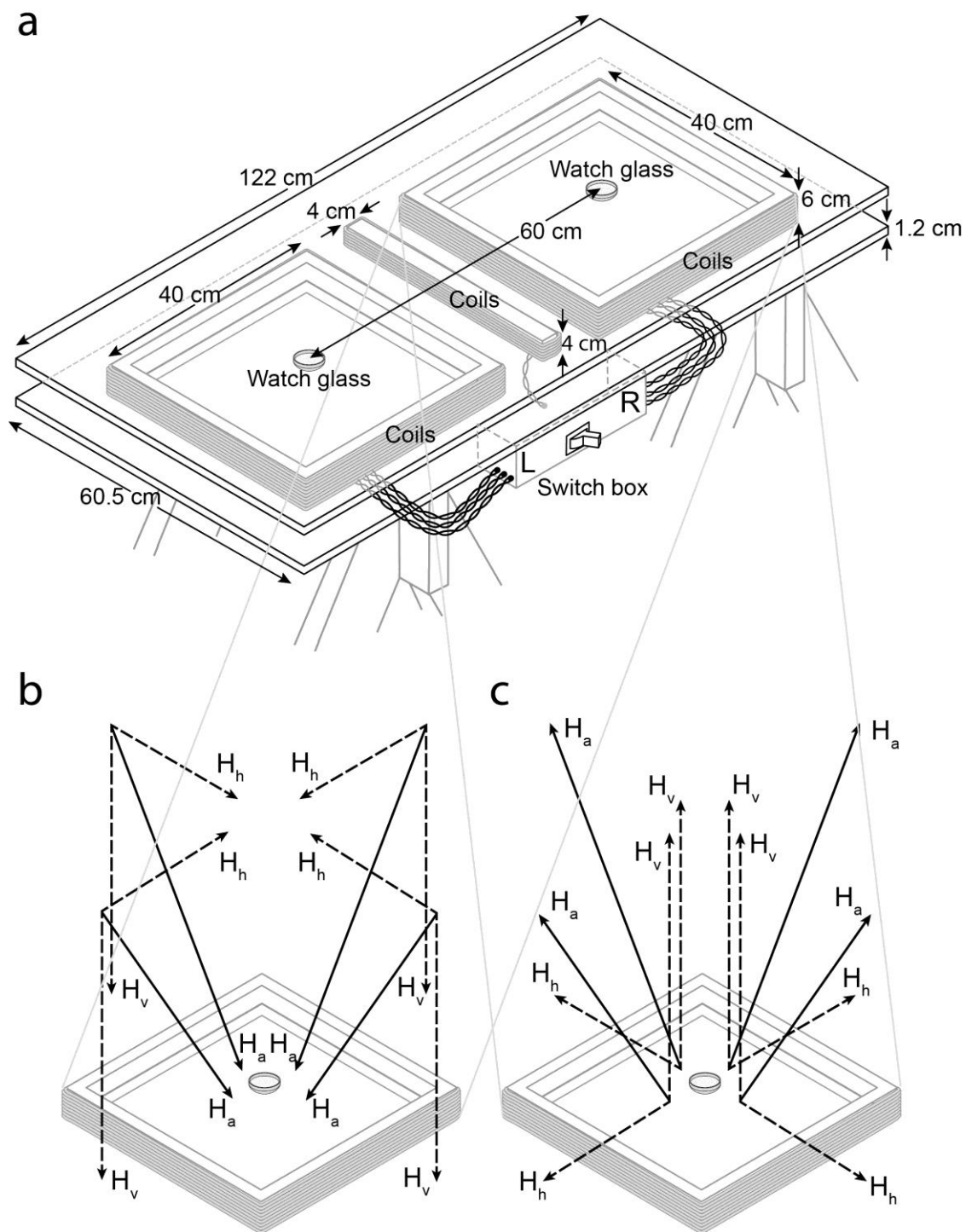


Figure 4.2. (a) Custom-built, two-choice bioassay table. An anomaly in the local magnetic field is generated by one lateral coil or the other. The central coil, in conjunction with the opposite lateral coil, minimize perturbations to the magnetic field on the other side of the table (adapted from Lambinet et al., 2017); (b, c) Schematic representations of the magnetic field in the vicinity of the watch glass when an anomaly is generated, showing decomposition of the field \vec{H}_a associated with the anomaly into vertical (H_v) and horizontal (H_h) components. During training sessions and the control experiment (b) the polarity of the field is such that the magnetic field accessible to the bees generally converges toward the watch glass. During the treatment experiment (c) the polarity of the anomaly is inverted and the magnetic field accessible to the bees generally diverges away from the watch glass. (The full magnetic field produced by the bioassay table is of course a continuous three-dimensional function of position.)

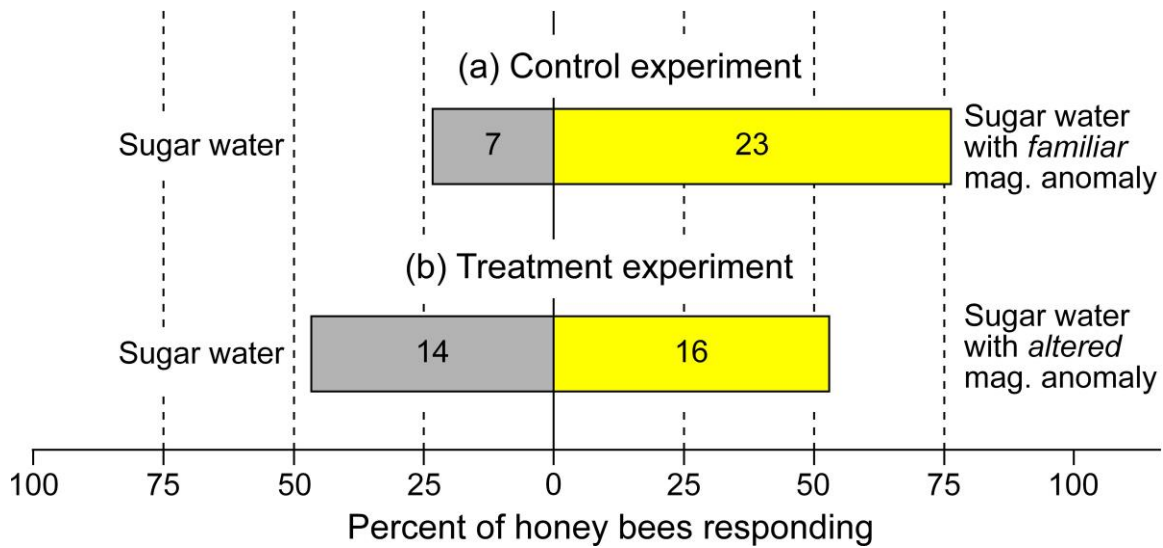


Figure 4.3. Responses of honey bees that were first trained to associate a magnetic anomaly converging toward a watch glass with a sugar reward (Fig. 2a,b). Each trained bee was then subjected to two different experiments, conducted in pseudo-random order. In the control experiment (a), the polarity of the magnetic anomaly was the same (“familiar mag. anomaly”) as it was during the training session. In the treatment experiment (b), the polarity was reversed (“altered mag. anomaly”). Bees continued to respond to the magnetic anomaly in the control experiment (χ^2 test, $p = 0.0035$) but did not respond to it in the treatment experiment (χ^2 test, $p = 0.72$).

Chapter 5.

Evaluation of magnetometry data acquired from elongated samples

A very similar version of this chapter has been published: Michael E Hayden, Veronika Lambinet, Surath Gomis, Gerhard Gries (2017) Review of Scientific Instruments 88: (5) 056106

5.1. Abstract

We document and validate an analytic expression for the flux integral characterizing the response (or sensitivity) of a magnetometer equipped with second-order axial gradiometer coils to long, thin, uniformly magnetized samples. We then demonstrate an application inspired by this analysis, in which magnetometric sensitivity to weak magnetic signatures is readily enhanced simply by increasing the sample volume (and hence the quantity of analyte) well beyond conventional limits.

5.2. Main text

Magnetometers are widely employed for materials characterization. The literature describing their design, operation, and application to diverse systems is correspondingly extensive. Our focus here is on the role of sample geometry in magnetometry experiments. In particular, we examine the interpretation of data acquired from long thin samples that are homogeneously magnetized parallel to their axes. This problem has been addressed previously in a variety of ways, including via brute force techniques in which extended samples are modelled numerically as arrays of point dipoles (Zieba, 1993, Ausserlechner et al., 1998, Stamenov and Coey 2006, Pereira et al., 2011). Our approach is more direct, and is amenable to implementation by casual or non-expert users of magnetometers. We exploit the simple fact that the flux integral characterizing the magnetometer response to long thin uniformly magnetized samples is analytic, and thereby obtain a model function that can be fit to data.

We first review the response of a magnetometer to a point source, when detection is accomplished using second-order axially symmetric gradient coils. This

standard response is then extended to the case of interest: a uniform line source of arbitrary length, magnetized parallel to its axis. Next, data from two experiments are presented. The first validates the line source model, and illustrates the manner in which it can be used to extend the capabilities of a commercial magnetometer. The second highlights an application wherein an unconventional choice of sample geometry enables a significant improvement in sensitivity to weak magnetic signatures.

Consider a point magnetic dipole \mathbf{m} that is coaxial with four identical, series-connected, circular coils of wire arranged in a second-order gradiometer configuration, as shown in Figure 5.1(a). The magnetic flux Φ_i through each coil is equal to the line integral of the vector potential \mathbf{A} evaluated around its periphery. That is

$$\Phi_i = \oint \mathbf{A} \cdot d\mathbf{l}' \quad (1)$$

where

$$\mathbf{A} = \frac{\mu_0}{4\pi} \frac{\mathbf{m} \times \mathcal{R}}{\mathcal{R}^3} \quad (2)$$

μ_0 is the permeability of free space, and $\mathcal{R} \equiv \mathbf{r} - \mathbf{r}'$ denotes the vector displacement between the source point \mathbf{r}' (the location of the dipole) and the field point \mathbf{r} (at which \mathbf{A} is evaluated). The net flux encircled by the coils is thus

$$\Phi = \sum_{i=1}^4 \Phi_i = \frac{\mu_0 m}{2R} F_0(z), \quad (3)$$

where

$$F_0(z) = 2f_0(z) - [f_0(z + \Lambda) + f_0(z - \Lambda)] \quad (4)$$

and

$$f_0(z) = \frac{R^3}{[R^2 + z^2]^{3/2}} \quad (5)$$

are dimensionless response (or ‘sensitivity’) functions, z is the displacement of \mathbf{m} from the gradiometer 2Λ is the axial extent of the gradiometer, and R is the coil radius. In dc magnetometry applications, gradiometer coils made from superconducting wire are employed, and are coupled to a superconducting quantum interference device (SQUID)

via a flux transformer. Changes in the strength, orientation, or position of \mathbf{m} cause changes in persistent currents in the gradiometer coils, which are ultimately registered as changes in the SQUID output voltage. Typically, one measures this voltage as a function of axial sample position and then fits a model equation based on $F_0(z)$ to the data in order to extract an amplitude. This amplitude can then be converted into an absolute value for m , as long as the overall gain of the system is known. Analogous conclusions are reached when other inductively-coupled magnetometry systems (such as vibrating sample magnetometers) and/or other coil configurations (such as first- or higher-order axial gradiometers) are subjected to this analysis.

If instead of the point source discussed above, the same dipole moment \mathbf{m} is uniformly distributed over a line of length 2Δ along the gradiometer axis Figure 5.1b, then

$$\Phi = \frac{\mu_0 m}{2R} \frac{1}{2\Delta} \int_{-\Delta}^{\Delta} F_0(z + \xi) d\xi = \frac{\mu_0}{2R} m F_{\Delta}(z), \quad (6)$$

where

$$F_{\Delta}(z) = 2f_{\Delta}(z) - [f_{\Delta}(z + \Delta) + f_{\Delta}(z - \Delta)] \quad (7)$$

and

$$f_{\Delta}(z) = \frac{R}{2\Delta} \left[\frac{(z + \Delta)}{\sqrt{R^2 + (z + \Delta)^2}} - \frac{(z - \Delta)}{\sqrt{R^2 + (z - \Delta)^2}} \right] \quad (8)$$

are again dimensionless response functions, analogous to $F_0(z)$ and $f_0(z)$. In fact, the latter are simply the limiting forms of $F_{\Delta}(z)$ and $f_{\Delta}(z)$ as $\Delta \rightarrow \infty$. Deviations between $F_{\Delta}(z)$ and $F_0(z)$ are of order 1% or less for $\Delta/R < 0.1$ and 5% or less for $\Delta/R < 0.2$. Importantly, and in contrast to Eqs. 3, 4, and 5 for a point source, no restrictions on sample length are implied by Eqs. 6, 7, and 8. These analytic expressions, and their application to sample magnetometry, are the focus of this note.

Figure 5.2 shows the relative signal amplitude $S(z) \propto (2\Delta / R) F_{\Delta}(z)$ expected for long thin samples of length 2Δ interrogated using second order axial gradiometer coils with aspect ratio $\Delta / R = \sqrt[4]{6}$, as is the case for the *Quantum Design* Magnetic Property Measurement System (MPMS) which has $\Delta = 1.519$ cm and $R = 0.97$ cm (Quantum

Design, 2002). Each sample is assumed to have the same uniform linear magnetization density. Thus, the signal amplitude $S(0)$ at $z = 0$ increases in proportion to the quantity of magnetized material present when Δ/R is small. It reaches a maximum when the sample length is equal to the coil diameter, and then drops to zero as the sample becomes long compared to the gradiometer. This progression is accompanied by a broadening of the response function, and a splitting of the central peak $\Delta/R \gtrsim 1.7$.

To validate this model, we used a model XL-7 MPMS to measure magnetic moments of crystalline ferrous ammonium sulphate hexahydrate powder samples encapsulated in custom fabricated quartz sample holders (cf. Figure 5.3a). Each sample holder consisted of a precision bore quartz tube (15 cm x 4.21 mm; 6.35 mm outer diameter) into which close fitting quartz rods could be inserted from either end, to define a centrally positioned cylindrical void or sample space. The ends of these rods were cut at right angles and cleaned with nitric acid to remove surface impurities. Sample material was pressed into the holder during assembly until a mechanically stable (jammed) configuration was achieved, and then rigidly sealed in place by fusing both ends of the quartz rod and tube. A threaded glass-filled polycarbonate adaptor (Quantum Design, 4096 - 395) was then aligned with the axis of this assembly and bonded to one end using Stycast 1266 epoxy (Henkel Corporation). Seven such $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \times 6\text{H}_2\text{O}$ samples were prepared at a 77(1) % packing fraction and as constructed axial lengths 2Δ spanning the range 1.2 mm to 4.96 cm. An eighth sample holder with a 1.01-cm long sample space was left empty and used to infer the susceptibility of the quartz.

Measurements were performed at 295 K, at magnetic fields H spanning the range $\pm 2\text{kOe}$ ($1.6 \times 10^5 \text{ A/m}$), and at azimuthal sample orientations spanning 360° . At each field, the magnetometer response was recorded as a function of sample position, and a model equation of the form $A+B(z-z_0) + CF_\Delta(z-z_0)$ was fit to the data with A , B , C and z_0 as free parameters. Invariably, the quality of fits so obtained was excellent; representative examples are shown in Figure 5.3. Also shown are the results of a parallel analysis in which $F_0(z)$ was substituted for $F_\Delta(z)$ in the model equation. Consistent with the manufacturer's recommended operating procedures (Quantum Design, 2000), these analyses yield virtually identical results for sample lengths $2\Delta \gtrsim 5$ mm where the point dipole approximation is accurate. Systematic deviations between the two become evident as the sample length is increased.

Absolute values of magnetic moments m were derived from fit parameters C , using manufacturer specified range-dependent and instrument-specific calibration and gain factors, then the MPMS software employs a model function based on $F_0(z)/R^3$ rather than $F_0(z)$. A factor of R^2 (cf. Eqs. 3 and 4), with R in cm, is thus embedded in the overall system gain). These moments were then used in conjunction with sample geometry to calculate magnetization densities M and ultimately to extract volume magnetic susceptibilities $\chi \equiv dM/dH$. Data acquired from the empty sample holder yield the volume magnetic susceptibility of the quartz from which it was manufactured. We find $-1.11(1) \times 10^{-5}$ (SI units; dimensionless). This in turn permits small corrections to magnetic moments observed in connection with the remaining samples, to account for the fact that measurements are made relative to a weakly diamagnetic background.

Figure 5.3c shows the inferred volume magnetic susceptibility χ of $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \times 6\text{H}_2\text{O}$ as a function of sample length. Note that the range of sample lengths spans a factor of 40, and that the longest sample exceeds the recommended limit for satisfying the point dipole approximation by an order of magnitude. The fact that the same susceptibility is observed to within $\pm 2\%$ over this range is strong evidence in support of the underlying model. Residual scatter results from imperfect alignment of samples with the gradiometer axis (Miller, 1996), inhomogeneity of the applied field over the scan length, and variations in the linear density of analyte. For contrast, Figure 5.3c also shows susceptibilities inferred from a conventional analysis in which samples are naïvely treated as point dipoles.

Finally, to illustrate a natural application of the methods discussed above, we turn to a problem that has intrigued biologists for decades: the observation of magnetic remanence in honey bees, and the potential association between the material responsible for that remanence and the known ability of these insects to sense magnetic fields (magnetoreception) (Lambinet et al., 2017). Insofar as magnetometry is concerned, the challenge is that the magnetic moments of interest are very small. They are much weaker than the 10^{-4} emu threshold below which careful attention to sample handling and mounting procedures is essential if artifacts are to be avoided (Garcia et al., 2009, Pereira et al., 2011). More critically, and particularly so below 10^{-6} emu, they can be small enough to be confounded with hysteretic effects that are instrumental in origin (Sawicki and Stefanowicz, 2011, Pereira et al., 2011). One approach to combatting these issues is to increase the quantity of analyte. As an example, in one

experiment we pressed 19 lyophilized honey bee abdomens into a 4 - mm dia. x 1.8 - cm long cylindrical pellet to maximize the signal amplitude, cf. Figure 5.2. We subjected that pellet to analysis by SQUID magnetometry, observed a hysteresis loop, and resolved a net remanent magnetic moment of $4.8(4) \times 10^{-6}$ emu, or an average of $2.5(2) \times 10^{-7}$ emu per bee. This procedure clearly achieves an order of magnitude improvement in moment resolution compared to the result one would expect from a measurement performed on a single bee. It also boosts the magnitude of the remanent magnetic moment out of the 10^{-7} emu range where instrumental sources of hysteresis might compete with, or obscure, the signal of interest. Further examples of data acquired and analyzed in this manner can be found in Lambinet et al. (2017). This strategy is readily adapted to other weakly magnetic systems, as long as sufficient quantities of analyte are available.

In summary, we have described and validated a simple, readily implemented, and useful approach to evaluating magnetometry data acquired from long thin samples.

5.3. Acknowledgement

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5.4. References

A. Zieba, Rev. Sci. Instrum. 64, 3357 (1993).

U. Ausserlechner, P. Kasperkovitz and W. Steiner, Meas. Tech. Sci. 9, 989 (1998).

P. Stamenov and J.M.D. Coey, Rev. Sci. Instrum. 77, 015106 (2006).

L.M.C. Pereira, J.P. Araújo, M.J. Van Bael, K. Temst, and A. Vantomme, J. Phys D: Appl. Phys. 44, 215001 (2011).

Quantum Design, Inc. San Diego CA 92121, USA; Application Note 1014-213 (Feb 4, 2002).

Quantum Design, Inc. San Diego CA 92121, USA; part 4096-395.

Henkel Corporation Loctite; formerly Emerson and Cuming.

Quantum Design, Inc. San Diego CA 92121, USA; Application Notes, 1096-306 (Mar 15, 2016) & 1014-201 (Nov 1, 2000).

M.A. Garcia, E. Fernandez Pinel, J. de la Venta, A. Quesada, V. Bouzas, J. F. Fernandez, J.J. Romero, M.S. Martín González, and J.L. Costa-Krämer, J. Appl. Phys. 105, 013925 (2009).

L.L. Miller, Rev. Sci. Instrum. 67, 3201 (1996).

V. Lambinet, M.E. Hayden, K. Reigl, S. Gomis, and G. Gries, Proc. R. Soc. B 284, 20162873 (2017) and refs. therein.

M. Sawicki, W. Stefanowicz, and A. Ney, Semicond. Sci. Technol. 26, 064006 (2011).

5.5. Figures

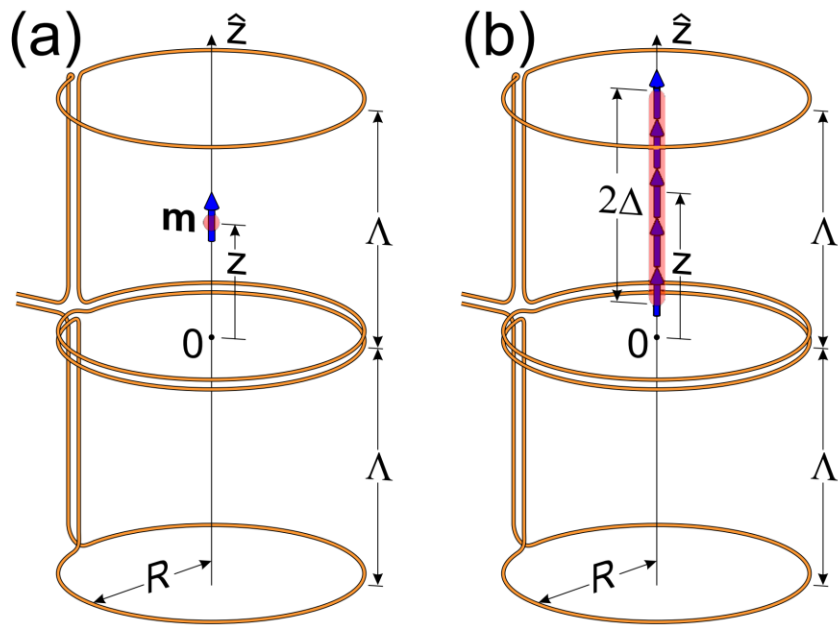


Figure 5.1. Point dipole (b) line sources on the axis of a second – order gradiometer. Note that the outer Coils are counter – wound relative to the inner pair. In (b), z denotes the distance between the midpoints of the source and the coils

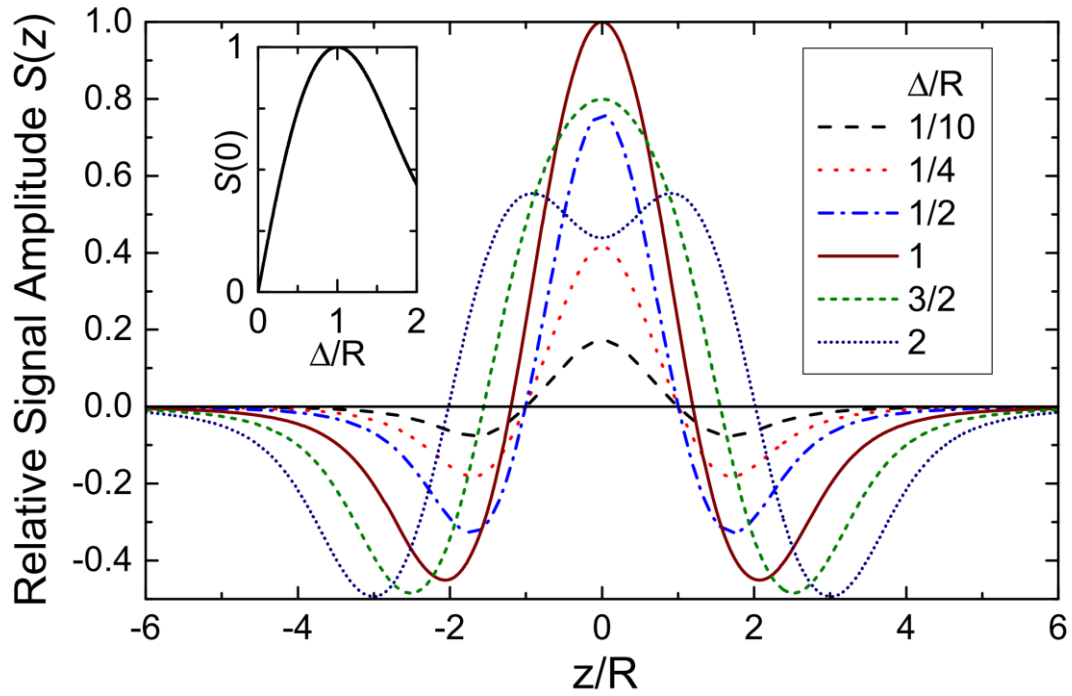


Figure 5.2. Signal amplitude as a function of sample position z , calculated for long thin samples in a gradiometer with $\Lambda / R = \sqrt[4]{6}$. Normalization is with respect to the maximum response, which occurs when the sample length (2Δ) is equal to the coil diameter ($2R$). For the *Quantum Design* MPMS this occurs when the sample is 1.94 cm long. The linear magnetization density of all samples is assumed to be the same. Inset: Response at $z = 0$ as a function of Δ/R .

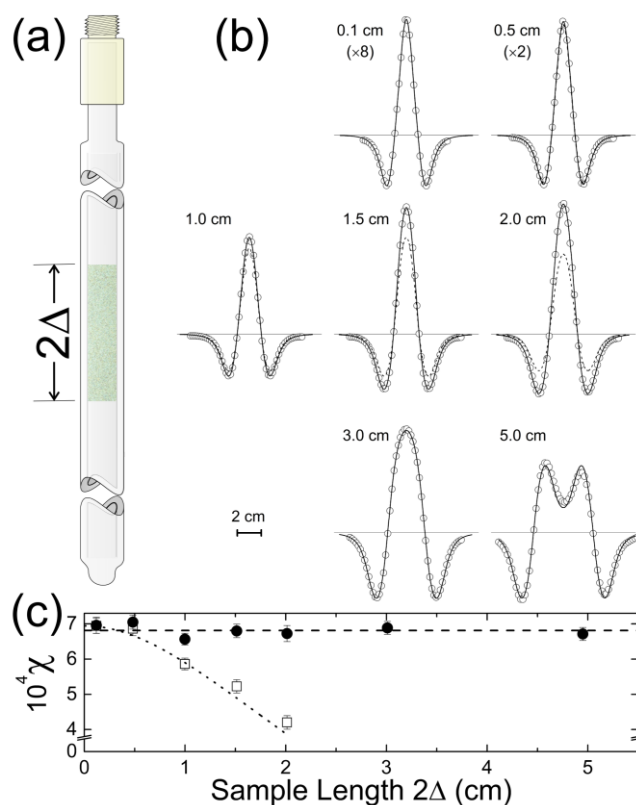


Figure 5.3. Sample and sample holder geometry; (b) Examples of magnetometer response data as a function of sample position (points) and corresponding fits of model equations based on $F_{\Delta}(z)$ (solid lines) and $F_0(z)$ (dashed lines), for samples of various lengths. Background and offset corrections have been applied; (c) Volume magnetic susceptibility χ of $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \times 6\text{H}_2\text{O}$ (SI units; dimensionless) obtained by treating the sample as a uniformly magnetized line source (solid symbols; dashed line showing mean) or as a point source (open symbols; dotted line showing trend). By design, demagnetizing effects are negligible in these experiments.

Chapter 6. Concluding summary

Pertinent background information for my research, and the main findings and conclusions that can be drawn from it, are as follows:

6.1. Chapter 2

- When a forager bee waggle dances on a vertical comb inside the hive, she informs her hive mates about a rich food source. Directional information pointing to that food source relative to the sun's azimuth is encoded in the angle between the waggle run line (the straight segment of her waggle dance) and a reference line. Gravity was implicated as the natural basis for this reference line following the discovery of a gravity-sensing organ in bees. However, it was also conceivable that the local (ambient) geomagnetic field (LGMF) serves as the reference line. Like gravity, the LGMF is uniform and stable over long distances.
- In field experiments, I tested the effect of the LGMF on the recruitment success of waggle dancing forager bees by placing observation hives inside large Helmholtz coils, and then either reducing the LGMF to 2% or shifting its apparent declination from North to East.
- Neither cancelling the ambient magnetic field nor shifting its declination had any measurable effect on the recruitment success of waggle-dancing forager bees. These results likely eliminate the LGMF as a reference for the waggle-run alignment of a dancing bee that attempts to inform her hive mates about a food source. These results also implicate, yet again, gravity as the more plausible reference line, which together with the waggle-run line of the dancing bee appears to form the angle that encodes the direction to a food source.

6.2. Chapter 3

- Previous studies of magnetoreception in honey bees have focused on the identification of magnetic material, its formation, the location of the receptor, and potential underlying sensory mechanisms. A 2016-study has linked proboscis extensions of honey bees to their sensing of magnetic stimuli, and has traced the origin of the neuronal signal triggering the proboscis reflex to the abdomen. This study convincingly demonstrates that the magnetoreceptor of honey bees is located in the abdomen, but does not reveal its material characteristics. In my thesis research, I directly link the magnetic material of bees to a magnetoreceptive function. I demonstrate that ferromagnetic material consistent with magnetite plays an integral role in the bees' magnetoreceptor.

- My experimental approach entailed lyophilizing and pelletizing bee tagmata and subjecting tagma samples to analyses by a Superconducting Quantum Interference Device (SQUID). My analyses revealed a distinct hysteresis loop for the abdomen but not for the thorax or the head of bees, indicating the presence of ferromagnetic material in the bee abdomen.
- Moreover, I show that magnetic remanence of abdomen pellets produced from bees that I did, or did not, expose to the field of a NdFeB magnet while alive differed, indicating that magnet-exposure altered the magnetization of this magnetite in live bees.
- The magnetoreceptive function of the magnetite in the abdomen of honey bees became particularly obvious in two-choice field experiments. Following exposure of live bees to the same NdFeB magnet employed in laboratory studies, these magnetized bees, unlike sham-treated control bees, failed to sense, or respond to, the presence of a magnetic anomaly. This demonstrates a functional connection between magnetite in the abdomen and the magnetoreceptor, and temporary or permanent disablement of the receptor through magnet-exposure.
- It seems conceivable that a chain of single-domain ferromagnetic magnetite crystals is part of the honey bees' magnetoreceptor, and that this chain could crumble or buckle upon exposure to a magnetic field substantially larger than its coercive field. Studies addressing whether exposure to intense magnetic fields renders the magnetoreceptor of honey bees temporarily or permanently dysfunctional would provide insight into the microstructure of the receptor and its ability, or not, to effect repairs. SQUID-based measurements of remnant magnetic moments performed on abdomen pellets of bees that were, or were not, exposed to the fields of a magnet while they were still alive seem to indicate that applied fields reconfigured envisaged chains of ferromagnetic magnetite particles associated with a magnetoreceptor.

6.3. Chapter 4

- Honey bees exploit parameters of the geomagnetic field for orientation during foraging and for alignment of their combs within a hive or nest cavity. They are capable of detecting natural and engineered magnetic anomalies against the geomagnetic background. However, the functional mode of the magnetoreceptor in honey bees was still unknown.
- In general, potential mechanisms of magnetoreception include electromagnetic induction, light-dependent chemical magnetoreception as well as magnetite-based magnetoreception. Terrestrial animals in the "magnetite-based mechanism" group are deemed capable of sensing the polarity of the geomagnetic field. Because honey bees possess ferromagnetic particles (magnetite) in their abdomen that are an integral part of the bees' magnetoreceptor (Chapter 3), I hypothesized that honey bees belong to the "magnetite-based mechanism" group and thus perceive the polarity of a magnetic field.

- To test this hypothesis, I designed a magnetic anomaly that resembled the dipole field of a bar magnet, analogous to the geomagnetic field crudely. In behavioral field studies, I trained bees to associate this anomaly with a sugar water reward. I then subjected trained bees in random order to a control or a treatment experiment. In each experiment, I presented a sugar water reward in two separate watch glasses, placing one reward in the center of the anomaly that I either kept the same as during bee training (control experiment) or that I altered by reversing its polarity (treatment experiment).
- My findings that bees continued to recognize the magnetic anomaly when its polarity was kept unaltered, but failed to recognize the anomaly when its polarity was reversed, support the hypothesis that honeybees have a polarity-sensitive magnetoreceptor.
- The geomagnetic sensitivity of honey bees had been interpreted as evidence that honey bees may possess a polarity compass. In my study, I explicitly tested the hypothesis, and report conclusive experimental data, that honey bees indeed possess a polarity-sensitive magnetoreceptor.

6.4. Chapter 5

- Bee tagmata revealed only weak evidence for ferromagnetism which was further obscured by the diamagnetic response of bee body tissues to applied magnetic fields. I addressed the challenge of detecting ferromagnetism in samples through three approaches: (1) I minimized the diamagnetic component of the signal by lyophilizing bee tagmata to reduce water content; (2) I maximized the signal amplitude by compressing lyophilized bee body tissue into pellets; and (3) instead of choosing sample dimensions in the order of a few millimeters that could be treated as a point source in SQUID measurements (as typically done), I intentionally produced large cylindrical pellets, and then explicitly accounted for sample dimensions in data analyses.

I conclude that my thesis research has significantly advanced our understanding of the magnetic sense in honey bees.