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Effect of a near-zero magnetic field on development and flight of oriental armyworm (*Mythimna separata*)

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Abstract

The geomagnetic field affects all living organisms on the Earth. In this study we investigated the developmental and behavioral effects of rearing *M. separata* in a near-zero magnetic field (<500 nT) compared to the local geomagnetic field (approximately 50 μT). The near-zero magnetic field produced by a Helmholtz coil system significantly lengthened larval and pupal development durations, increased male longevity, and reduced pupal weight, female reproduction, and the relative expression level of the vitellogenin (Vg) gene in newly emerged females. Moreover, the near-zero magnetic field had a considerable negative effect on the mating ratio of *M. separata* adults. In addition, the moths in the near-zero magnetic field displayed less flight activity late in the night than those in the Earth's normal geomagnetic field, indicating that the flight rhythm of *M. separata* may be affected by the near-zero magnetic field. Reduction in magnetic field intensity may have negative effects on the development and flight of oriental armyworm, with consequent additional effects on its migration.

Keywords: development, flight activity, geomagnetic field, *Mythimna separata*, near-zero magnetic field

1. Introduction

Since life on the Earth originated approximately 3.5 billion years ago, the geomagnetic field has been an important part of the environment (Shles 1985). Geomagnetic field intensity increases from less than 30 μT at the magnetic equator to over 65 μT at the magnetic poles. The presence of the geomagnetic field protects the Earth and its biosphere from the solar wind, deflecting most of the charged particles carried by its currents (Occhipinti *et al.* 2014). Numerous studies have shown that many biological processes are affected by magnetic fields (Belyavskaya 2004; He *et al.* 2018). Some species of fish (Quinn 1980; Quinn and Brannon 1982), amphibians (Lohmann *et al.* 2004), mollusks (Lohmann and Willows 1987), birds (Wiltschko and Wiltschko 1988), reptiles (Lohmann 1991), arthropods (Walker and Bitterman 1989; Gegear *et al.* 2010; Buehlmann

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et al. 2012) and mammals (Mather and Baker 1981) perceive and use the Earth's geomagnetic field for navigation.

Magnetoreception is a widespread but poorly-understood sensory ability. Despite the remarkable progress that has occurred during the past three decades, the transduction mechanisms underlying magnetoreception and magnetic orientation remain largely unclear (Johnsen and Lohmann 2008; Nordmann *et al.* 2017; Henrik 2018). Near-zero magnetic fields, also known as hypomagnetic fields, are often applied experimentally by eliminating the local geomagnetic field to obtain insight into the effects of magnetic field intensity changes. Experiments have elucidated the effects of shielding the local geomagnetic field on many species, including humans (Sarimov *et al.* 2008; Mo *et al.* 2013), other animals (Wan *et al.* 2014; Mo *et al.* 2015) and plants (Xu *et al.* 2012, 2013). Such effects include modifications of behavior (Mo *et al.* 2015), genetics (Mo *et al.* 2014), development (Wan *et al.* 2014) and neural conduction (Sarimov 2008).

Mythimna separata (Walker), the oriental armyworm, is a typical long-distance migratory insect that is also a major agricultural pest. It is distributed in east Asia and Australia, and has a broad host range (Sharma and Davies 1983; Farrow and McDonald 1987). *Mythimna separata* exhibits obvious orientation behavior during migration (Shi *et al.* 2010). Recent studies show that *M. separata* becames disoriented in an extremely weak magnetic field (500 nT), and that a strong magnetic field (1.8 T) changed the flight vector of adult moths (Xu J J *et al.* 2017; Wan *et al* 2018). These findings infer that *M. separata* has a magnetic sense that detects geomagnetic information for navigational guidance. Although much is known about the growth, development, flight and reproduction of *M. separata* under Earth's natural geomagnetic field, there is a lack of research under different magnetic field strengths (Luo *et al.* 1999; Jiang *et al.* 2011). In this study, we investigated the biological effects of a near-zero magnetic field on the development and flight of *M. separata*, and thus, by inference, clarified the effects of the natural geomagnetic field.

2. Materials and methods

2.1. Experimental set-up

A near-zero magnetic field was produced by three perpendicular pairs of Helmholtz coils, connected to two sources of high-precision adjustable direct current power. The diameter of each group of coils was 1 m. A near-zero magnetic field (<500 nT) was generated in a volume of 50 cm ×50 cm×50 cm by adjusting the electrical current in the central area of the Helmholtz coils. Two separate Helmholtz coils systems were established in the same room to avoid deviations in the device, temperature, illumination and humidity; and one system was used to generate a near-zero magnetic field, while the other system was used for sham exposure. The *M. separata* individuals used in this study were reared in the central area of the Helmholtz coils. During the experiments, the magnetic flux densities of the near-zero magnetic field and geomagnetic field were measured using a fluxgate (CH-330F; Beijing Cuihai-Hall Electronic Device Co., Ltd., China; sensitivity ±1 nT). The variance of the near-zero magnetic field did not exceed 500 nT.

An insect autonomous flight monitoring system (Cheng and Luo 2011) was used to determine the effects of a near-zero magnetic field on the free flight of adult *M. separata* reared as larvae under the normal geomagnetic field. This system contains a photoperiod control mechanism, a monitoring container, a video recorder (25 frames s^{-1}), and an image motion recognition and analysis module. A flight parameter extraction module and a statistical module formed the data analysis software (Metlab2010). This system can monitor the free-flight activity of several insects simultaneously by recognizing the position of each individual in each video frame. Accumulated flight frequency (number of independent flights between periods of resting) and accumulated flight duration were recorded for each hour over the full 12-h test period. We used a novel variable, 'flight action', developed by Qin *et al.* (2018) to reflect the extent of relative flight activity. This variable is calculated as the square root of the sum of squared differences in pixel values between one video image frame and the next. Consequently, a flight action value has no units, but a higher value indicates greater flight activity of the moth than a lower value (Qin *et al.* 2018).

2.2. Insect stock and determination of developmental parameters

The experimental insects were obtained from a continuous multigeneration colony maintained in the laboratory, with a stocking density of 10 individuals per bottle. For this colony, the relative humidity of the feeding environment was approximately 70%, the temperature was (26±1)°C, and the photoperiod was 14 h L:10 h D. In this experiment, 310 and 300 freshly laid eggs were transferred into arenas exposed to the near-zero magnetic field and geomagnetic field, respectively. After hatching, the larvae were fed on artificial diet. Mature larvae were transferred to glass bottles with soil (water content of 15%), and mortality up to that point was recorded. Forty-eight hours after pupation, the pupae were removed from the soil and weighed using an automatic electronic balance (XS205, DualRange, Mettler-Toledo International Inc., Greifensee, Switzerland) with an accuracy of ±0.01 mg. The dates of pupation and emergence were recorded. After emergence, the adults were identified to sex and paired. Each pair was placed in a cylindrical cage (20 cm tall, 5 cm in diameter) containing oviposition paper, and provided with 10% glucose water until death, at which point the date was recorded. Females were dissected immediately after death, and the number of spermatids was observed to determine the number of times they had mated (Lu and Chang 1963). At least 40 individuals were dissected for each treatment.

2.3. Quantitative real-time PCR (qPCR) analysis of vitellogenin (Vg) gene expression

Total RNA from newly emerged adult females was extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. A NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA, USA) was used to determine the concentration and quality of the total RNA. Total RNA was subjected to reverse transcription using the 15–18 primer designed by Beacon Designer Software (Premier Biosoft) and the SuperScript First-Strand Synthesis System (Invitrogen) in a 20 μL reaction volume. A total of 2 μL cDNA solution was used for qPCR in a thermal cycler (Bio-Rad CFX96, Bio-Rad Laboratories, USA) with 35 cycles for 30 s at 95°C, 40 s at 60°C, and final extension for 32 s at 72°C. 18S and *Actin* were chosen as the housekeeping genes in the qPCR analysis. The primer pairs used for the PCR were as follows: Vg: VgF (5´-TGGCCCAGATGACCCATGAT-3´), VgR (5´-ACTGAGCTGCCCTAGACAGG-3´); Actin: ActinF (5´-AACTTCCCGACGGTCAAGTCAT-3´), ActinR (5´-TGTTGGCGTACAAGTCCTTACG-3´); and 18S: 18SF (5´-GGAAGGATTGACAGATTAACA-3´), 18SR (5´-GCTCCACCAACTAAGAAC-3´).

2.4. Free flight in the near-zero magnetic field

All experimental moths were reared under local geomagnetic field conditions. Six 2-day old adults, three females and three males, were randomly chosen and placed into the insect free-flight monitoring container. The monitoring containers were placed in the central area of the Helmholtz coils, while the other components of the autonomous flight monitoring system were located outside of the Helmholtz coils. Moths were tested under the near-zero magnetic field and the sham exposure on the same day to minimize experimental variation. The dark period began at 22:00 and extended until the next day at 08:00, and video recording took place from 22:00 until 10:00 the next day. Flight action, flight frequency and flight duration were used to evaluate the flight activities of adult *M. separata*. We analyzed and compared the total flight and the hourly flight parameter values of *M. separata* under the two different magnetic fields during the 12-h test period.

2.5. Data analysis

All data are shown as the mean±standard error (SE). Durations of the egg, larval and pupal developmental stages, pupal weight and relative Vg transcript levels and data from the free-flight experiments were compared using one-way analysis of variance (ANOVA). Adult longevity was analyzed using two-way ANOVA, with the presence of a magnetic field as the main factor and sex as the subfactor. Survival and mating rate data were analyzed using the Chi-square test. All data were analyzed using SPSS 22.0 Software (IBM Inc., Armonk, NY, USA). Significance was defined as $P<0.05$.

3. Results

3.1. Effect of a near-zero magnetic field on the durations of different developmental stages

The type of magnetic field did not significantly affect the duration of egg ($F_{1,\ 473}$=0.167, P=0.683) or adult female stages ($F_{1,\ 81}$=3.251, P=0.075), however, it significantly affected the duration of larval ($F_{1,\ 304}$=90.142, P<0.001), pupal ($F_{1,\ 344}$=16.38, P<0.001) and adult male stage ($F_{1,\ 90}$= 9.208, P=0.003) of *M. separata*. Moreover, there was an significant interaction between sex and type of magnetic field on the duration of adult longevity ($F_{1,\ 172}$=14.135, P=0.001) (Table 1). Compared to the local geomagnetic field, the near-zero magnetic field significantly extended the duration of the larval stage by 1.3 days, the pupal stage by 0.2 days, and the adult male longevity by 1.7 days (Fig. 1). In addition, the longevity of females showed no significant decreasing tendency under the near-zero magnetic field compared to the local geomagnetic field ($F_{1,\ 81}$=3.251, P=0.075).

3.2. Effect of a near-zero magnetic field on pupal weight

The type of magnetic field significantly affected the pupal weight of *M. separata* reared under either the near-zero magnetic field or the local geomagnetic field ($F_{1,\ 344}$=29.34, P<0.001). Compared with the local geomagnetic field, the near-zero magnetic field significantly decreased the pupal weight of *M. separata* by 0.02 g (P<0.05; Fig. 2).

3.3. Effect of a near-zero magnetic field on female fecundity and the relative expression level of Vg

Compared to individuals reared under the local geomagnetic field, newly emerged adult females reared under the near-

Table 1 ANOVA results for the effect of the magnetic field on the different developmental stages pupal weight, fecundity and relative expression level of Vg of *Mythimna separata*

Parameter	Magnetic field			Sex			Magnetic field×Sex		
	df	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
Egg duration	1, 473	0.16	0.68						
Larval duration	1, 304	90.14	<0.01						
Pupal duration	1, 344	16.38	<0.01						
Female moth longevity	1, 81	3.21	0.07	1,172	226.36	<0.01	1,172	14.135	0.001
Male moth longevity	1, 90	9.20	<0.01						
Pupal weight	1, 344	29.34	<0.01						
No. of eggs per female	1, 75	0.34	0.56						
Relative expression level of Vg	1, 5	85.72	<0.01						

The blank space in the table indicates that there is no relevant data for the corresponding treatment.

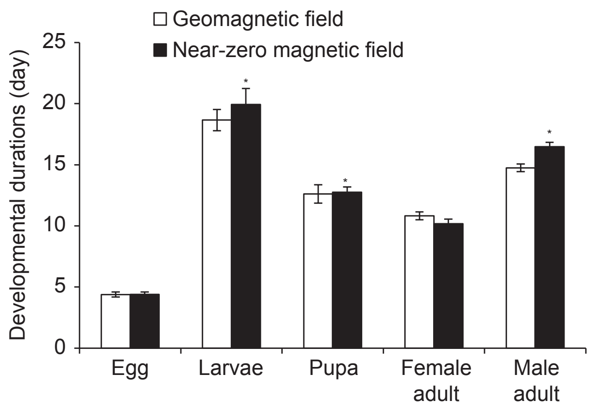

Fig. 1 Effects of a near-zero magnetic field on the different developmental stages of *Mythimna separata*. Data are mean±SE. * indicates a significant difference (P<0.05). n=230 and 244 for eggs, n=157 and 148 for larvae, n=171 and 174 for pupae, n=47 and 36 for adult female moths, and n=51 and 40 for adult male moths under geomagnetic field and near-zero magnetic field, respectively.

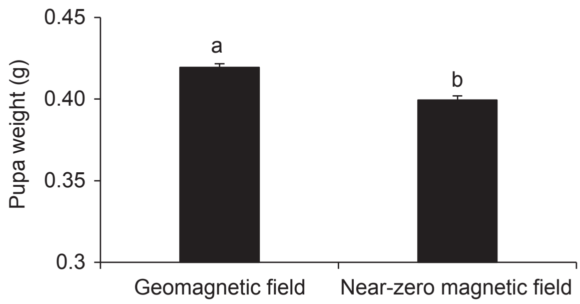

Fig. 2 Pupal weight of *Mythimna separata* reared under the local geomagnetic field and near-zero magnetic field. Data are mean±SE. Different lowercase letters indicate significant differences (P<0.05) between the treatment means. n=171 and 174 for the pupae under near-zero magnetic field and geomagnetic field, respectively.

zero magnetic field showed a significantly lower expression level of the Vg gene ($F_{1, 5}$=85.72, P<0.001) (Fig. 3-B). However, female fecundity was not significantly affected by exposure to the near-zero magnetic field in comparison to the local geomagnetic field ($F_{1, 75}$=0.34, P=0.562) (Fig. 3-A).

3.4. Survival and mating rates under a near-zero magnetic field

There were no significant differences in the survival rates of *M. separata* for any of the instar stages exposed to different magnetic fields (P>0.05; Table 2). The mating rate of paired adults under the near-zero magnetic field was significantly lower than that under the geomagnetic field (χ^2=23.38, P<0.001).

3.5. Effect of a near-zero magnetic field on free flight

The peak of *M. separata* flight activity under the near-zero magnetic field lasted approximately 3 h, spanning the 6-,7- and 8-h after dark, whereas it continued to increase to a peak between 9–10 hours after dark under the local geomagnetic field (Fig. 4). Flight activity in both near-zero and local geomagnetic fields decreased rapidly after the beginning of the light period. At 10 h after dark, flight action, flight frequency and flight duration were all significantly greater under the geomagnetic field than under the near-zero magnetic field ($F_{1, 9}$=6.751, P<0.001; $F_{1, 9}$=6.319, P<0.001; $F_{1, 9}$=4.138, P=0.001) (Fig. 4). Total free-flight frequency, duration and flight action showed apparent but nonsignificant decreases under the near-zero magnetic field compared with those under the local geomagnetic field ($F_{1, 9}$=1.1916, P=0.191; $F_{1, 9}$=3.875, P=0.073; $F_{1, 9}$=4.271, P=0.061) (Table 3).

4. Discussion

The geomagnetic field is believed to function like the Global Positioning System for animal navigation (Wiltschko and Wiltschko 1988). However, the specific biological effects of magnetic fields on insects have rarely been studied.

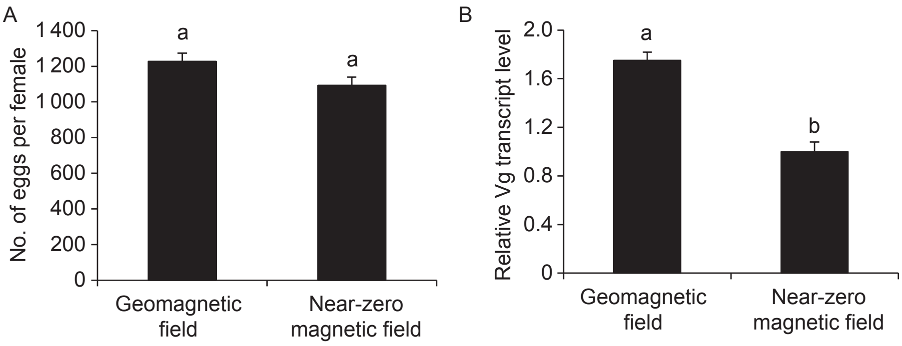

Fig. 3 Fecundity (A) and relative Vg transcript levels (B) of *Mythimna separata* females maintained under the local geomagnetic field or near-zero magnetic field. Data are mean±SE. Different lowercase letters indicate significant differences ($P<0.05$) between treatment means. n=36 and 40 for the female moths under near-zero magnetic field and geomagnetic field, respectively.

Table 2 Survival rates of different instars and adult mating rates of adult *Mythimna separata* maintained under different types of magnetic field

Development stage	Geomagnetic field			Near-zero magnetic field		
	n	Survival rate (%)	Mating rate (%)	n	Survival rate (%)	Mating rate (%)
egg	300	95.48		310	96.67	
1st instar	286	89.52		299	86.55	
2nd instar	256	80.00		259	85.66	
3rd instar	205	86.36		222	87.90	
4th instar	177	94.21		195	89.90	
5th instar	166	94.97		175	94.70	
6th instar	158	93.53		165	90.68	
pupa	148	89.34		149	86.93	
Adult	43		79.07	42		69.04*

* indicates a significant difference between means under geomagnetic and near-zero magnetic fields ($P<0.05$). The blank space in the table indicates that there is no relevant data for the corresponding treatment.

A near-zero magnetic field can be used to elucidate the function of the geomagnetic field (Xu *et al*. 2012; Wan *et al.* 2014). Following exposure to a near-zero magnetic field, planthoppers exhibited a longer duration of the nymph stage than under the local geomagnetic field (Wan *et al.* 2014). A near-zero magnetic field also had an adverse effect on *M. separata,* extending the duration of larval and pupal stages, decreasing pupal weight, and significantly reducing both fecundity and Vg expression level of the planthoppers (Wan *et al.* 2014). In our study, the near-zero magnetic field similarly reduced Vg expression in *M. separata* females, but the average number of eggs laid was not affected. Likewise, the fecundity of cotton bollworm (*Helicoverpa armigera* Hübner) was not affected by near-zero magnetic field (Dong and Ge 2013).

Since magnetotactic bacteria were first discovered, an increasing number of organisms have been shown to sense magnetic fields, while the mechanism underlying magnetoreception is still poorly understood (Nordmann *et al.* 2017; Henrik 2018). Two main theoretical models of magnetoreception, the radical-pair model and magnetite-based model, are generally recognized. The radical-pair mechanism, first proposed by Schulten *et al.* (2008), suggests that pigment molecules absorb photons of light energy, causing the transient formation of radical pairs whose spin states can be affected by an external magnetic field. As a photopigment, cryptochrome has been identified as a magnetoreceptor in this model. The magnetite-based magnetoreception model holds that permanent tiny crystals of magnetic material inside living organisms are sensitive to external magnetic fields (Fleissner *et al.* 2007). However, these two mechanisms do not entail a comprehensive list, since magnetic effects are also known to exist in nonmagnetotactic bacteria, such as *Escherichia coli* cells and cell cultures (Belyaev 2011; Binhi and Prato 2017).

Multiple studies have shown that a moderate magnetic field (1 mT–1 T) affects membrane channels, such as metal ion channels (Rosen 2003). Research on strong magnetic fields (>1 T) has mainly focused on the potential harm to the human body. In the case of weak magnetic fields (<1 mT), most of the attention has focused on behavioral responses. Recently, scientists have been increasingly interested in near-zero magnetic fields (Mo *et al.* 2012, 2013; Xu *et al.* 2012; Wan *et al.* 2014, 2015; Xu C X *et al.* 2017). For

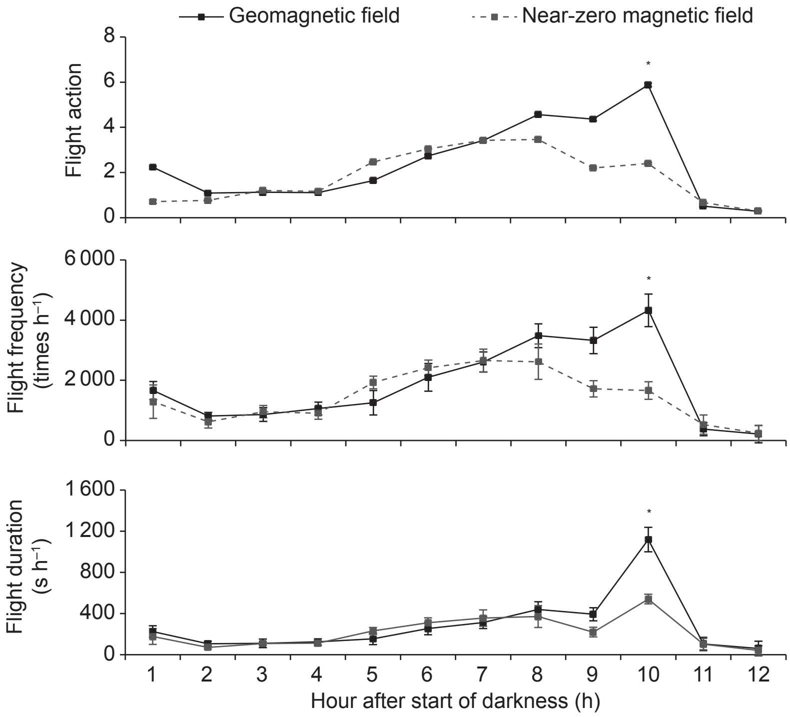

Fig. 4 Average 1-h autonomous flight activity of *Mythimna separata* after the start of darkness. The vertical bars indicate SE. * indicates a significant difference (P<0.05) between the geomagnetic and near-zero magnetic field treatment means.

Table 3 Influence of a near-zero magnetic field on autonomous flight of adult *Mythimna separata*

Magnetic field	*n*	Flight action	Flight frequency (times h^{-1})	Flight duration (s h^{-1})
Geomagnetic field	30	31.0±2.27	353.83±73.97	1 890.02±235.35
Near-zero magnetic field	30	23.6±2.79	200.83±23.94	1 503.87±149.72

Data are mean±SE.

example, 2-h exposure to a near-zero magnetic field can affect the development of *Xenopus* (Amphibia) embryos at the cleavage stage (Mo *et al.* 2012). Shielding the geomagnetic field accelerates the proliferation of human neuroblastoma by promoting G1-phase progression (Mo *et al.* 2013). Elimination of the geomagnetic field may interfere with animal brain functions. Under long-term exposure to a near-zero magnetic field, memory in *Drosophila melanogaster* was impaired (Zhang *et al.* 2004). Elimination of the geomagnetic field influenced the viability and mitochondrial activity of mouse skeletal muscle cells (Fu *et al.* 2016). Tubulin assembly is sensitive to a decrease in the geomagnetic field, which likely prevents tubulin assembly into microtubule-like structures (Wang *et al.* 2008).

The blue light receptor protein cryptochrome-1 mediates magnetic sensing in the plant *Arabidopsis thaliana* (Ahmad *et al.* 2007). Magnetic particles exist in birds (Wiltschko *et al.* 2010) and insects (Pan *et al.* 2015). In vertebrates, two types of magnetoreception may be involved in orientation, and the visual system may play a vital role in sensing magnetic fields in birds (Fleissner *et al.* 2007; Wiltschko *et al.* 2010). Both the cryptochrome-1 and cryptochrome-2 genes of rice planthoppers respond to near-zero magnetic field, and two types of magnetoreception may simultaneously operate in this species (Wan *et al.* 2015). There is compelling evidence that many magnetic field-dependent phenotypes occur in fruit flies, and binary choices in mazes (Gegear *et al.* 2008, 2010), circadian timing (Yoshii *et al.* 2009; Fedele *et al.* 2014a), locomotor activity (Fedele *et al.* 2014a), geotaxis and gravitaxis (Fedele *et al.* 2014b; Bae *et al.* 2016; Wan *et al.* 2016), seizure response (Marley *et al.* 2014) and courtship activity (Wu *et al.* 2016) are all mediated by cryptochromes. When exposed to a >20 Gauss static magnetic field, male flies significantly increased courtship activities compared to those under 0 Gauss static magnetic field. Magnetic fields may affect male courtship activity by altering neural activity *via* a cryptochrome-dependent pathway (Wu *et al.* 2016). Similarly, in our study, paired *M. separata* adults under a near-zero field showed a lower mating rate than those under the local geomagnetic field. Thus, it seems possible that a near-zero magnetic field may reduce the mating rate by disturbing male courtship activities.

Some studies have shown decreased flight activity and decreased insect foraging in the vicinity of power lines (Orlov and Babenko 1987; Bindokas *et al.* 1988; Orlov 1990). A static magnetic field generated by direct current powered coils can attract brown-banded cockroaches (*Supella longipalpa)* (Wijenberg *et al.* 2013). Hermann *et al* (1988) reported that flight activity of honeybees can be reduced by a horizontal magnetic field, and that decreasing the magnetic field to 2% of the Earth's magnetic field reduced the dance tempo of honeybees. However, Neumannn (1988) demonstrated that a magnetic field usually has no effect on honeybee rhythmicity. For many years, magnetic fields were discussed as a possible zeitgeber for circadian clocks (Close 2014). Furthermore, several studies have shown that the geomagnetic field influences the circadian systems of various organisms, including humans (Wever 1968). The circadian rhythm of fiddler crabs can respond to a small change in the intensity of Earth's magnetic field (Muraveiko *et al.* 2013). The circadian activity of house sparrows can be entrained to a cycle of change in the intensity of Earth's magnetic field (Bliss and Heppner 1976). Adult male mice show a prolonged alteration of their circadian drinking rhythm and a reduction in general activity under a hypomagnetic field environment (Mo *et al.* 2015). Yoshii *et al.* (2009) found that cryptochrome mediated the free-flight rhythm of *Drosophila* in a weak magnetic field by altering circadian clock oscillation. Cryptochromes are also encoded by core circadian genes that participate in complex molecular feedback loops to generate the circadian oscillation (Ceriani *et al.* 1999; Busza *et al.* 2004; Sheeba *et al.* 2008). Whether cryptochromes mediate free-flight activity in *M. separata* should be further elucidated. For migratory organisms, a sense of time is vital for orientation. *Mythimna separata* adults exhibit 24-h circadian rhythms in flight activities, which occur at night. Flight rhythms can be changed by temperature, photoperiod, and food availability (Sharma and Chandrashekaran 2005). In our study, *M. separata* adults displayed a flight activity peak an hour before the morning light period under the local geomagnetic field. A sharp reduction in flight activity was observed after dawn, consistent with the flight activities of *M. separata* in the field (Lin 1990). In contrast, the near-zero magnetic field disturbed the flight rhythm of the *M. separata* adults as evidenced by the elimination of the late-night peak in flight activity. Because of our small sample size, we could not examine any differences between males and females.

During the migration of oriental armyworm, in addition to the temperature, humidity and photoperiod change, the magnetic field intensity increases with latitude. Our results indicated that a decrease of magnetic field intensity had negative effects on the development, reproduction and flight activity of *M. separata*. Therefore, it is possible that the increased geomagnetic field intensity in the north may enhance the developmental rate of immigrant moths in northern China, increasing the risk of population outbreaks and damage compared to the southern source regions. Although fecundity was not affected by the near-zero magnetic field in this study, Vg gene transcription, and thus presumably vitellogenesis and oogenesis, was negatively affected. *Mythimna separata* migrants exhibit the oogenesis-flight syndrome (Johnson 1969; McNeil *et al.* 1995), where egg development and mating are delayed until the termination of migration (Jiang *et al.* 2011), consistent with lower rates of vitellogenesis and mating at lower latitudes before long-distance northward migration in the spring. Therefore, it is interesting that migrating *M. separata* females captured over the Bohai Gulf in northern China during the summer were mostly mated and sexually mature (Zhao *et al.* 2009). Though quite indirectly, our data suggest that the strength of the geomagnetic field at different latitudes may affect the rate of reproductive development in a way that contributes to the observed differential display of the oogenesis-flight syndrome among migrant *M. separata* in different seasons originating from different latitudes. Testing this hypothesis will require very thorough experimentation, however, such as determining the developmental and behavioral effects of relevant geomagnetic field strengths across the latitudes experienced by breeding populations during different seasons in China.

5. Conclusion

In summary, this study demonstrated that the elimination of the geomagnetic field results in delayed pupation and adult emergence, prolonged adult male longevity, and decreased female Vg transcript levels. Adults exhibited a lower mating rate under the near-zero magnetic field, which also caused a decrease in the free flight activities of adult *M. separata*.

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