Extending the analogy of the hot water bottle, the inside of the bottle corresponds to the apical surfaces of the squamous and columnar epithelia.

Nearly all research into imaginal disc development has been focused on the columnar cells of the disc proper — after all, they make almost all of the adult organs. But it is becoming clear that the peripodial epithelium cannot be ignored: it is essential for the eversion and fusion of the discs. There is also evidence that peripodial cells are required for growth, patterning and morphogenesis of the disc proper. So we can begin to think of the two-layered disc as a more complex tissue in its own right, with interactions between the layers driving development of the structures that will become the final adult organs.

What is the future for imaginal disc studies? Of course we can't know what will happen in the future. But recent advances make it clear that the live observation of the previously described processes is on the horizon and this will make a big difference. After all, the processes that transform an undifferentiated bag of 50 cells into a complex three-dimensional adult organ are highly dynamic; the ability to study them in real time in future, rather than by snapshots of fixed tissue, should have a dramatic effect on the next few years of imaginal disc research.

Where I can find out more?

Bates, M. and Martínez-Arias, A. (1993). The Development of *Drosophila melanogaster*. (New York: Cold Spring Harbor Laboratory.)
García-Bellido, A., Ripoll, P. and Morata, G. (1973). Developmental compartmentalisation of the wing disc of Drosophila. Nature New Biol. 245, 251–253.
Gibson, M.C., and Schubiger G. (2000). Peripodial cells regulate proliferation and patterning of Drosophila imaginal discs. Cell 103, 343–350.
Held, L.I. (2002). Imaginal Discs, the Genetic and Cellular Logic of Pattern Formation. (Cambridge: Cambridge University Press.)
Hartenstein, V. (1993). Atlas of *Drosophila*. (New York: Cold Spring Harbor Laboratory.)

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Magnetoreception

James L. Gould

Few subjects in animal behavior have more exotic mystery than magnetic-field sensitivity. A force we cannot sense, generated by events no one completely understands, creates field lines that pass through our bodies without any evident effect on us or on them. It is an energy felt as much by migrating lobsters on the sea floor as by ocean-crossing birds thousands of meters overhead, transduced in generally poorly understood ways. Despite the blindness of humans, modern life depends on this invisible, ghostlike field. Aside from lights and heaters, nearly every electrical device we own makes use of electromagnetism, and that same magnetism is essential in generating the power these new-found necessities consume. But for many animals, the reliance is far older and more basic: their life-or-death ability to find their way around in the world depends on correctly interpreting the earth's magnetic field.

Magnetism

As any survivor of introductory college physics knows, a flow of electrons generates a magnetic field around it, while a changing or travelling magnetic field induces movement in nearby electrons. Each electron is a tiny magnetic-field generator: it has a spin (of which there are two quantum-mechanical states, 'up' and 'down') plus, if it is part of an atom, an orbital motion relative to its nucleus. The spinning produces roughly twice the field strength of orbiting. These two fields are not normally directionally correlated. For most biomagnetic effects, spin is the key element.

The precise origin of the earth's field is still controversial, though the broad outlines seem clear (by exclusion if for no better reason). The earth's outer core consists of a flowing but viscous metallic liquid. The convective transfer of heat from the radioactive inner core along with the rotation of the earth keeps the molten iron core in motion, probably in part through the Coriolis effect. The resulting irregular convection cells and gyres are aligned roughly north–south and generate most of the field felt by animals at the earth's surface. Because of the geometry of the field, the magnetic force lines at most locations slant down toward the nearer pole at an angle called the 'dip'.

As might be expected of an electromagnet created in such a haphazard way, the earth's magnetic field varies over time in average strength, direction, and even polarity. Near the short-term end of the scale, the north magnetic pole has wandered about 1.4° north and 3.6° west just in the last five years; this corresponds to a migration of about 175 m per day. Shifting cells and gyres are presumably at fault. At the long-term extreme, complete reversals occur (very) roughly every 300,000 years, though the last was 780,000 years ago; a reversal now would be extremely inconvenient to humans and other animals alike. Clearly, at least some creatures needing to make use of such a fickle field must be able to calibrate themselves to the field strength and direction at least once in their lifetime; some do so daily [1].

While this dynamo theory of circulating metals helps account for more than 95% of the magnetism, there is a much more variable residual component with clear short-term effects on at least homing pigeons and honey bees. The earth's jet streams capture ions from the ionosphere and move them eastward at up to hundreds of kilometers per hour. As moving electrons, they generate their own magnetic field. The jet streams shift north and south on a daily basis simply as a consequence of the warming and cooling (and consequent expansion and contraction) of the atmosphere. From day to day and week to week they can migrate across hundreds of kilometers of latitude, changing not only the weather below but the strength and direction of the small magnetic field they induce. During magnetic 'storms' the sun delivers huge numbers of new ions to the jet streams, briefly and unpredictably amplifying this induced field [2].

Maps and compasses

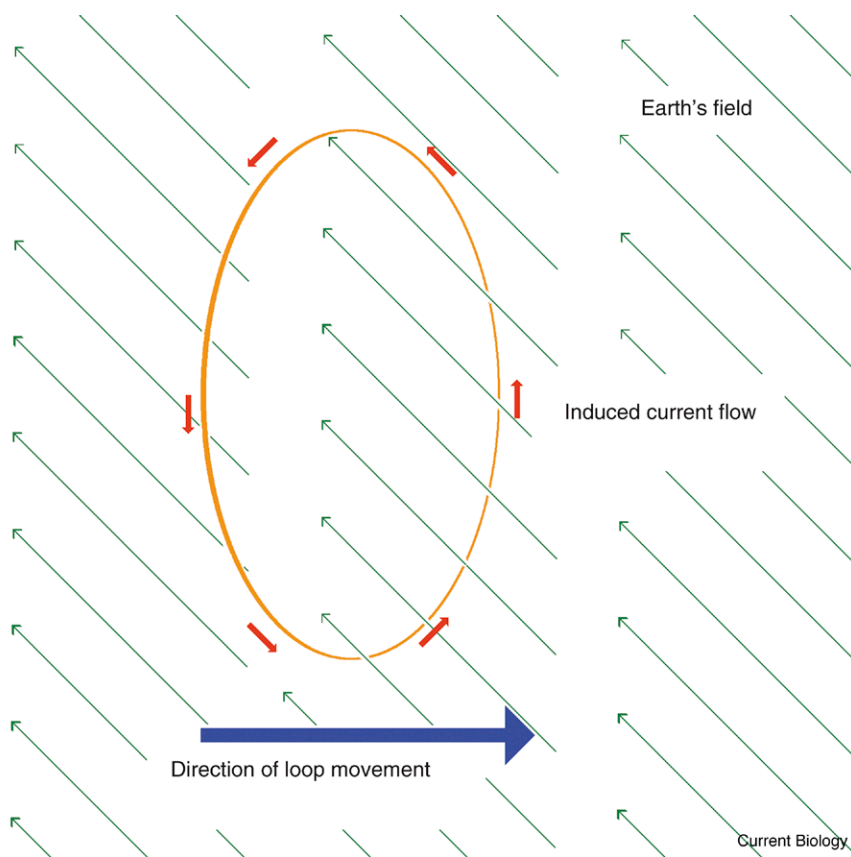Aside from evidence that honey bees can use the diurnal variations

Figure 1. Induction.

When a conducting loop moves through a magnetic field (or a field moves relative to the loop) an electrical current is induced. The flow depends on velocity, the geometry of the movement relative to the field, the cross-sectional area of the loop, the conductance of the loop, and the strength of the field.

of the induced field to recalibrate their circadian clocks, animals are thought to use magnetic field parameters primarily to determine direction and relative location, as well as an often indirect way of recalibrating alternative celestial compasses. Each putative use has its own challenges.

With regard to compasses, the conventional view is that the earth's magnetic field is used as a backup to celestial (sun, polarization-based, or star) compasses [3]. There are some species, however, for which the magnetic compass appears to be the primary tool. The experiments that originally led to this view involved clock shifts: pigeons kept on six-hour-fast or six-hour-slow regimens were compared to birds maintained under the same artificial conditions but without shifting. When released on sunny days at noon, the fast group acts as though it is 6 pm, while the late group

interprets the time to be 6 am. Using the southern sun as their compass, the late group departs roughly 90° clockwise of home; the slow group instead judges the sun to be in the east and flies off approximately 90° counter-clockwise of home. Under overcast conditions, however, the birds are accurately oriented; clearly, they are using a secondary compass [4]. Applying a strong static magnetic field disrupts the homing on cloudy days, but not when the sun — the primary compass — is visible [5,6].

The most obvious challenge for animals is the 160 km discrepancy between the earth's axis of rotation and corresponding north magnetic pole. (The south magnetic pole is much farther from its geographic counterpart. The rotational and effective magnetic axes of the earth are offset by about 11.5° overall.) This discrepancy means that at most locations on the earth's surface

there is an angular difference (declination) between the geographic and magnetic poles. This error is typically greatest at high latitudes, but it can be as large as 30° even in the temperate zone.

The easiest theoretical solution is to measure the declination, using the pole point as a guide — the spot in the sky around which the stars, the sun, or the polarization of the daytime sky appear to rotate. This would be an especially serious challenge for aquatic animals, with their restricted access to celestial cues. Worse yet, this solution is location specific: migrating species moving through latitude or longitude may experience major changes in local declination. The ability to recalibrate would be essential.

The theoretical possibility that components of the earth's magnetic field strength might be used as a map has been evident since the time of Edmond Halley (when he proposed the idea as a way of determining longitude). The total intensity changes by a factor of two from the magnetic equator to the magnetic poles. Vertical intensity — and the corresponding dip angle, the angle of the magnetic field lines relative to the earth's surface — varies in a somewhat different and more extreme pattern over the planet. Total intensity and vertical intensity/ dip angle are each highest at the poles. The two parameters form a skewed grid, intersecting at roughly 24° in the northeastern US [7].

Taking advantage of a magnetic map would require substantially greater sensitivity than merely determining compass direction; it would also necessitate more elaborate calibration. At home or another reference location an animal must measure the local total- and vertical-field strengths. It must then measure the gradient for both components — the rate at which they change over distance—as well as the direction of each gradient. Say, for instance, that the total intensity at home is 45,000 nT and the vertical intensity is 25,000 nT; imagine too that the total intensity gradient is 5nT/km to the NW and the vertical intensity gradient is 10 nT/km to the NNW. An animal finding itself at a location with a total intensity of 44,287 nT and a vertical intensity of 24,235 nT would infer that it is 200 km due east of home [7].

This example demonstrates that the sensitivity of any field-strength measurement would need to be very high. To account for the apparent accuracy of vision-impaired homing pigeons, a breathtaking 10 nT resolution would be just adequate [8]. Another problem is the varying induced field generated overhead. Animals would need to compensate for these slow changes in a way analogous to the adjustments birds (and human pilots) make to the ever-varying barometric pressure — a cue important in judging altitude. Solar storms would prove more difficult, as evidence from homing pigeons suggests is indeed the case. Magnetic anomalies — places where the field strength is enhanced, usually because of shallow iron deposits — are another challenge; pigeons seem to deal with this problem by simply flying magnetically downhill until they find a normal gradient, when they appear to determine relative location [9].

Induction

If animals had a set of copper coils and an ammeter they could simply move across the field and measure the current this motion induces in the circuit (Figure 1). The amplitude of the resulting flow of electrons would depend on the cross-sectional area of the coil, the speed of the organism, the angle between the direction of travel and the field, and the resistance of the coil. For an accurate measure of compass direction the creature would need to compare the flow in two or more directions at least 60° apart — that is, it would probably do well to circle, as homing pigeons do upon release.

Theoretical calculations have never been very encouraging for the coil/ammeter model: animals move too slowly, are too small to have sufficiently large detectors, and have too much resistance in the fluids that could serve as conductors. Nevertheless, at least some elasmobranchs use induction to judge direction—or, at least, they can be trained to do so in the lab. They employ their ampullae of Lorenzini — jelly-filled canals which apparently evolved as electroreceptors designed to detect the vanishingly weak electrical emanations of potential prey. These
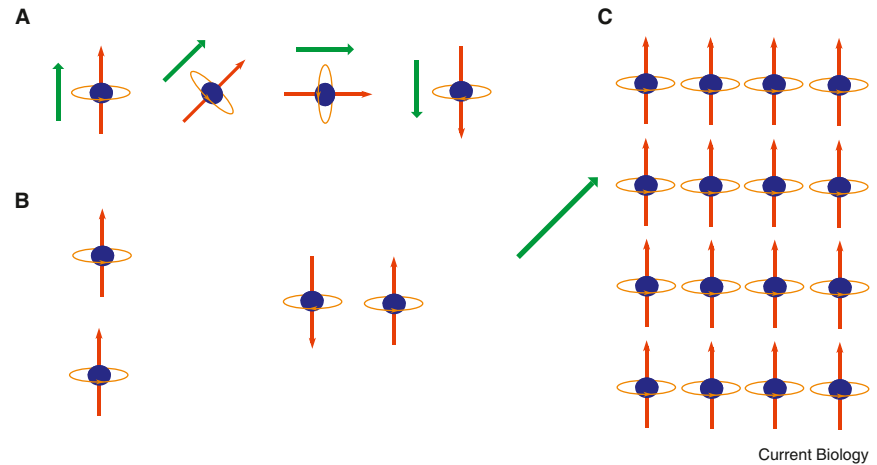

Figure 2. Electron spins.
(A) Isolated electrons will align their spins with any outside field — most often the earth's (the large green arrow to the left); this is a simple paramagnetic interaction. (B) Just like magnets, pairs of electrons close enough together will generally align their fields either end to end or side to side in an antiparallel arrangement; the latter is more stable, but creates no net field. This is a superparamagnetic interaction. (C) In certain substances with a special set of lattice angles and spacings the aligned end-to-end spacing of atoms with unpaired electrons can dominate over the antiparallel side-to-side spacing, producing (if the crystal is large enough) a permanent magnet.

are not coils, but when combined with the surrounding seawater — which provides a huge return path for induced electrical flow — they operate in the same way [10].

Paramagnetism

Each electron is a miniature dynamo, generating its own very local field. Every filled electron shell in an atom has a complete set of electron pairs. Because each pair includes a spin-up and a spin-down electron, their personal magnetic fields cancel one another out. But unpaired electrons are free to interact with external fields — the earth's, typically. Many materials (particularly bismuth) are weakly diamagnetic; they produce a slight field in the opposite direction of the external field. Much stronger is the paramagnetic interaction of unpaired electrons with any applied magnetic force: the electron spins become statistically aligned and thus amplify the external field. (I say "statistically" because thermal noise, kT, is acting at the same time to randomize the orientations.) Changing the direction of the external field causes the electrons to realign almost instantly (Figure 2A); canceling the field allows them to lose their alignment immediately, and with it the small net paramagnetic field they had been generating [11].

While paramagnetic alignment by itself seems useless, it could in theory be used to modulate another source of energy — most usefully, light. The first suggestion along these lines invoked optical pumping, in which a photon of the appropriate wavelength raises an unpaired electron to a higher energy level. The earth's field would bias the spin direction, modulating the strength of the effect. A later elaboration of this photon/electron-spin interaction is the radical-pair hypothesis, which has attracted considerable notice. In this model, light generates two radicals (atoms with a supernumerary unpaired electron); the spins of these extra electrons are correlated (parallel or antiparallel) and decay back to the normal state along a time course that depends on the external field [12].

The key signatures of an optical/paramagnetic effect are: first, a dependence on a specific wavelength of light (and corresponding lack of sensitivity to other colors, in complete darkness, or when the optic nerve is severed); second, a sensitivity to jamming with a particular frequency of radio waves; and third, an insensitivity to polarity — that is, the putative detector could determine the horizontal direction and dip angle
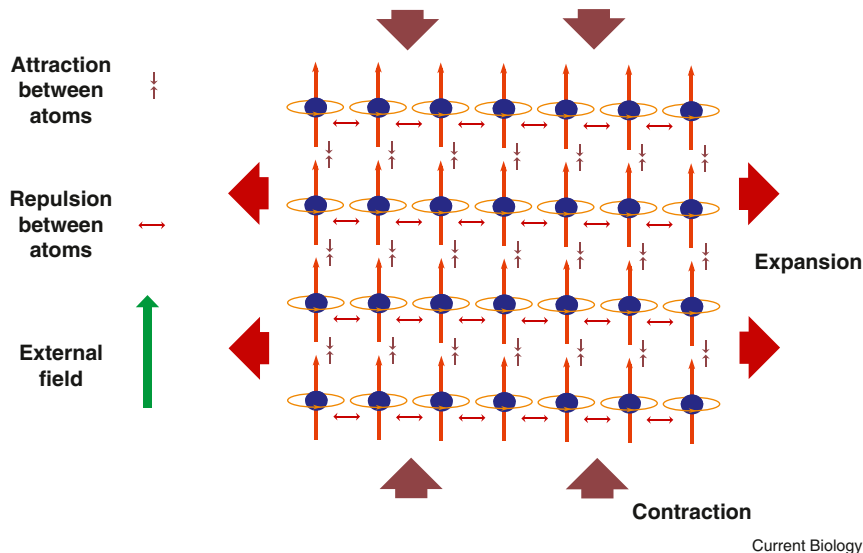
Figure 3. Internal forces in a superparamagnetic crystal.

The spins of the unpaired electrons in a crystal too small to have a permanent, self-stable field orient themselves to be parallel with the external (earth's) field (green arrow). Atoms with spins aligned end to end attract each other, causing the crystal to contract along the N–S axis. Atoms to each side, on the other hand, repel one another, leading to expansion in the other two axes.

of the field lines, but would confuse north with south.

A variety of tests on migrating birds and some invertebrates indicate that their compass sense is indeed blind to polarity and requires at least dim light. The action spectrum of their magnetic sensitivity roughly corresponds to the absorption of cryptochrome, an all-purpose blue/UV pigment that underlies the germination response and photoperiodism in many plants, as well as circadian rhythms in animals. Elegant genetic work shows that inactivating the cryptochome system knocks out most of the compass response in birds and some insects. Curiously enough, in birds, only the right eye seems to be involved. More recent work, however, while still supporting a paramagnetic cryptochrome-based compass, casts considerable doubt on the radical-pair mechanism [13].

Superparamagnetism

Many substances are paramagnetic, interacting with an external field such as the earth's. A much smaller group can form crystals with a particular alignment and spacing that allows the individual paramagnetic atoms to also reliably 'feel' and respond to the fields of unpaired electrons in adjacent

atoms. In the absence of an external field, the spins can align themselves fleetingly in chains or as antiparallel pairs (Figure 2B); for a given separation, the chain orientation is stronger — that is, it better resists the randomizing effects of thermal noise. The most familiar substance capable of forming superparamagnetic crystals is magnetite ($FeO \cdot Fe_2O_3$) in grains (technically 'domains') smaller than about 0.05 µm (the exact size depends on geometry and temperature).

In the simplest case, applying an external field to an idealized superparamagnetic crystal (Figure 3) aligns the unpaired spins. Atoms whose spins are aligned head to tail will attract one another, much as the North end of a bar magnet will attract the South end of another:

NS →← NS →← NS →← NS →← NS

Atoms to the side with aligned paramagnetic fields will tend to repel one another:

N ←→ N ←→ N ←→ N ←→ N
S ←→ S ←→ S ←→ S ←→ S

In theory, then, the crystal should experience a contraction along the axis of the external field and

an expansion across this axis (Figure 3). (The actual geometry of superparamagnetic crystals is more complex than this, with 'hard' and 'soft' alignment susceptibilities and — usually — two interdigitated lattices, but the basic principle is the same.) Like paramagnetic models, a superparamagnetic system ought not to distinguish magnetic polarity [11].

The best candidate for ownership of superparamagnetic detectors are honey bees, which are so sensitive to magnetic fields that they can track small changes in the induced field — changes which alter their dances and judgement of time. They can also be trained in the lab to differentiate minute differences in field strength [14]. While some bees have permanently magnetic grains as well (discussed below), all have vast numbers of uniformly sized superparamagnetic domains localized in a specific class of innervated cells. In theory, bees have enough of these crystals to measure field-strength changes far, far weaker than the minimum necessary to account for their behavior [15].

Superparamagnetic crystals are classified as either ferromagnetic (the case illustrated in Figure 3, and typical of alloy magnets) or ferrimagnetic (in which atoms with weak antiparallel spins in one lattice alternate with the more dominant unpaired electrons in another). The best known ferrimagnetic substance is magnetite, which is synthesized biologically by a variety of organisms. When small, magnetite crystals are superparamagnetic. As they grow, their self-reinforcing internal fields become strong enough to maintain magnetic moment independent of the external field — that is, they become permanent magnets (Figure 2C). And at this same instant the crystals become sensitive to the polarity of the earth's field, and instead of producing a compression/expansion of the crystal, an external field induces a torque as the domain seeks to align itself [11].

Permanent magnets

As a permanently magnetic crystal grows further, it spontaneously reorients its electron spins to create two magnetic domains with opposite polarities. This arrangement is more stable because the two fields

cancel one another and there is no longer any net magnetic moment. This process continues with more domains forming as the crystal continues to enlarge [11]. By applying a strong external field, however, all the domains in a crystal (ferromagnetic) or the primary sublattice (ferrimagnetic) become aligned; this is the way most commercial magnets are made, as well as being the trick used for storing information on magnetic media such as recording tapes and hard drives.

Another way to align the domains is to construct a long thin crystal. The magnetic moment of the first domain will align itself with the external field, with a strong bias toward the long axis of the domain. As a second forms in the chain the most likely (most stable) alignment will be along the axis of the chain. Each additional domain strengthens this tendency, producing what is to all intents a long bar magnet.

The first organism found to employ an aligned chain of permanent magnets was a species of sediment-dwelling, low-oxygen bacteria [16]. The magnetic material is magnetite, the densest substance made biologically. Quite possibly synthesis of a magnetite grain evolved to provide a weight — an element of gravity bias: a high-density crystal near the head would induce a small net tendency to point downwards. A chain of permanent magnets more reliably (but still passively) rotates the organism into alignment with the earth's dip angle, aiming it back to the safety of the bottom [14].

Analogous chains of magnetite are evident in salmon, a migratory species with evident magnetic sensitivity. They are found in the ethmoid sinuses, small elements of the paranasal sinus complex located between the olfactory and optic nerves, and innervated by a branch of the trigeminal nerve [17]. This is the same location in which enormous numbers of permanent-domain magnetite crystals are found in homing pigeons [18], migratory birds, sea turtles, dolphins, and other vertebrates. Localized concentrations of magnetite are found in a variety of invertebrates as well.

Diagnostic characteristics of a magnetite-based compass are polarity sensitivity, light independence, long-lasting disruption by strong magnetic pulses (which, properly applied, can serve to reverse the polarity of some or all domains), independence from radio-frequency jamming and, in vertebrates, loss of function when the trigeminal nerve is anesthetized or severed. It is particularly well suited to animals without access to blue/UV light such as hive-dwelling, subterranean, nocturnal, or deep-sea creatures.

Maps based wholly or in part on magnetic-field-strength information are implicated in a variety of animals, both vertebrates and invertebrates [7]. The typical pattern is to find that very small changes in field strength have enormous effects on homing, whether or not the primary (celestial) compass is visible [3]. Implausibly large changes are ignored. Strong magnetic pulses (which only affect permanent magnets) often impair or destroy orientation for hours or days. In experimentally convenient species, where the several field parameters can be precisely controlled, the resulting orientation corresponds with the magnetic-map predictions [7] (though most tests have manipulated only latitude cues; displacement of the same species by up to 5000 km in longitude results in equally precise reorientation [19]).

Magnetite-based sensors are the only plausible candidates with the requisite sensitivity to judge map-level changes in field strength and direction [11]. One increasingly popular model is that many migrating birds use the paramagnetic system in the eye to judge compass direction and the permanent-magnet system in the ethmoid as the basis of their location sense [20]. One conjecture is that the magnet-based mechanism evolved first as a compass, and then reinvented itself as a map when the cryptochrome-centered approach to direction sensing evolved.

In the end, however, the only detailed mechanisms and processing strategies that can be tied to a magnetic-orientation behavior with absolute certainty are the induction-based conditioning of elasmobranchs and the passive rotation of bacteria with magnetite chains. Everything else has some degree — often quite a large degree — of conjecture still attached to it. It is in the nature of wide-ranging animals to create extraordinary technical hurdles, but one suspects that there is more subtlety to the maps and compasses than our current philosophy imagines.

References

1. Gould, J.L. (1995). Constant compass calibration. Nature 375, 184.
2. Keeton, W.T., Larkin, T.S., and Windsor, D.M. (1974). Normal fluctuations in the earth's magnetic field influence pigeon orientation. J. Comp. Physiol. 95, 95–103.
3. Gould, J.L. (1980). The case for magnetic sensitivity in birds and bees. Am. Sci. 68, 256–267.
4. Keeton, W.T. (1969). Orientation by pigeons: is the sun necessary? Science 165, 922–928.
5. Keeton, W.T. (1971). Magnets interfere with pigeon homing. Proc. Natl. Acad. Sci. USA 68, 102–106.
6. Walcott, C., and Green, R.P. (1974). Orientation of homing pigeons is altered by a change to the direction of an applied magnetic field. Science 184, 180–182.
7. Lohmann, K.J., Lohmann, C.M.F., and Putman, N.F. (2007). Magnetic maps in animals: nature's GPS. J. Exp. Biol. 210, 3697–3705.
8. Schmidt-Koenig, K., and Walcott, C. (1978). Tracks of pigeons homing with frosted lenses. Anim. Behav. 26, 480–486.
9. Wagner, G. (1976). Das Orientierungsverhalten von Brieftauben im erdmagnetisch gestörten Gebiete des Chasseral. Revue Suisse Zoologie 83, 883–890.
10. Kalmijn, A.J. (1978). Experimental evidence of geomagnetic orientation in elasmobranch fishes. In Animal Migration, Navigation, and Homing, K. Schmidt-Koenig and W.T. Keeton, eds (Washington DC: U.S. Government Printing Office), pp.345–353.
11. Kirschvink, J.L., and Gould, J.L. (1981). Biogenic magnetite as a basis for magnetic field detection in animals. BioSystems 13, 181–201.
12. Ritz, T., Adern, S., and Schulten, K. (2000). A model for photoreceptor-based magnetoreception in birds. Biophys. J. 78, 707–718.
13. Gegear, R.J., Foley, L.E., Casselman, A., and Reppert, S.M. (2010). Animal cryptochromes mediate magnetoreception by an unconventional photochemical mechanism. Nature 463, 804–807.
14. Walker, M.M. and Bitterman, M.E. (1989). Honey bees can be trained to respond to very small changes in geomagnetic field intensity. J. Exp. Biol. 145, 489–494.
15. Gould, J.L., Kirschvink, J.L., and Deffeyes (1978). Bees have magnetic remanence. Science 201, 1026–1028.
16. Blakemore, R.P. (1975). Magnetotactic bacteria. Science 190, 377–379.
17. Kirschvink, J.L., Walker, M.M., Chang, S.-B., Dizon, A.E., and Peterson, K.A. (1985). Chains of single-domain magnetite particles in chinook salmon. J. Comp. Physiol. A 157, 375–381.
18. Walcott, C., Gould, J.L., and Kirschvink, J.L. (1979). Pigeons have magnets. Science 205, 1027–1029.
19. Gould, J.L. (2008). Animal navigation: the longitude problem. Curr. Biol. 18, R214–R216.
20. Wiltschko, W., and Roswitha Wiltschko, R. (2008). Magnetic orientation and magnetoreception in birds and other animals. J. Comp. Physiol. A 191, 675–693.

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