SUPPLEMENARY MATERIALS

**Change in geomagnetic field intensity alters migration-associated traits in a migratory insect**

Guijun Wan, Ruiying Liu, Chunxu Li, Jinglan He, Weidong Pan, Gregory A. Sword, Gao Hu, Fajun Chen\*

**Detailed Material and methods**

**(a) Insect stock**

Adult *N. lugens* were collected from paddy fields at Nanjing, Jiangsu province of China during their south-to-north migration in the summer and reared indoors on susceptible Taichung Native 1 rice seedlings at 25±1℃, 70-80% RH and 14:10 h light: dark cycle (Dark during 20:00-06:00) to establish a lab colony. The colony was maintained for ten generations before they were allocated to the two experimental magnetic treatment groups.

**(b) Magnetic fields and insect exposures**

The GMF intensity generally ranges from ~25μT at the magnetic equator to ~65μT at the magnetic poles. We use two three-axis DC-type Helmholtz coil systems (External diameter: 1200 mm) to mimic the GMF intensity of two points on the migration route of *N. lugens*, Zhanjiang city (21°12' 29" N, 110° 21' 11" E; mimic intensity: 45000 ± 255 nT, GMF45μT) and Nanjing city (32° 3' 42" N, 118° 46' 40" E; mimic intensity: 50000 ± 239 nT, GMF50μT) indoors. To exclude potential effects of directional information of the GMF on insect migration-associated traits, which was not the intent of this study, the same declination (−5.7 ± 1.32°) and inclination (50.2 ± 1.23°) between GMF50μT vs. GMF45μT treatments were generated by adjusting the current of the three independent coil pairs composing the Helmholtz coil systems. The GMF was altered in effective homogeneous (< 2% heterogeneity) areas of 300 × 300 × 300 mm3 inside each coil system, and a Faraday cage inside each coil was used to shield the experimental insects from potential anthropogenic electromagnetic noise. The parameters of the simulated magnetic field were monitored daily with a fluxgate magnetometer (Model 191A, HONOR TOP Magnetoelectric Technology Co., Ltd., Qingdao, China; sensitivity: ±1 nT). To ensure uniform environmental factors, the two coils systems were located in the same room separated by 6.5 m to avoid interference with each other. The position of tested *N. lugens* within the effective areas of the two coil systems was randomly changed in the same way daily. Following an established rearing protocol, rice planthoppers were exposed to the GMF50μT vs. GMF45μT treatments for one generation from mated F0 females to newly emerged F1 adults that were used in the assays [1]. We exposed *N. lugens* to the different GMF intensities for one generation because the insects were expected to undergo at least one more long-distance migration after F0 generation. The animals were maintained under corresponding magnetic conditions throughout the experiments and sampling before quickly killed in liquid nitrogen for the molecular assays. All experiments were conducted indoors at Nanjing Agricultural University.

**(c) Wing dimorphism and behavioural assays**

Once the F1 adults emerged, individuals from each GMF group were identified to sex and wing form. Knowing that only macropterous adults can migrate, and most migrants are unmated [2], macropterous unmated adults from the GMF50μT and GMF45μT groups were respectively tested for flight capacity and positive phototaxis. Given that 2-day-old adult *N. lugens* begin to take off for migration from dusk to dawn [3], 2-day-old (D2) adults were investigated for flight capacity from 18:00–08:00 covering the time period of dusk, night and dawn. In addition, according to the responses of the closely-related migratory species *S. furcifera* to GMF nullification [1]*,* newly emerged to 3-day-old (D1-D3) adults were investigated for positive phototaxis also from 18:00–08:00.

To compare flight distances between the two GMF treatments, an infrared-beam based 8-channel flight mill system was constructed with clear acrylic plexiglass and non-magnetic metals (brass and tungsten) to avoid any anomalies triggered by ferromagnetic materials. The rice planthoppers could make a circular flight (diameter: 120 mm) while affixed to flight arm made from twisted copper wire (diameter: 2 mm) with the vertical axis for the attachment of the adult’s mesotergum as previously described [1]. Eight insects could be tested each day. An equal number of adults from each GMF treatment were distributed to corresponding flight mills at 17:45 (allowing 15 min for acclimation). Any insects that appeared unhealthy or escaped from their flight arms were excluded for further analyses. A total of fifteen 2-day-old adult *N. lugens* of each sex were tested in each GMF treatment group.

Positive phototaxis was assessed for each treatment group under their respective magnetic field conditions using the experimental apparatus depicted in Figure S1. The left main body of the apparatus (excluding the lamp) was placed within the Faraday cage made of red copper mesh during the test. A lamp (PS-15II type; Jiaduo Science, Industry and Trade Co. Ltd., Hebi, China) which is commonly used for pest monitoring and control, was installed as the light source (15 W; λ = 320-680 nm) and positioned in the middle of the two Helmholtz coil systems. Thus, insects treated under GMF50μT and GMF45μT groups used the same light source for the positive phototaxis test. To ensure that the only difference between the groups was the imposed magnetic field and considering that *N. lugens* did not all emerge on the same day, ten female or male adults of the same post-emergence age (D1, D2 or D3) were selected from GMF50μT or GMF45μT treatments and assigned to each channel of the phototaxis testing apparatus under corresponding magnetic field conditions at 17:45 (allowing 15 min for acclimation). The lamp was switched on from 18:00–08:00 each day. Once the light was off at the end of the trial, the number of adults that moved to the right-hand bottles toward the light (including the adults that moved beyond openings of the funnel-shaped transparent parts in the opaque test channels but did not reach the right-hand bottles) for each magnetic field treatment was checked and recorded. Insects that failed to move beyond openings of the funnel-shaped transparent parts were excluded for the following tests. The ratios of macropterous adults that moved towards the light on the 1st-3rd days and on the first 3 days after adult emergence were calculated to assess adult positive phototaxis. The final sample size for each D1-D3 test group was at least 90 (see exact sample sizes in the caption of Figure 1 in the main text).

 

**Figure S1.** Experimental apparatus to test for positive phototaxis of brown planthoppers. The left four-channel main body of the apparatus is made of glass, plastic and cardboard. The length of each test channel including the bottles is 1200 mm with a diameter of 120 mm. The length between the bottom of the left bottle and the opening of the funnel-shaped plastic part designed to prevent backward movement of insects for each channel is 300 mm. The cross-sectional area of the four test channels is within 300 × 300 mm2 to ensure the exposure of insects to the effective homogeneous area within the Helmholtz coil system. The light shielding part is used to shelter the bottles from light interference. The light source was a widely used light trap for brown planthoppers with the wavelength of 320-680 nm.

**(d) Molecular and biochemical analysis**

We selected the multifunctional *Crys* (*Cry1* and *Cry2*) genes in 1 to 3-day-old macropterous unmated adults for transcript expression analysis by quantitative real-time polymerase chain reaction (qRT-PCR) using *Arginine kinase* and *alpha 2-tubulin* as reference genes. Nine biologically independent pools, each containing ten adults, were frozen at the same time of 18:00 in liquid nitrogen (collected at 17:45 hours and acclimated for 15 min) for each group divided by sex, magnetic field intensity and age, just before conducting the positive phototaxis experiment. Total RNA isolation, quality control, and concentration determination for total RNA, cDNA synthesis, and qRT-PCR were conducted as previously described [1]. Primer information can be found in Table S1. The primary flight fuels, including hemolymph triglyceride (Triglyceride detection kit; Jiancheng Bioengineering Institute, China) and whole-body trehalose (Trehalose detection kit; Suzhou Comin Biotechnology Co. Ltd., China) were measured using commercially available test kits [4, 5]. Samples were collected in nine biologically independent pools (each containing ten 2-day-old adults) for each group divided by sex and magnetic field intensity and frozen at the same time of 18:00 in liquid nitrogen (collected at 17:45 hours and acclimated for 15 min) just before the flight capacity test.

**Table S1.** Primers used to measure the transcript expression of *cryptochrome1* and *cryptochrome2* of unmated macropterous adult *Nilaparvata lugens* in the qRT-PCR assays.

|  |  |  |  |
| --- | --- | --- | --- |
| **Primer** | **Sequence (5’–3’)** | **Description** | **Genebank** |
| *NLAK*-F | TGCACATCAAAGTCCCCAAG | *Arginine kinase* (Reference gene) | KU365925.1 |
| *NLAK*-R | TCGTAGACACCGCCCTCG |
| *NLTub*-F | TGACCGAGTTCCAGACTAACCT | *Alpha 2-tubulin* (Reference gene) | FJ810204.1 |
| *NLTub*-R | AGACAACTGCTCGTGGTAGG |
| *NLCry1*-F | CAGACATGGGCTTCGATTTCA | *Cryptochrome 1* | KM108579.1 |
| *NLCry1*-R | ACCAGCACTTTCTCCGTCAAAT |
| *NLCry2*-F | CGCATACTCTCTACAGACTTGAT | *Cryptochrome 2* | KM108578.1 |
| *NLCry2*-R | CACCGTCTGGAATTTGCGATAC |

**(e) Statistical analysis**

All data were analyzed using SPSS 20 (IBM Inc., Armonk, NY, U.S.A.). Associations among categorical variables were assessed with the two-sided Cochran-Mantel-Haenszel chi-square test, while continuous variables were analyzed with general linear models (GLM). For continuous variables, Shapiro–Wilk test was used to test for normality (*P* > 0.05) and Levene's test for the homogeneity of variances (*P* > 0.05), before an analysis of variance (ANOVA). In this study, all variables were separated by sex to investigate the main effect of GMF intensity. Therefore, we did not take sex as a fixed factor in GLM, and no post-hoc multiple comparison tests for the sampling time or test day were performed. We used a two-sided Cochran-Mantel-Haenszel test to investigate the association between GMF intensity (GMF50μT or GMF45μT) and positive phototaxis stratified by test day (D1, D2, D3), and a two-way ANOVA to analyze the effects of the GMF intensity, sampling time (D1, D2, D3) and their interactions on gene expression levels of *Cry1* and *Cry2* (log(x + 1) transformation) at α = 0.05 for both females and males. When we found a significant association between the two categorical variables, a follow-up chi-square test (two-tailed) with Yates's correction was performed to detail the association between GMF intensity and the positive phototaxis for females or males during the three test days. If a significant effect of GMF intensity or of interactions between the GMF intensity and sampling time was found on the gene expression levels, we used follow-up one-way ANOVA to compare the means for GMF50μT and GMF45μT at α = 0.05. A chi-square test (two-tailed) with Yates's correction was performed to investigate the association between GMF intensity and wing dimorphism. A one-way ANOVA was used to test for the effect of GMF intensity on flight distance, as well as the content of primary flight fuels for female or male adults at α = 0.05, respectively. Effect sizes were estimated using Cohen's *w* and partial *η*2 for chi-square test (small effect: *w* = 0.1; medium effect: *w* = 0.3; large effect: *w* = 0.5) and ANOVA (small effect: partial *η*2 = 0.01; medium effect: partial *η*2 = 0.06; large effect: partial *η*2 = 0.14), respectively, based on the benchmarks of Cohen (2013) [6].

**References**

[1] Wan, G., Yuan, R., Wang, W., Fu, K., Zhao, J., Jiang, S., Pan, W., Sword, G.A. & Chen, F. 2016 Reduced geomagnetic field may affect positive phototaxis and flight capacity of a migratory rice planthopper. *Anim Behav* **121**, 107-116. (doi:10.1016/j.anbehav.2016.08.024).

[2] Zheng, D., Hu, G., Yang, F., Du, X., Yang, H., Zhang, G., Qi, G., Liang, Z., Zhang, X., Cheng, X., *et al.* 2014 Ovarian development status and population characteristics of *Sogatella furcifera* (Horváth) and *Nilaparvata lugens* (Stål): implications for pest forecasting. *J. Appl. Entomol.* **138**, 67-77. (doi:10.1111/jen.12067).

[3] Cheng, X., Wu, J. & Ma, F. 2003 *Brown planthopper: occurrence and control*. Beijing, China, China Agricultural Press.

[4] Zhao, Z. & Zera, A.J. 2002 Differential lipid biosynthesis underlies a tradeoff between reproduction and flight capability in a wing-polymorphic cricket. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 16829-16834. (doi:10.1073/pnas.262533999).

[5] Arrese, E.L. & Soulages, J.L. 2010 Insect fat body: energy, metabolism, and regulation. *Annu. Rev. Entomol.* **55**, 207-225. (doi:10.1146/annurev-ento-112408-085356).

[6] Cohen, J. 2013 *Statistical Power Analysis for the Behavioral Sciences*. 2nd Edition ed. New York, Routledge. (doi:10.4324/9780203771587)