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# Behavioral evidence for geomagnetic imprinting and transgenerational inheritance in fruit flies

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**Certain long-distance migratory animals, such as salmon and sea turtles, are thought to imprint on the magnetic field of their natal area and to use this information to help them return as adults. Despite a growing body of indirect support for such imprinting, direct experimental evidence thereof remains elusive. Here, using the fruit fly as a magnetoreceptive model organism, we demonstrate that exposure to a specific geographic magnetic field during a critical period of early development affected responses to a matching magnetic field gradient later in life. Specifically, hungry flies that had imprinted on a specific magnetic field from 1 of 3 widely separated geographic locations responded to the imprinted field, but not other magnetic fields, by moving downward, a geotactic behavior associated with foraging. This same behavior occurred spontaneously in the progeny of the next generation: female progeny moved downward in response to the field on which their parents had imprinted, whereas male progeny did so only in the presence of these females. These results represent experimental evidence that organisms can learn and remember a magnetic field to which they were exposed during a critical period of development. Although the function of the behavior is not known, one possibility is that imprinting on the magnetic field of a natal area assists flies and their offspring in recognizing locations likely to be favorable for foraging and reproduction.**

magnetic imprinting | geomagnetic field | fruit fly | transgenerational inheritance | magnetoreception

The ability to sense geomagnetic fields (GMFs), and use this information to guide movement across a broad range of spatial scales is widespread among animals (1–3). The GMF provides animals with 2 potential types of information. The simplest is directional or compass information, which enables an animal to maintain a consistent heading in a particular direction such as north or south (2, 4). Some animals can also derive positional or "map" information from the GMF about different geographic locations (5). This information is thought to be used to follow complex migratory routes (6, 7) or facilitate navigation toward a specific target area (8, 9).

Several magnetically sensitive animals, such as salmon and sea turtles, leave their natal areas when young, migrate long distances, and then return to reproduce as adults. Growing evidence suggests that salmon and sea turtles imprint on their natal magnetic field (MF) and use this information to return on maturity (6, 10–13). Until now, however, there has been no direct experimental evidence that animals are truly capable of geomagnetic imprinting; in other words, that they can learn and remember a MF to which they were exposed during a critical period of development.

The *Drosophila* fruit fly can orient magnetically and is a useful model system for studying magnetoreception (14–17). Recent studies have reported that a static MF can disrupt innate negative geotactic behavior in flies (18), and an earth-strength MF was found to be a sensory cue for positive geotaxis of starved fruit flies in a food-conditioning assay, modulating vertical food-search movement (19). The present study investigated whether the experience of developing in a MF characteristic of a particular geographic area altered fly behavior therein as adults. Our results indicated that the specific MF experienced by flies during a critical period before hatching later elicits positive food-searching geotaxis in starved adult flies, whereas other fields do not. This change in behavior was also shown to be transmitted to the next generation under certain conditions.

## Results

**Food-Seeking Geomagnetic Imprinting Behavior in Flies.** We used a tube-positioning assay as described previously (19) to determine whether flies imprinted on a specific earth-strength MF during different developmental stages. The MF used for this imprinting was the local field at Daegu, Republic of Korea, as estimated by the International Geomagnetic Reference Field model (version IGRF-12) (20) (*SI Appendix*, Table S1). Because of the steel beams in the laboratory building, the Daegu field differed significantly from the field measured in the laboratory (*SI Appendix*, Table S1). Hereafter, the ambient MF in the laboratory to which the flies were preexposed is referred to as the sham field.

Eggs, larvae, and pupae were exposed to the Daegu MF for specific, limited periods during development (Fig. 1 *A*, *Upper*). Geotactic responses in starved adult flies were subsequently

## Significance

**Although migratory animals, such as salmon and sea turtles, are thought to imprint on their natal magnetic field and use this information to return as adults, direct experimental evidence thereof remains elusive. This study experimentally demonstrates that organisms can imprint a magnetic field and that this imprinting can be inherited by their progeny. Prenatal exposure to a specific geographic magnetic field during development affected adult responses to the matching field gradient through downward movements associated with foraging. This same behavior occurred spontaneously in the progeny of the next generation. These results suggest that imprinting on the magnetic field of a natal area assists magnetoreceptive organisms and their offspring in recognizing locations favorable for foraging and reproduction.**

Author contributions: K.-S.C. initiated the study; H.-J. Kim and K.-S.C. designed research; I.-T.O., H.-J. Kwon, and K.-S.C. performed research; S.-C.K. and K.-S.C. contributed new reagents/analytic tools; I.-T.O., H.-J. Kim, and K.-S.C. analyzed data; and H.-J. Kim, K.J.L., and K.-S.C. wrote the paper.

The authors declare no competing interest.

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Data deposition: The raw data of this study are publicly available at Blackfynn Discover, https://www.blackfynn.com/academia/ (https://app.blackfynn.io/N:organization:7c104f5e-1b21-4c88-8ea9-5fc2288135ea/datasets/N:dataset:1d7e94e9-0e6c-458b-92be-c6a68d6d7d4e/overview).

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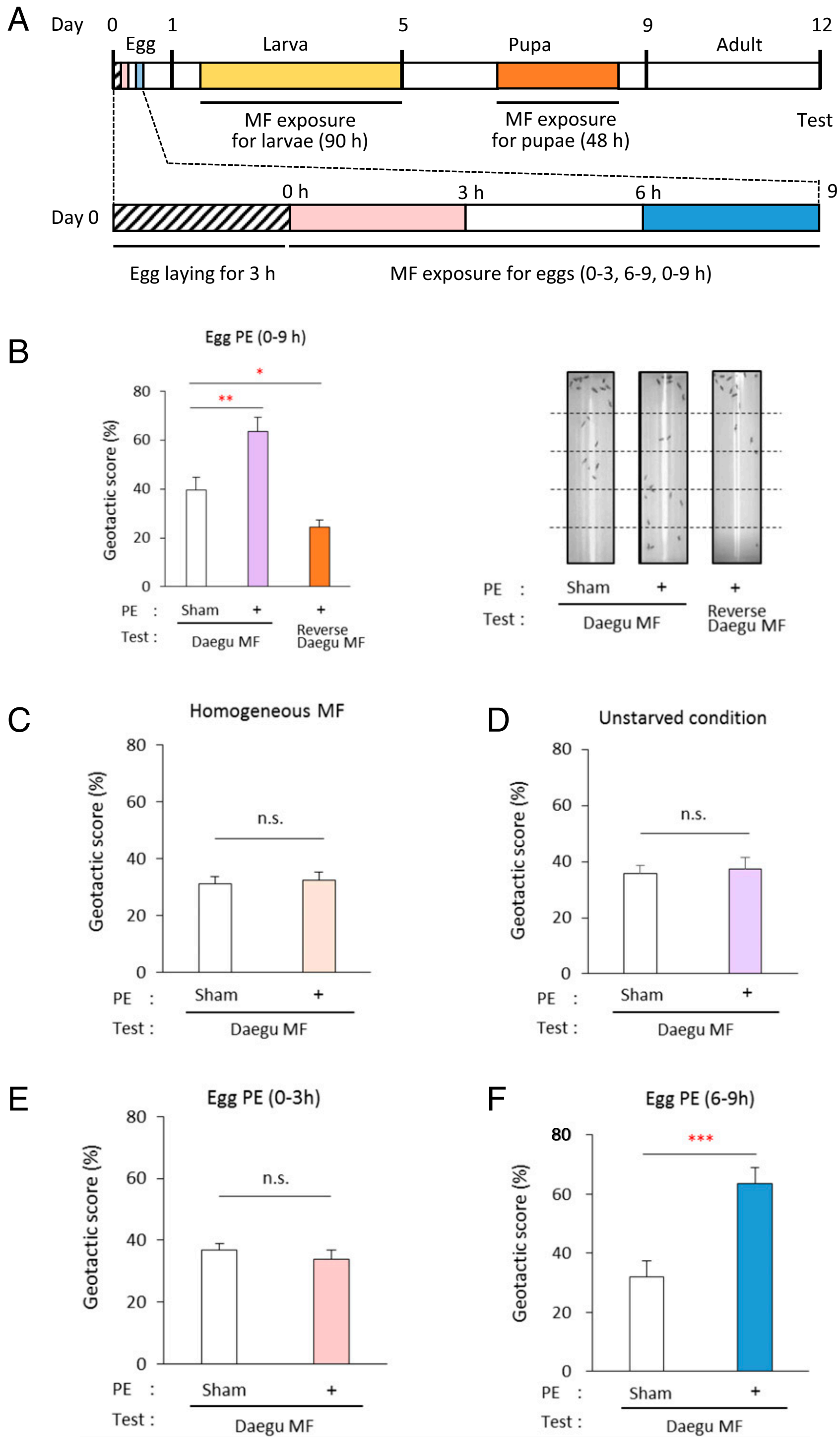


**Fig. 1.** Food-seeking geotactic magnetic imprinting behavior in flies. (*A*) Schematic of the timeline for MF exposure. (*A*, *Upper*) Life cycle of fruit flies at 25 °C and MF exposure timeline for egg, larval, and pupal stages. (*A*, *Lower*) Detailed timeline for MF exposure during egg stage (*Methods*). (*B*) Geotactic responses of flies were determined by tube-positioning assay (*Methods*). (*B*, *Left*) Imprinted downward response of flies exposed to Daegu MF before hatching (0 to 9 h). Flies prenatally exposed to sham (ambient GMF in the coils) or Daegu MF and tested under a gradient of Daegu MF or a reverse gradient of Daegu MF. (*B*, *Right*) Representative images of geotactic responses under Daegu MF and reverse Daegu MF. Dashed lines denote divisions for geotactic scoring. (*C*) Lack of geotactic imprinting response in flies from Daegu MF-exposed eggs in a homogeneous Daegu MF during the test. Whole test tubes were placed in the Daegu MF at 99% homogeneity. (*D*) Lack of geotactic imprinting response in unstarved flies from Daegu MF-exposed eggs. (*E*) Lack of imprinting downward behavior of flies exposed to the Daegu MF during the first 3 h (0 to 3) after laying. (*F*) Imprinted downward response of flies exposed to Daegu MF during hours 6 through 9 after laying. PE, prenatal exposure; +, exposure to Daegu MF; error bars, SEM. *$P < 0.05$; **$P < 0.01$; ***$P < 0.005$ by Student's *t* test or one-way ANOVA Tukey's test; n.s., not significant. $n = 10$ trials for all experiments.

tested in 2 different gradient MFs to mimic the continuum of GMF intensity by altitude and across different natural regions (*SI Appendix*, Fig. S1 and Table S2). Flies from eggs exposed to the Daegu MF showed significant downward ($P < 0.01$) or upward ($P < 0.05$) movement (Fig. 1*B*) under a Daegu MF gradient or reverse gradient, respectively (*SI Appendix*, Fig. S2), whereas flies from eggs exposed to the sham field did not noticeably respond to the Daegu MF gradient (Fig. 1*B*). As observed in our previous study (19), most of the flies moved up and down several times before settling on a position, which typically occurred during the first 3 min. Note that most of the sham-preexposed control flies moved upward in the test tube (geotactic score, ∼38%), and that an increase in geotactic score represents an increase in downward movement and vice versa. Importantly, there was no notable downward response when starved Daegu MF-preexposed flies were tested under a homogenous application of the Daegu MF across the test tube (Fig. 1*C*), indicating that the flies tested under the Daegu MF gradient (Fig. 1*B*) tended to move downward toward the bottom part of the tube, an area where the field was most similar to the Daegu MF. In addition, unstarved Daegu MF-preexposed flies did not show notable downward response under the same Daegu MF gradient as in Fig. 1*B* (Fig. 1*D*), indicating that downward movement was observed under starvation only. In contrast, the starved flies from the Daegu MF-preexposed larvae and pupae did not show a noticeable downward response under the Daegu MF gradient (*SI Appendix*, Fig. S3 *A* and *B*). Taken together, these results demonstrate that prenatal exposure to a specific, earth-strength MF affects how these flies respond to a gradient with the same field at the top or bottom of the tube as adults.

We elucidated the specific sensitive period for MF imprinting by selectively exposing eggs to the Daegu MF during either the 0- to 3-h or 6- to 9-h time window after laying (Fig. 1 *A*, *Lower*). As before, adult flies were tested under starvation and in the same Daegu MF gradient. Flies exposed to the Daegu MF during the 6- to 9-h period of egg development moved downward in response to the Daegu MF gradient, whereas flies exposed to the Daegu field during the 0- to 3-h period of egg development did not (Fig. 1 *E* and *F*). These results suggest that the 6- to 9-h period of egg development is critical for MF imprinting.

**Geographic MF-Specific Magnetic Imprinting Behavior.** Behavioral imprinting typically exhibits stimulus specificity during the imprinting period and for the imprinted behavior afterward (21). Geotactic responses in flies, including nongeotactic or negative/positive geotactic responses, were dependent on a particular combination of GMF parameters (19). The extent of MF specificity in magnetic imprinting was evaluated by testing flies in several different MFs representing different global locations. For this work, we used the IGRF model (20) to determine the MFs that exist in Vancouver, Canada, and Madrid, Spain (*SI Appendix*, Table S1). Flies that had not been exposed to any of these MFs (Daegu, Vancouver, or Madrid) during development had nearly identical geotactic responses as adults when tested in Daegu, Vancouver, and the sham fields, and showed an upward geotactic response under the Madrid MF (Fig. 2*A*). Conversely, flies exposed to one of these fields during development showed enhanced geotactic responses when tested in the field to which they had previously been exposed. Specifically, flies exposed to the Daegu MF during the critical imprinting period did not show a noticeable downward response in the Vancouver or the sham MFs, but did move downward in response to the Daegu MF (Fig. 2*B*). Similarly, flies exposed to the Vancouver MF during the imprinting period showed significant downward movement when tested in the Vancouver MF, but not when tested in the Daegu or sham MF (Fig. 2*C*). Flies exposed to the Madrid MF at the critical imprinting period showed a significant downward response when tested in the Madrid MF, but not when tested in the other MFs (Fig. 2*D*). Note that in each test under the MFs from the different locations, there was a gradient, with the specified field at the bottom of the tube. These data demonstrate that enhanced downward movement occurs when starved adult flies are placed in a gradient of same MF to which they were exposed during a critical period of development.

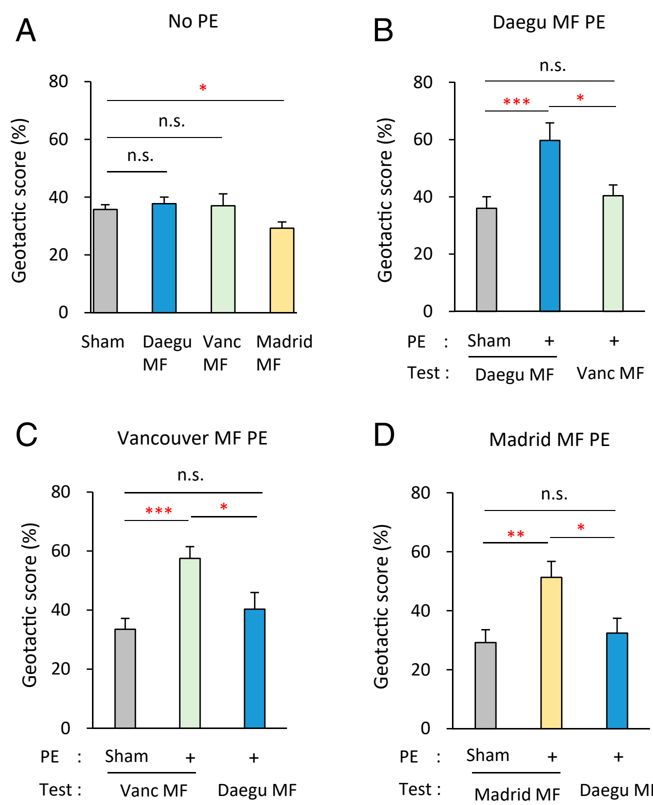


**Fig. 2.** MF specificity in magnetic imprinting behavior. (*A*) Comparison of the geotactic responses in flies reared without prenatal exposure to MF, under nongeotactic Daegu MF, Vancouver MF, or negative geotactic Madrid MF. Note that the Madrid MF elicited a negative geotactic response. (*B*) Imprinted downward response of flies exposed to Daegu MF during the imprinting period, positive in the Daegu MF but not the Vancouver MF. (*C*) Differential imprinting behavior of flies exposed to the Vancouver MF during the imprinting period. Flies tested in the Vancouver MF showed a significant downward response and no response to the Daegu MF. (*D*) Imprinted downward behavior of flies exposed to Madrid MF during the imprinting period, when under the same MF, with no response to Daegu MF. PE, prenatal exposure; +, exposure to Daegu MF, Vancouver MF, or Madrid MF. Error bars, SEM. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.005$ by one-way ANOVA Tukey's test; n.s., not significant. $n = 10$ trials for all experiments.

**Sex-Dependent Magnetic Imprinting Behavior.** Potential differences in responses according to sex were investigated by again rearing flies from eggs exposed to the sham or Daegu MF. Adults were tested in all-male groups, all-female groups, and mixed groups of males and females (male:female, 1:1.1; Fig. 3*A*). Based on a directionality metric (*Methods*; Fig. 3*B* and *SI Appendix*, Fig. S4*A*), the female and mixed groups showed significant downward behavior when exposed to the matching MF gradient from their egg stage. Conversely, the all-male groups did not, although the male flies in the mixed group did show a downward response, suggesting their behavior might be influenced by female flies. These results raised the question of whether prenatal exposure to the MF was required for male flies to show MF imprinting behavior in the mixed group, or whether males merely followed female flies as they moved downward. Subsequent experiments involving groups of mixed flies separated by prenatal exposure lineage (Fig. 3*C*) indicated that prenatal exposure to the MF was

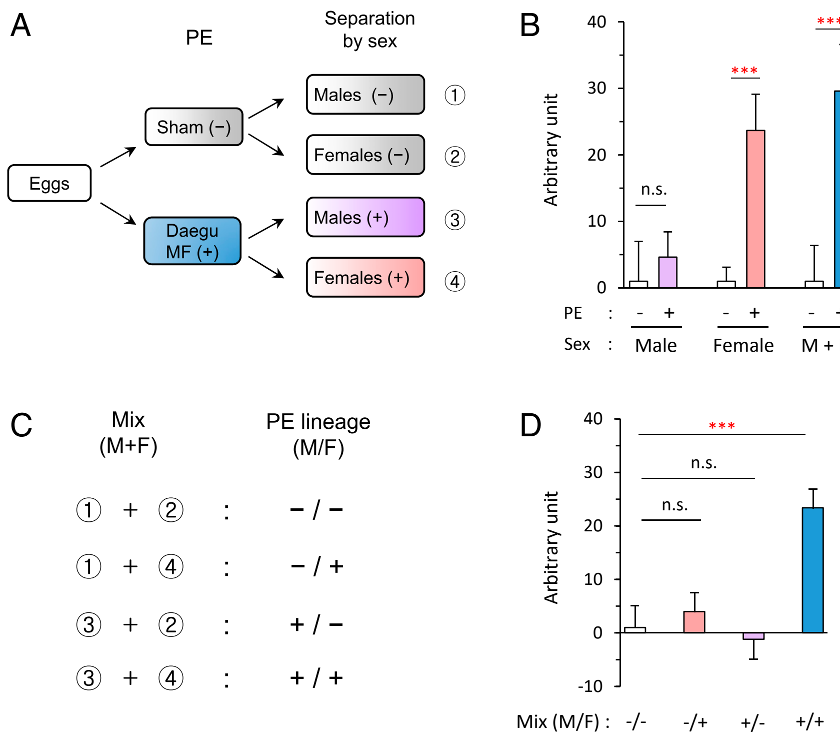


**Fig. 3.** Female vs. male geotactic magnetic imprinting. (*A*) Schematic of MF exposure and separation of exposed flies by sex. Flies from eggs exposed to sham (−) or Daegu MF (+) were separated into males (1 or 3) and females (2 or 4). (*B*) Sex-dependent geotactic imprinting behavior in flies exposed to Daegu MF during the imprinting period. Females showed a significant downward imprinting response similar to that of the mixed group flies; males alone did not demonstrate this response. Data are represented in arbitrary units for normalized comparison with all data for the sham-exposed flies shifted to 1 for normalization (*Methods*). (*C*) Mix of male and female flies preexposed to sham or the Daegu MFs. Numbers 1–4 are the same as referenced in *A*. (*D*) Prenatal exposure lineage-dependent geotactic imprinting behavior in mixed flies. Only the group in which both male and female parent flies were exposed to the Daegu MF showed a significant downward imprinting response to the Daegu MF gradient. PE, prenatal exposure; −, exposure to sham; +, exposure to Daegu MF; M, male; F, female; M/F, indicator of whether prenatal exposure lineage was male or female and exposed to sham (−) or Daegu MF (+). Error bars, SEM. ***$P < 0.005$ by the Student's $t$ test or one-way ANOVA Tukey's test; n.s., not significant. $n = 10$ trials for all of the experiments.

indeed necessary for male flies to express the MF imprinting response when together with female flies (Fig. 3*D* and *SI Appendix*, Fig. S4*B*). Taken together, the results imply that males and females are similar, in that both must be exposed to a particular MF during the egg stage to move downward in the imprinted field gradient as adults. The 2 sexes differed in that MF-exposed adult females showed a strong tendency to move downward in the field regardless of the behavior of other flies, whereas MF-exposed males moved downward only in the presence of females that were also moving downward.

**Transgenerational Inheritance of Magnetic Imprinting.** We then assessed whether Daegu MF imprinting by parent flies could be transmitted to subsequent generations. Progeny from the parent flies for which no additional exposure was provided were tested for geotactic responses. Starved F1 flies showed a significant downward response under the Daegu MF gradient ($P < 0.01$), although the extent was slightly attenuated compared with parent flies (Fig. 4*A* and *SI Appendix*, Fig. S5*A*). Conversely, the generations that followed (F2 to F5) did not show any significant downward movement in the MF. These results indicate that the MF experienced by a parent fly during a critical period of its development influences the behavioral responses of its F1 offspring to the matching MF gradient.

Finally, we examined sex dependency on the inheritance of MF imprinting behavior in F1 flies. F1 progeny were obtained by the combinatorial mating of parent flies that were derived from sham or Daegu MF preexposed eggs (Fig. 4*B*). The 3 different groups of F1 flies were tested as described previously. In the mixed group, the F1 flies (+/+) from the Daegu MF-preexposed parents showed significant ($P < 0.01$) downward behavior, whereas the F1 progeny (−/+) from the parents for which only the females were prenatally exposed to the MF only demonstrated a marginal increase in downward responses (Fig. 4*C*). In the female-only and male-only groups, all of the lineages of F1 flies (−/+, +/−, and +/+) from the combinatorial mating of the parents failed to show a downward response relative to F1 control flies (−/−; Fig. 4*D* and *SI Appendix*, Fig. S5*B*). These results suggest that both male and female parent flies must imprint on an MF for the inheritance of the MF imprinting behavior in mixed F1 progeny, implying that male and female parent flies synergistically contribute to the inheritance of MF imprinting by the next generation.

## Discussion

Results presented here represent experimental evidence of MF imprinting and transgenerational inheritance thereof in a magnetoreceptive species. Our findings suggest that an imprinted or inherited MF is 1 of the 2 essential components (the other being magnetic compass) in the conceptual scenario for MF-directed navigation in adults or progeny among magnetoreceptive migratory animals. Evidence obtained on MF imprinting in experimental laboratory conditions may differ from the experiences of flies in nature, and the reasons for this MF sensitivity remain unclear, but we speculate that parent and offspring flies use MF imprinting and transgenerational inheritance thereof to recognize favorable areas for foraging. In the absence of olfactory and visual cues for food searching, local magnetic anomalies from mountains, rocks, and even underground volcanic deposits (20, 22, 23) may provide useful sensory cues to flies. According to our data (*SI*

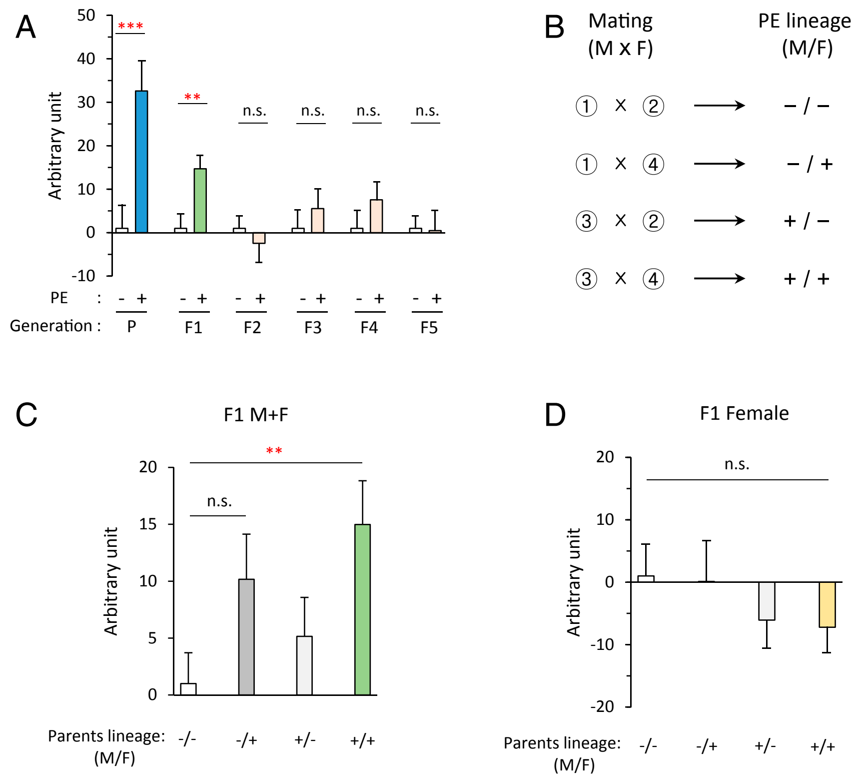


**Fig. 4.** Transgenerational inheritance of magnetic imprinting and sex dependency. (*A*) Comparison of the geotactic responses of parent flies exposed to sham or Daegu MF during the imprinting period and their progeny (F1 to F5). Progeny flies were not exposed to sham or Daegu MF before the test. Only parent and F1 flies showed significant downward imprinting behavior. (*B*) Parent flies schematic for sex dependency experiment on F1 progeny. The male and female parent flies (1–4) indicated in Fig. 3*A* were mated in various combinations to produce progeny flies with different exposure lineages. (*C* and *D*) Differential geotactic magnetic imprinting behavior depending on sex and exposure lineage in F1 progeny. F1 flies were from lineages of parent flies exposed to sham or Daegu MFs during development (6 to 9 h after laying). In the F1 mixed group (F1 M+F), only progeny flies from parent flies (+/+) wherein both sexes were exposed to the Daegu MF showed significant downward imprinting response (*C*). No significant geotactic response was observed in the F1 female group from any exposure lineage (*D*). PE, prenatal exposure; –, exposure to sham; +, exposure to Daegu MF; P, parent flies; M, male; F, female; M/F, symbol for the exposure lineage. Error bars, SEM. ***P* < 0.01; ****P* < 0.005 by Student's *t* test or one-way ANOVA Tukey's test; n.s., not significant. *n* = 10 trials for all experiments.

*Appendix*, Fig. S1 and Table S2), the GMFs at the different regions were not homogeneous and varied measurably by altitude. Intensity of the GMF was proportional to or inversely proportional to the altitude, depending on the location within the regions. Taken with our data, this suggests that a continuum in GMF intensity in nature would result in similar imprinted responses demonstrated in the gradient MFs in the laboratory. If this scenario does apply in nature, gradient, rather than homogeneous, MF might be more recognizable to flies, which would then navigate up or down via imprinted geotactic response, whereas homogeneous MF might be more useful as a static factor for modulation of altitude in flies (19). This possibility has also been demonstrated in studies on oasis-return flights of flies in deserts and the innate ability of progeny to recapitulate these navigational paths and identify horizontal/vertical directions for returning to the place where they were feeding (24, 25). Similar to MF imprinting behavior found in flies, natal homing has also been suggested in magnetoreceptive female loggerhead sea turtles and both sexes of salmon. GMF imprinting may help female parent flies and both sexes of progeny flies select a suitable mating area by returning to the place used by parent flies. In female flies, GMF imprinting may enable selection of an optimal egg laying site to provide hatchling larvae with plentiful food for survival. Indeed, a broad taxonomic range of animals including flies, fish, and birds are known to prefer habitats similar to those they encounter while young (26), and may be memorizing the MF that exists in that area and exploiting it for habitat preference.

Our results suggest that MF information was sensed and memorized by the primitive sensory brain system of developing flies before hatching and then retrieved in adulthood; hence the imprinting behavior we observed. This idea is supported by the observation that GMF-like MFs appeared to be imprinted in the 6- to 9-h period after egg laying, corresponding to developmental stages 13 to 15 (27), when their primitive nervous systems begin to organize and function. It was unexpected that the application of an MF to eggs acted as a conditioning stimulus for starved flies to exert downward movement therein as adults against their innate upward preference. This seems to be a molecular-level mechanism designed to direct food foraging behavior during food scarcity. We speculate that this MF information was internalized by the sensory brain system (28, 29) in association with the process of yolk metabolism in the embryo, and was stored in the developing nervous system until adulthood. This information was subsequently transduced through an unknown pathway from the nervous system to germline cells in developing gonads (27) that were destined to produce gametes during the reproductive process, a process that would lead to the MF imprinting behavior in adults and their progeny under certain conditions. A potential molecular basis for inheritance thereof across one generation could be epigenetic modification such as methylation of related genes in the parent gametes resulting in germline imprinting (30). Based on the experimental evidence for MF imprinting and its transgenerational inheritance in the present study, elucidation of molecular mechanisms underpinning magnetoreception and MF learning and imprinting at the egg stages, and transgenerational inheritance thereof, may provide further insight into the biological significance and genetic and developmental aspects of magnetic sensing in animals.

## Methods

**Fly Stock.** Flies from the Canton-S strain were obtained from the Bloomington *Drosophila* Stock Center (Indiana University, Bloomington, IN) and reared on a standard cornmeal-yeast-agar diet, as described in previous studies (17, 19, 31). Briefly, rearing conditions were 25 °C ± 0.5 °C, 60 ± 2% relative humidity, and a 12 h/12 h light/dark cycle under a full-spectrum (350 to 800 nm) light-emitting diode light (~500 lx = $3.03 \times 10^{14}$ photons/cm$^2$/s), which was turned on and off at 0900 and 2100 (local time), respectively. Ambient GMF parameters in the rearing room are described in *SI Appendix*, Table S1, and all MF components were measured relative to true north. Both sexes of flies were used together for experiments unless otherwise specified.

**MF Modulation.** A rectangular, double-wrapped (32) Helmholtz coil system modified from previous studies (17, 19, 31) and consisting of 3 pairs of parallel coils arranged orthogonally for the 3 axes was used to generate GMF-like MFs. Briefly, the coil for the *X*-axis (north-south) was aligned with true north so that the *Y*-coil (east-west axis) could be modulated for the *Y* component of the GMF. Pairs of coils for each axis were connected to a DC power supply (E3631A; Agilent Technologies). For sham MF, current flowed in the antiparallel direction: the coils were double-wound, and the same amount of current was put through the 2 coils in opposite directions so that the 2 MFs generated from the 2 coils cancelled one another out. Modulated MF parameters are indicated in *SI Appendix*, Table S1. The homogeneity of the MF varied by experiment and was measured using a magnetometer (MGM 3AXIS; ALPHALAB). Plastic flasks containing fruit fly eggs were placed in the center of the coil system for prenatal exposure, where homogeneity of the MF intensity was 99%. For the tube-positioning assay, the test tube containing fruit fly adults was placed at the periphery of the coil system, where the field was ~90% homogeneous (*SI Appendix*, Fig. S2). For example, in tests under a Daegu MF gradient, the MF was modulated so that the intensity at the top of the tube was slightly stronger (~5 μT) than that at the bottom of the tube (e.g., ~50 μT for the Daegu MF), such that the MF in the lower part of the tube was closer to the Daegu MF, with a slight MF gradient inside the tube (*SI Appendix*, Fig. S2*B*). In tests with the same Daegu MF used for preexposure, the tube was placed at the center of the coil system, where the intensity of the Daegu MF (50 μT) was ~99% homogeneous, resulting in a virtually homogenous MF across the entire tube. In the experimental assay area, the temperature was maintained at 25 °C ± 0.3 °C and monitored with a USB Data logger 98581 (MIC Meter Industrial Company). The ambient 60-Hz magnetic and electric fields were less than 3 μT, as monitored by a TES 1390 (TES Electrical Electronic) and less than 1.20 V/m as monitored by a 3D NF Analyzer NFA 1000 (Gigahertz Solutions), respectively (19). Intensity of the GMF and altitude of 3 flatlands and 3 mountains in local regions (*SI Appendix*, Fig. S1 and Table S2) were measured every 4 s, using ExpoM-ELF (Fields at Work GmbH) by an experimenter who walked through the measurement routes with the magnetometer on his left waist.

**MF Imprinting.** MF imprinting was conducted by exposing prenatal fly samples at different developmental stages to a particular MF for a certain period and then rearing them to adulthood under normal laboratory conditions. Eggs laid by 1- to 3-d-old mixed flies were exposed to the Daegu MF during the 0- to 3-, 6- to 9-, or 0- to 9-h period after egg laying. Once the optimal imprinting window of 6 to 9 h after laying was established, additional imprintings using the Vancouver or Madrid MFs were conducted on other flies during this same developmental period. The effect of MF exposure on larvae and pupae was evaluated by separating and exposing ~0.75-d-old larvae or ~1.6-d-old pupae to the Daegu MF for 90 or 48 h, respectively. The sham exposures were carried out in the sham field within the coils, and not in the rearing room.

MF imprinting was tested as follows: flies were placed in a plastic flask without food for 24 h and without water for another 6 h before each test. Tests were conducted using 20 ± 2 flies in a transparent plastic test tube between Zeitgeber time (ZT) 5 and ZT 8. The test was performed with mixed-sex groups of flies unless otherwise specified. In a pilot test, 2 tubes were evaluated simultaneously by inverting test tubes containing flies for 1 min and then gently placing the tube bottom down in a cube located at the periphery or the center of the Helmholtz coil. Vertical movement of the flies in the test tubes was recorded for 11 min, and quantified as described in the subsequent data analysis section. To prevent miscalculations, such as 2 flies being in the same place in 5 consecutive photos, another set of photos from a 90° counterclockwise angle were taken for comparison in initial experiments, decreasing the likelihood thereof to about 1%, for which no statistical differences were observed (19).

Geotactic imprinting in parent flies was examined by testing 1- to 3-d-old flies from exposed eggs, larvae, or pupae to the sham or Daegu MF conditions specified here, using the same tube-positioning assay. To examine sex-associated effects of MF imprinting, virgin male and female flies hatched from the sham MF- and Daegu MF-exposed eggs were separately collected by sex under $CO_2$ anesthesia and placed as depicted in Fig. 3 *A* and *C* into different groups for testing.

All experiments were performed in a double-blinded manner; that is, the experimenter who conducted the tube-positioning assay did not know which flies were being tested, the MFs to which tested flies had been exposed, or the developmental stages during which the flies were exposed. Another experimenter calculated the geotactic scores for flies in the captured photos, also without knowledge of which fly samples were represented therein. This experimenter conducted the initial MF exposures and MF test modulations. All tests were performed by the interspersion of treatments in a random sequence on the experimental days. A random sequence might be as follows: flies prenatally exposed to the Daegu MF were first tested in the Daegu MF gradient, then the Vancouver MF gradient, followed by the Daegu MF, the Vancouver MF gradient, the Daegu MF gradient, and so on. All experiments were performed 10 times.

**MF Imprinting Inheritance Assay.** Potential imprinting behavior in progeny was evaluated by mating parent flies of both sexes that demonstrated imprinting behavior to produce F1 flies. This same reproduction procedure was consecutively carried out up to the F5 generation. The flies in each generation (F1 to F5) were reared without prenatal MF exposure and tested under the same Daegu MF gradient as the parent (F0) flies. Sex dependency of MF imprinting inheritance in F1 progeny was examined by collecting and separating virgin male and female parent flies from sham-exposed and Daegu MF-exposed eggs under $CO_2$ anesthesia, and F1 progeny were reproduced by combinatorial mating of these flies as described earlier and in Fig. 4*B*. F1 flies were then tested in female-only, male-only, and mixed groups.

**Data Analysis.** Geotactic responses were evaluated by measuring and scoring the vertical positioning of the flies in the test tubes, as described previously (19). Briefly, geotactic scores were quantified using the average of the scores from 5 consecutive 5-s interval photographs with this equation: ([number of flies at the lowest 4 sections of a test tube equally divided into 5 sections/total number of flies] × 100%). For normalized comparisons between sample groups, geotactic imprinting responses were arbitrarily represented by an arithmetic adjustment to 1 of the geotactic score of a sample that was not prenatally exposed to an MF in accordance with an adjustment of the geotactic score of the corresponding exposed sample by the same arithmetical addition or subtraction. Statistical analyses were performed using Student's *t* test or one-way ANOVA Tukey's test, and results were calculated with Origin software. Statistical values are presented as mean ± SEM. All experiments were performed 10 times. A *P* value of less than 0.05 was considered statistically significant. A profile of local GMF intensity is presented via VWorld map (33), using *QGIS* 3.4.4 (34).

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