Geomagnetic field intensity may be a cue for the regulation of insect migration

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Abstract

The geomagnetic field (GMF) intensity can help animal migrants to determine position during their migration. However, its potential roles in mediating other migration-related phenotypes remain mostly unknown. Here, by simulating the GMF total intensity of two points ($GMF_{50\mu T}$ vs. $GMF_{45\mu T}$) in the migration route of a nocturnal insect migrant, brown planthopper *Nilaparvata lugens*, we investigated their magnetic responses of three crucial migration-involved performance including wing dimorphism, flight capacity and positive phototaxis to only a ~5 μT change in field intensity after one generation exposure. Our results showed that all the three phenotypes of *N. lugens* could respond to the small changes in field intensity between the mimic northern vs. southern region ($GMF_{50\mu T}$ vs. $GMF_{45\mu T}$) in a way potentially benefitting their south-to-north expanding and securing a thriving population. Consistent magnetic response patterns of phototaxis-related *Drosophila-like cryptochrome 1* (*Cry1*) and two primary energy substances during flight, including triglyceride and trehalose, were also found. Our findings indicate the potential importance of GMF intensity in the regulation of insect migration and highlight the unique role of magnetoreception in helping insect adapt to the environment.

Keywords

Geomagnetic field intensity; Brown planthopper; Magnetoreception; Wing dimorphism; Flight capacity; Positive phototaxis

1. Introduction

Organisms on the earth are generally surrounded in the geomagnetic field (GMF) all the time. Many animals have evolved the ability to sense or even utilize the GMF components of field intensity, direction, or both, although the details of the biophysical mechanisms are still largely unknown [1, 2]. Two mechanisms, including magnetite-based magnetoreception and radical-pair-based magnetoreception, have been mostly focused on for terrestrial animals [3-5]. Especially for the radical-pair-based magnetoreception, which is mainly based on quantum coherent evolution of the different spin states of radical pairs under the effects of magnetic interactions and spin-selective reactions, has gained more and more positive evidence pointing the cryptochrome (Cry) as a potential agent for the radical-pair-based magnetoreception in a light-dependent way [3, 4, 6-8].

Despite the use of GMF for orientation and navigation, animals were also found to show other physiological and behavioral responses to changes in magnetic field intensity [9-15], even though few works have focused on responses of animals to geomagnetic level changes in field intensity [12, 16]. However, more studies on bioeffects triggered by shielding or compensating the GMF have been conducted in the last decade, and thus validated the importance of GMF in maintaining organism homeostasis [13, 17]. Our work with migratory white-backed planthopper *Sogatella furcifera* showed that the GMF absence can affect essential migration-related behaviour such as positive phototaxis (critical for the ascent process of a long-distance insect migration) and flight capacity (massive energy-consuming process and the main parameter for assessing the migration ability) potentially through a Cry-mediated hormone signaling [9, 18]. Besides, previous work in migratory birds showed that changes in GMF information (both intensity and direction) over the subjective migration route could affect their fuelling decision and migratory restlessness [19-22]. It is, therefore, speculated that GMF intensity change might also contribute to the regulation of insect migration chronically.

Migratory insects can conduct long-distance migration within a short time [23, 24]. Therefore,

possible responses of their migration-related performance to the difference in GMF intensity between the emigration and immigration areas deserve attention. The nocturnal migratory brown planthopper *Nilaparvata lugens* has an annual long-distance migration in East Asia. Generally, their northward migration is initiated in spring from tropical and subtropical areas in Indochina to South China, followed by several further waves of migration during the summer from South to North China, as well as Japan and Korea Peninsula. Moreover, as they cannot overwinter in temperate East Asia, they will then conduct a southward return migration through similar routes in Autumn [25, 26]. *N. lugens* also showed physiological magnetic responses under NZMF vs. GMF. Notably, both candidate magnetite crystals [27] and characterization of potential magnetic field targets, including Cry1, Cry2 [28], and iron-sulfur cluster assembly1 (IscA1) [29], have been systematically explored in *N. lugens*. Together with the advantage that *N. lugens* draft genome sequence is available[30], it is a promising insect model to investigate magnetoreception. Adult *N. lugens* exhibit wing dimorphism, including macropterous with functional wings for seasonal migration and brachypterous with vestigial wings for enhanced fecundity[31]. Combining the investigation of magnetic field effects on wing dimorphism, positive phototaxis, and flight capacity, we reported the first responses of migration-involved performance to a field intensity change caused by long-distance insect migration.

2. Material and methods

Insect Stock

The migratory adults of brown planthopper, *N. lugens* were collected from the paddy fields at Nanjing, Jiangsu province of China during their south-to-north migration in summer (tested also in summer), and reared indoors on susceptible Taichung Native 1 rice seedlings to expand the colony at 25±1□, 70-80% RH and 14:10 h light: dark cycle (Dark during 1700-0800 hours).

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 DC-type Helmholtz coils 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, $GMF_{45\mu T}$) and Nanjing city (32° 3' 42" N, 118° 46' 40" E; mimic intensity: 50000 ± 239 nT, $GMF_{50\mu T}$) at same declination (−5.7 ± 1.32°) and inclination (50.2 ± 1.23°) within the effective homogeneous areas of 300 × 300 × 300 mm^3. The parameters of the simulated magnetic field were measured and adjusted with a fluxgate magnetometer (Model 191A, HONOR TOP Magnetoelectric Technology Co., Ltd., Qingdao, China; sensitivity: ±1 nT) daily. To ensure uniform environmental factors, the two coils systems were located in one room with a distance of 6.5 m between each other (to avoid interference with each other), and the position of tested *N. lugens* within the effective areas of the two coils systems was randomly changed in the same way daily. Follow the previous workflow and criterion for a one-generation exposure of rice planthoppers (from mated F0 females to newly emerged F1 adults before the following tests) [9]. We conduct the one generation exposure here because *N. lugens* can generally make another long-distance migration after at least one generation.

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 conduct migration, and most migrants are unmated [32], macropterous unmated adults from the $GMF_{50\mu T}$ and $GMF_{45\mu T}$ groups were respectively tested for positive phototaxis, and flight capacity. According to the responses of the closely-related migratory species *S. furcifera* to GMF compensation, and given that 2-day-old adult *N. lugens* begin to take off for migration [25], newly emerged to 3-day-old adults and 2-day-old adults were investigated for positive phototaxis and flight capacity from 1700–0800 hours (subjective dark period), respectively. To ensure that only difference between the groups is the imposed magnetic field, same numbers of ten female or male adults at the same age randomly selected from $GMF_{50\mu T}$ or $GMF_{45\mu T}$ were assigned to each channel of the phototaxis testing apparatus under corresponding magnetic field

conditions at 1645 hours (another 15 min for acclimation). The positive phototaxis testing procedure is the same as the previous description except that we do not reuse the ones failing to move to the light at the night before in this study [9]. The infrared-beam based multi-channel flight mill system within the coils system (granted patent: ZL201620774681.1) was developed to measure the adult flight distance under the two GMF groups. The rice planthoppers would make a circular flight (diameter: 120 mm) with the help of a flight arm made from twisted copper wire (diameter: 2 mm). All adults were randomly distributed to corresponding flight mills at 1645 hours (another 15 min for acclimation). Any insects that appeared unhealthy or escaped from their flight arms were excluded for further analyses.

Molecular and biochemical analysis

We selected the multifunctional *Crys* (*Cry1* and *Cry2*) in newly emerged to 3-day-old macropterous unmated adults for transcript expression analysis (*Arginine kinase* and *alpha 2-tubulin* as reference genes) by quantitative real-time polymerase chain reaction (qRT-PCR). Nine biologically independent pools containing ten adults frozen at the same time of 1700 hours by liquid nitrogen (collected at 1645 hours and acclimated for 15 min) just before the positive phototaxis assay daily were used for gene expression analysis, and we found similar developmental changes in gene expression of adult *Cry1* and *Cry2* with the previous study [28]. Total RNA isolation, quality control, and concentration determination for total RNA, cDNA synthesis, and qRT-PCR were conducted as previously described [9]. Primer information can be found in the supplementary material (table S1). The content of primary flight fuels, including triglyceride (Jiancheng Bioengineering Institute, Nanjing, China) and trehalose (Suzhou Comin Biotechnology Co. Ltd., Suzhou, China) [33, 34], in 2-day-old adults frozen at the same time of 1700 hours by liquid nitrogen (collected at 1645 hours and acclimated for 15 min) just before the flight capacity test, has also been determined using commercially available test kits.

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 outcomes, 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 outcomes were directly separated by sex to investigate the main effect of GMF intensity. Therefore we didn't take sex as a fixed factor in GLM, and no post-hoc multiple comparison test 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 ($GMF_{50\mu T}$ or $GMF_{45\mu T}$) and positive phototaxis stratified by test day (D1, D2, D3), and a two-way analysis of variance (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 $\alpha = 0.05$ for females or males, respectively. 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 $GMF_{50\mu T}$ and $GMF_{45\mu T}$ at $\alpha = 0.05$. Besides, the chi-square test (two-tailed) with Yates's correction and one-way ANOVA were also performed to investigate the association between GMF intensity and wing dimorphism, and the effect of GMF intensity on the flight distance as well as the content of primary flight fuels for female or male adults at $\alpha = 0.05$, respectively. Effect sizes were estimated using Cohen's w, partial η^2, Cohen's d for chi-square test (small effect: $w = 0.1$; medium effect: $w = 0.3$; large effect: $w = 0.5$), ANOVA (small effect: partial $\eta^2 = 0.01$; medium effect: partial $\eta^2 = 0.06$; large effect: partial $\eta^2 = 0.14$) and Mann-Whitney U-test (small effect: $d = 0.2$; medium effect: $d = 0.5$; large effect: $d = 0.8$), respectively, based on the benchmarks of Cohen (1988).

3. Results and discussion

We found similar magnetic field effects on the selected three migration-involved performance with that in *S. furcifera* under NZMF. Significantly 128.57% ($X^2_{1, 323}$ = 27.21, P < 0.001, w = 0.30) and 51.23% ($X^2_{1, 367}$ = 17.24, P < 0.001, w = 0.22) more macropterous female and male adult *N. lugens* were respectively found under $GMF_{50\mu T}$ vs. $GMF_{45\mu T}$ (figure 1*a*.). The higher macropterous ratio, which means more migratory phenotype adults, can promote the south-to-north migration of *N. lugens*. On the other hand, with GMF intensity cues, *N. lugens* would 'know' where and how to balance the trade-off between flight capability and reproduction [31]. It is like that this magnetic response in wing dimorphism offered a link between GMF intensity and latitude areas. For *N. lugens*, the importance of reproductive capacity would rank higher for the reproductive-strategy *N. lugens* at a lower latitude, because lower latitude areas can be their overwinter base for the future migrations [25].

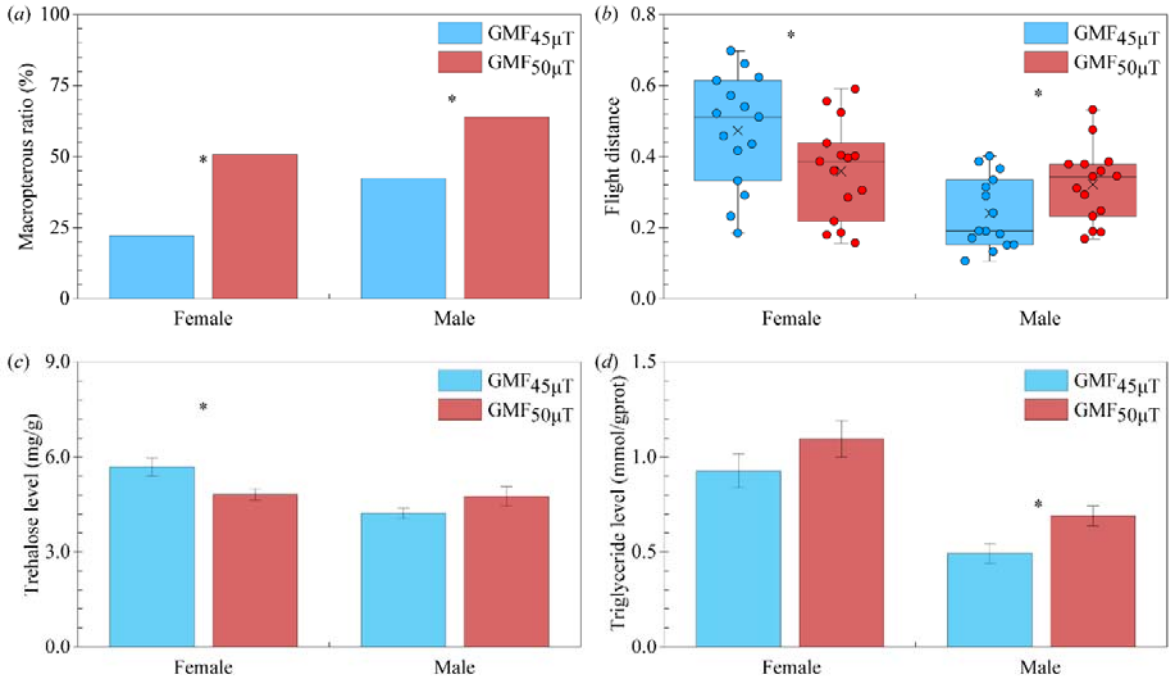

Figure 1. Wing dimorphism (*a*), flight distance (*b*), and main flight fuel trehalose and triglyceride

levels of *Nilaparvata lugens* under $GMF_{50\mu T}$ vs. $GMF_{45\mu T}$. In (*a*), N = 156 and 167 for females, and 180 and 187 for males under $GMF_{50\mu T}$ vs. $GMF_{45\mu T}$, respectively. In (*b*), N = 15 for each group of unmated 2-day-old macropterous adults. In (*c*) and (*d*), samples of nine biologically independent pools containing ten unmated 2-day-old macropterous adults were used. * denotes significant association between GMF intensity and wing dimorphism by chi-square test with Yates's correction (*a*), and significant differences between $GMF_{50\mu T}$ vs. $GMF_{45\mu T}$ by one-way ANOVA (*b*-*d*) for females or males at $p < 0.05$.

Interestingly, the simulated ~5μT GMF intensity change potentially caused by long-distance migration also showed the sexually dimorphic effect on 2-day-old adult flight capacity in *N. lugens*. Significantly extended (+33.84%; $F_{1,\ 28}$ = 4.74, P = 0.038, partial η^2 = 0.15) while shortened (-24.04%; $F_{1,\ 28}$ = 4.45, P = 0.044, partial η^2 = 0.14) flight distance in adult males and females was found under $GMF_{50\mu T}$ vs. $GMF_{45\mu T}$, respectively (figure 1*b*). As one of the most important performance during insect migration, our data suggested that adult male *N. lugens* could make a wider dispersal than females during the south-to-north emigration. One possible reason for this could be due to the critical role of females in reproduction, and higher dispersal ability of males may relieve intraspecific competition by avoiding competition to their progeny and optimizing the use of resources. As main energy substances for insect flight, consistent significantly decreased trehalose level in females (-15.18%; $F_{1,\ 16}$ = 5.89, P = 0.027, partial η^2 = 0.27; figure 1*c*) and increased triglyceride level in males (+40.46%; $F_{1,\ 16}$ = 6.33, P = 0.023, partial η^2 = 0.28; figure 1*d*) were found under $GMF_{50\mu T}$ vs. $GMF_{45\mu T}$, indicating that potential pathway regulating trehalose and triglyceride (e.g., adipokinetic, insulin and juvenile hormone signaling) is likely to be responsible for the sex-specific responses of flight distance to the GMF changes during migration[34, 35]. Taken together, the sexually dimorphic magnetic responses of flight capacity suggested the different adaptive strategies of females and males to the GMF. Furthermore, as *N. lugens* is a typical nocturnal

migrant, and we measured its flight capacity under darkness, potential magnetite crystal, electromagnetic induction within accessory structures or both may be the option here [36].

During the initial phase of migration, temporary inhibition of positive phototaxis was reported in *N. lugens* [37, 38]. Here, in the first three days after eclosion we monitored, unmated macropterous adults showed significantly weakened positive phototaxis under $GMF_{50\mu T}$ vs. $GMF_{45\mu T}$ (OR = 0.61, 95% CI: 0.50-0.76, $P < 0.001$, $w = 0.11$). Looking into details, compared with $GMF_{45\mu T}$, $GMF_{50\mu T}$ significantly decreased the percentage of unmated macropterous females and males that moved towards the light (λ = 320-680 nm) by 30.39% ($X^2_{1, 660} = 7.05$, $P = 0.008$, $w = 0.11$; figure 2*a*) and 19.49% ($X^2_{1, 880} = 7.92$, $P = 0.005$, $w = 0.10$; figure 2*b*) during the first 3 days, respectively. Notably, we found $GMF_{50\mu T}$ significantly decreased the move-to-light ratios of unmated macropterous females on the 2nd (-55.26%; $X^2_{1, 180} = 10.47$, $P = 0.001$, $w = 0.25$) and 3rd day (-36.59%; $X^2_{1, 220} = 4.206$, $P = 0.040$, $w = 0.15$; figure 2*a*), while males only on the 3rd day (-35.50%; $X^2_{1, 250} = 14.31$, $P < 0.001$, $w = 0.25$; figure 2*b*). Although the biological significance of positive phototaxis is still an open question, the weakened positive phototaxis under a higher GMF intensity may help adult *N. lugens* smoothly ascend to the sky after take-off for their south-to-north long-distance migration without distraction by light sources on the ground [38].

Table 1. Two-way ANOVAs with the geomagnetic field (GMF) as the main factor and sampling day as the sub-factor on the gene expression levels of *cryptochromes* (*Cry1* and *Cry2*) for the unmated macropterous female and male adults.

Sex	Genes	*df*	GMF[a]	*df*	Sampling day[b]	*df*[c]	GMF*Sampling day
Female	*Cry1*	1,48	11.77/0.001 (0.20)	2,48	5.64/0.006 (0.19)	2,48	1.37/0.264 (0.05)
	Cry2	1,48	6.14/0.017 (0.11)	2,48	98.15/<0.001 (0.80)	2,48	2.09/0.135 (0.08)
Male	*Cry1*	1,48	6.59/0.013 (0.12)	2,48	2.21/0.121 (0.08)	2,48	4.71/0.014 (0.16)
	Cry2	1,48	9.24/0.004 (0.16)	2,48	108.50/<0.001 (0.82)	2,48	7.35/0.002 (0.23)

Results are given as *F* value/*P* value (partial $\eta 2$).

[a] GMF - the $GMF_{50\mu T}$ vs. $GMF_{45\mu T}$.

[b] Sampling day - 17:00 on the 1st (D1), 2nd (D2) and 3rd (D3) day after the adult emergence.

[c] Ten individuals were randomly mixed as one sample.

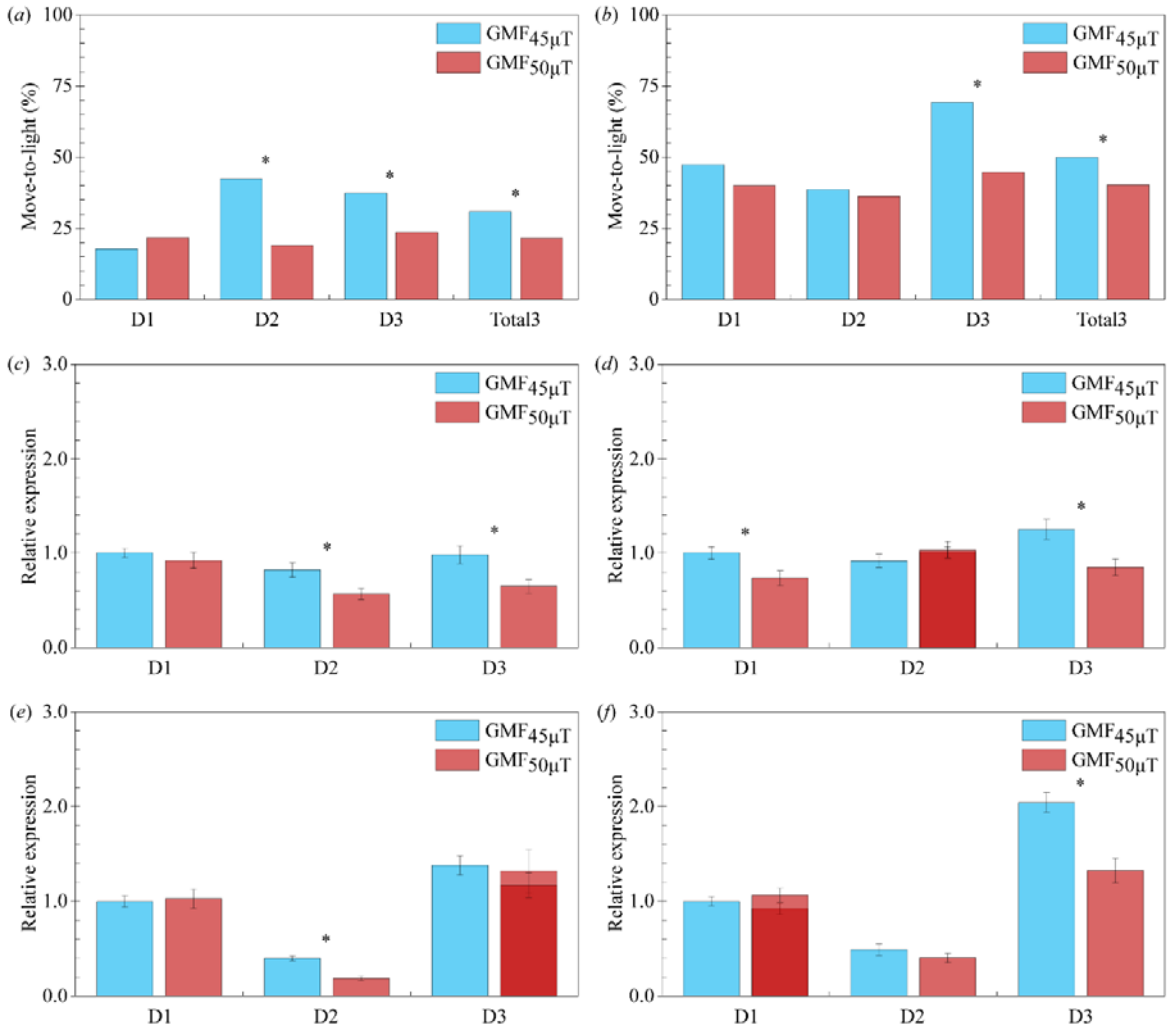

Figure 2. The positive phototaxis (*a*, *b*) and transcript expression analyses for the multifunctional *cryptochromes* (*Cry1* (*c*, *d*) and *Cry2* (*e*, *f*)) of unmated macropterous female (left panels) and male (right panels) *Nilaparvata lugens* under $GMF_{50\mu T}$ vs. $GMF_{45\mu T}$. For positive phototaxis, move-to-light percentage of adults on the 1st-3rd day (D1-D3) and in the first three days (Total3) after emergence were investigated. *N* = 130, 90, 110, 330, respectively, for the females in D1–D3 and Total3 under both $GMF_{50\mu T}$ and $GMF_{45\mu T}$; *N* = 130, 130, 130, 390, respectively, for the males in D1–D3 and Total3 under $GMF_{50\mu T}$; *N* = 220, 150, 120, 490, respectively, for the females in D1–D3 and Total3 under $GMF_{45\mu T}$. In (*c*) - (*f*), samples of nine biologically independent pools containing ten unmated macropterous adults were used. * denotes significant association between GMF intensity and positive phototaxis by chi-square test with Yates's correction (*a*, *b*), and significant differences

between $GMF_{50\mu T}$ vs. $GMF_{45\mu T}$ by one-way ANOVA (*c-f*) for each test (sampling) day at $p < 0.05$.

It is well established that multifunctional Crys were reported to involve magnetoreception, phototaxis, and the circadian clock [39, 40]. In our study, except for *Cry1* in males, the expression levels of *Crys* in both adult females and males on the 1st-3rd days (D1–D3) after emergence all varied significantly over time ($F_{2, 48} \geq 5.64$, $P \leq 0.006$, partial $\eta^2 \geq 0.19$). The significant interactions between the GMF intensity and sampling day on the expression levels of *Crys* were only found in males ($F_{2, 48} \geq 4.71$, $P \leq 0.014$, partial $\eta^2 \geq 0.16$), while the expression levels of *Crys* in both females and males were found significantly affected by the GMF intensity ($F_{1, 48} \geq 6.14$, $P \leq 0.017$, partial $\eta^2 \geq 0.11$; Table 1). Mazzotta *et al.* (2013) found that loss-of-function *Drosophila* Cry mutant can impair the phototaxis of fly [40]. Considering this, the consistent temporal expression pattern of Cry1 may be responsible for the weakened positive phototaxis. Going into details, for both females and males, significantly decreased gene expressions were found in 2- (-30.78%; $F_{1, 16} = 6.10$, $P = 0.025$, partial $\eta^2 = 0.28$) and 3-day-old (-34.09%; $F_{1, 16} = 7.00$, $P = 0.018$, partial $\eta^2 = 0.30$; figure 2*c*) females as well as 1- (-26.67%; $F_{1, 16} = 6.19$, $P = 0.024$, partial $\eta^2 = 0.28$) and 3-day-old (-32.17%; $F_{1, 16} = 7.76$, $P = 0.013$, partial $\eta^2 = 0.33$; figure 2*d*) males under $GMF_{50\mu T}$ vs. $GMF_{45\mu T}$. Significantly down-regulated *Cry2* expressions were also found in 2-day-old female (-52.86%; $F_{1, 16} = 29.36$, $P < 0.001$, partial $\eta^2 = 0.65$; figure 2*e*) and 3-day-old male (-35.24%; $F_{1, 16} = 16.75$, $P = 0.001$, partial $\eta^2 = 0.51$; figure 2*f*) adults. Not like *Drosophila*-like Cry1, Vertebrate-like Cry2 was reported as vestigial flavoproteins [41], and would unlikely to function as a magnetosensor by itself. Considering it is well established that *Drosophila*-like Cry1 is involved in magnetosensing, it is possible for *N. lugens* Cry1 to function by itself for magnetoreception [1, 3, 4]. The magnetic response of positive phototaxis we found here is likely to be a result of interactions between the roles of *Drosophila*-like Cry1 in phototaxis and magnetoreception [1, 3, 4, 40], even though Cry2 is also possible to function in a complex with photoreceptors or downstream the signaling for magnetoreception [42]. Moreover,

given that Cry2 is a component of the core circadian clock and it is reported that circadian clock is sensitive to changes in magnetic field intensity [11], potential timing mechanism for migration based on GMF intensity might also exist [43].

4. Conclusion

Although *N. lugens* can also conduct the north-to-south remigration, it is more vital for them to explore the non-overwintering higher latitude areas to broaden their niche breadth. Especially for females which is more critical in reproductive strategies, they can still potentially benefit from the changes in GMF intensity caused by north-to-south remigration due to the enhanced flight capacity and higher frequency of brachypterous adult which would possess increased fecundity. The magnetic responses we found here may be a balanced result after long-term adaptive evolution for the population homeostasis of *N. lugens*. Taken together, we presented the first evidence in a nocturnal insect migrant that crucial migration-involved performance can respond to changes in GMF intensity during long-distance migration, suggesting GMF as a potential cue for the complicated system in regulating animal migration.

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References

[1] Mouritsen, H. 2018 Long-distance navigation and magnetoreception in migratory animals. *Nature* **558**, 50-59. (doi:10.1038/s41586-018-0176-1).

[2] Chernetsov, N., Pakhomov, A., Kobylkov, D., Kishkinev, D., Holland, R.A. & Mouritsen, H. 2017 Migratory Eurasian reed warblers can use magnetic declination to solve the longitude problem. *Curr. Biol.* **27**, 2647-2651. (doi:10.1016/j.cub.2017.07.024).

[3] Hore, P.J. & Mouritsen, H. 2016 The radical-pair mechanism of magnetoreception. *Annu Rev Biophys* **45**, 299-344. (doi:10.1146/annurev-biophys-032116-094545).

[4] Ritz, T., Adem, S. & Schulten, K. 2000 A model for photoreceptor-based magnetoreception in birds. *Biophys. J.* **78**, 707-718. (doi:10.1016/s0006-3495(00)76629-x).

[5] Kirschvink, J.L., Walker, M.M. & Diebel, C.E. 2001 Magnetite-based magnetoreception. *Curr. Opin. Neurobiol.* **11**, 462-467.

[6] Maeda, K., Henbest, K.B., Cintolesi, F., Kuprov, I., Rodgers, C.T., Liddell, P.A., Gust, D., Timmel, C.R. & Hore, P.J. 2008 Chemical compass model of avian magnetoreception. *Nature* **453**, 387-390. (doi:10.1038/nature06834).

[7] Maeda, K., Robinson, A.J., Henbest, K.B., Hogben, H.J., Biskup, T., Ahmad, M., Schleicher, E., Weber, S., Timmel, C.R. & Hore, P.J. 2012 Magnetically sensitive light-induced reactions in cryptochrome are consistent with its proposed role as a magnetoreceptor. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 4774-4779. (doi:DOI 10.1073/pnas.1118959109).

[8] Liedvogel, M. & Mouritsen, H. 2010 Cryptochromes--a potential magnetoreceptor: what do we know and what do we want to know? *J R Soc Interface* **7 Suppl 2**, S147-162. (doi:10.1098/rsif.2009.0411.focus).

[9] Wan, G.J., Yuan, R., Wang, W.J., Fu, K.Y., Zhao, J.Y., Jiang, S.L., Pan, W.D., Sword, G.A. & Chen, F.J. 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).

[10] Fedele, G., Green, E.W., Rosato, E. & Kyriacou, C.P. 2014 An electromagnetic field disrupts negative

geotaxis in *Drosophila* via a CRY-dependent pathway. *Nat Commun* **5**, 4391. (doi:10.1038/ncomms5391).

[11] Yoshii, T., Ahmad, M. & Helfrich-Forster, C. 2009 Cryptochrome mediates light-dependent magnetosensitivity of *Drosophila*'s circadian clock. *PLoS Biol.* **7**, e1000086. (doi:10.1371/journal.pbio.1000086).

[12] Bae, J.E., Bang, S., Min, S., Lee, S.H., Kwon, S.H., Lee, Y., Lee, Y.H., Chung, J. & Chae, K.S. 2016 Positive geotactic behaviors induced by geomagnetic field in *Drosophila*. *Mol Brain* **9**, 55. (doi:10.1186/s13041-016-0235-1).

[13] Binhi, V.N. & Prato, F.S. 2017 Biological effects of the hypomagnetic field: an analytical review of experiments and theories. *PLoS ONE* **12**, e0179340. (doi:10.1371/journal.pone.0179340).

[14] Roman, A. & Tombarkiewicz, B. 2009 Prolonged weakening of the geomagnetic field (GMF) affects the immune system of rats. *Bioelectromagnetics* **30**, 21-28. (doi:10.1002/bem.20435).

[15] Zhang, X., Yarema, K. & Xu, A. 2017 *Biological Effects of Static Magnetic Fields*. Singapore, Springer.

[16] Lohmann, K.J. & Lohmann, C.M.F. 1996 Detection of magnetic field intensity by sea turtles. *Nature* **380**, 59-61. (doi:10.1038/380059a0).

[17] Choleris, E., Del Seppia, C., Thomas, A.W., Luschi, P., Ghione, G., Moran, G.R. & Prato, F.S. 2002 Shielding, but not zeroing of the ambient magnetic field reduces stress-induced analgesia in mice. *Proc. Biol. Sci.* **269**, 193-201. (doi:10.1098/rspb.2001.1866).

[18] Wan, G.J., Wang, W.J., Xu, J.J., Yang, Q.F., Dai, M.J., Zhang, F.J., Sword, G.A., Pan, W.D. & Chen, F.J. 2015 Cryptochromes and hormone signal transduction under near-zero magnetic fields: new clues to magnetic field effects in a rice planthopper. *PLoS ONE* **10**, e0132966. (doi:10.1371/journal.pone.0132966).

[19] Bulte, M., Heyers, D., Mouritsen, H. & Bairlein, F. 2017 Geomagnetic information modulates nocturnal migratory restlessness but not fueling in a long distance migratory songbird. *J. Avian Biol.* **48**, 75-82. (doi:10.1111/jav.01285).

[20] Fransson, T., Jakobsson, S., Johansson, P., Kullberg, C., Lind, J. & Vallin, A. 2001 Bird migration: magnetic cues trigger extensive refuelling. *Nature* **414**, 35-36 (doi:10.1038/35102115).

[21] Kullberg, C., Henshaw, I., Jakobsson, S., Johansson, P. & Fransson, T. 2007 Fuelling decisions in migratory birds: geomagnetic cues override the seasonal effect. *Proc. Biol. Sci.* **274**, 2145-2151. (doi:10.1098/rspb.2007.0554).

[22] Henshaw, I., Fransson, T., Jakobsson, S., Lind, J., Vallin, A. & Kullberg, C. 2008 Food intake and fuel deposition in a migratory bird is affected by multiple as well as single-step changes in the magnetic field. *J. Exp*.

Biol. **211**, 649-653. (doi:10.1242/jeb.014183).

[23] Chapman, J.W., Reynolds, D.R. & Wilson, K. 2015 Long-range seasonal migration in insects: mechanisms, evolutionary drivers and ecological consequences. *Ecol. Lett.* **18**, 287-302. (doi:10.1111/ele.12407).

[24] Dreyer, D., Frost, B., Mouritsen, H., Gunther, A., Green, K., Whitehouse, M., Johnsen, S., Heinze, S. & Warrant, E. 2018 The Earth's magnetic field and visual landmarks steer migratory flight behavior in the nocturnal Australian Bogong moth. *Curr. Biol.* **28**, 2160-2166. (doi:10.1016/j.cub.2018.05.030).

[25] Cheng, X.N., WU, J.C. & Ma, F. 2003 *Brown planthopper: occurrence and control*. Beijing, China, China Agricultural Press.

[26] Hu, G., Lu, M.H., Reynolds, D.R., Wang, H.K., Chen, X., Liu, W.C., Zhu, F., Wu, X.W., Xia, F., Xie, M.C., et al. 2019 Long-term seasonal forecasting of a major migrant insect pest: the brown planthopper in the Lower Yangtze River Valley. *J Pest Sci (2004)* **92**, 417-428. (doi:10.1007/s10340-018-1022-9).

[27] Pan, W., Wan, G., Xu, J., Li, X., Liu, Y., Qi, L. & Chen, F. 2016 Evidence for the presence of biogenic magnetic particles in the nocturnal migratory brown planthopper, *Nilaparvata lugens*. *Sci Rep* **6**, 18771. (doi:10.1038/srep18771).

[28] Xu, J.J., Wan, G.J., Hu, D.B., He, J., Chen, F.J., Wang, X.H., Hua, H.X. & Pan, W.D. 2016 Molecular characterization, tissue and developmental expression profiles of cryptochrome genes in wing dimorphic brown planthoppers, *Nilaparvata lugens*. *Insect Sci.* **23**, 805-818. (doi:10.1111/1744-7917.12256).

[29] Xu, J.J., Zhang, Y.C., Wu, J.Q., Wang, W.H., Li, Y., Wan, G.J., Chen, F.J., Sword, G.A. & Pan, W.D. 2019 Molecular characterization, spatial-temporal expression and magnetic response patterns of iron-sulfur cluster assembly1 (IscA1) in the rice planthopper, *Nilaparvata lugens*. *Insect Sci.* **26**, 413-423. (doi:10.1111/1744-7917.12546).

[30] Xue, J., Zhou, X., Zhang, C.X., Yu, L.L., Fan, H.W., Wang, Z., Xu, H.J., Xi, Y., Zhu, Z.R., Zhou, W.W., et al. 2014 Genomes of the rice pest brown planthopper and its endosymbionts reveal complex complementary contributions for host adaptation. *Genome Biol.* **15**, 521. (doi:10.1186/s13059-014-0521-0).

[31] Guerra, P.A. 2011 Evaluating the life-history trade-off between dispersal capability and reproduction in wing dimorphic insects: a meta-analysis. *Biol Rev Camb Philos Soc* **86**, 813-835. (doi:10.1111/j.1469-185X.2010.00172.x).

[32] Zheng, D.B., Hu, G., Yang, F., Du, X.D., Yang, H.B., Zhang, G., Qi, G.J., Liang, Z.L., Zhang, X.X., Cheng, X.N., 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).

[33] 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).

[34] 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).

[35] Xu, J., Sheng, Z. & Palli, S.R. 2013 Juvenile hormone and insulin regulate trehalose homeostasis in the red flour beetle, Tribolium castaneum. *PLoS Genet.* **9**, e1003535. (doi:10.1371/journal.pgen.1003535).

[36] Nordmann, G.C., Hochstoeger, T. & Keays, D.A. 2017 Magnetoreception-a sense without a receptor. *PLoS Biol.* **15**, e2003234. (doi:10.1371/journal.pbio.2003234).

[37] Hu, G., Lu, F., Lu, M.H., Liu, W.C., Xu, W.G., Jiang, X.H. & Zhai, B.P. 2013 The influence of Typhoon Khanun on the return migration of *Nilaparvata lugens* (Stål) in eastern China. *PLoS ONE* **8**, e57277. (doi:10.1371/journal.pone.0057277).

[38] Zhao, S.F. 1992 Behavioral mechanism of phototactic response of natural planthopper population (Sogatella furcifera and Nilaparvata lugens). *Jiangxi Nongye Daxue Xuebao* **4**, 74-79.

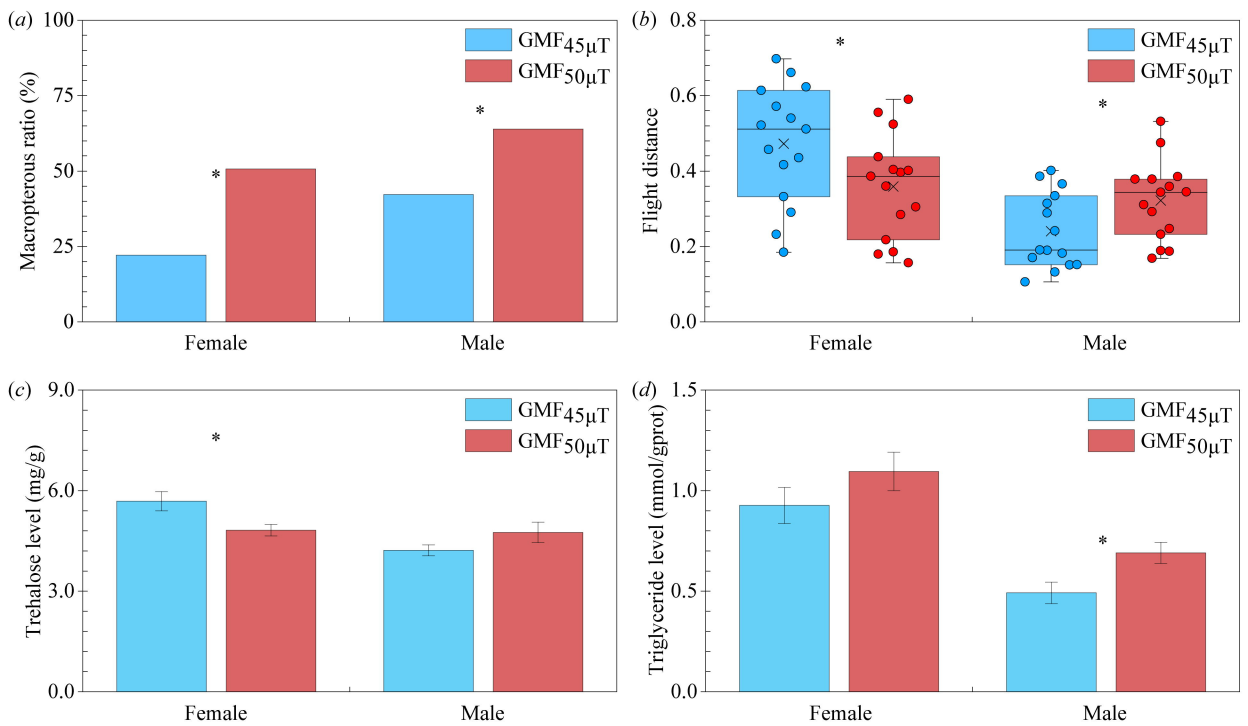
[39] Chaves, I., Pokorny, R., Byrdin, M., Hoang, N., Ritz, T., Brettel, K., Essen, L.O., van der Horst, G.T., Batschauer, A. & Ahmad, M. 2011 The cryptochromes: blue light photoreceptors in plants and animals. *Annu Rev Plant Biol* **62**, 335-364. (doi:10.1146/annurev-arplant-042110-103759).

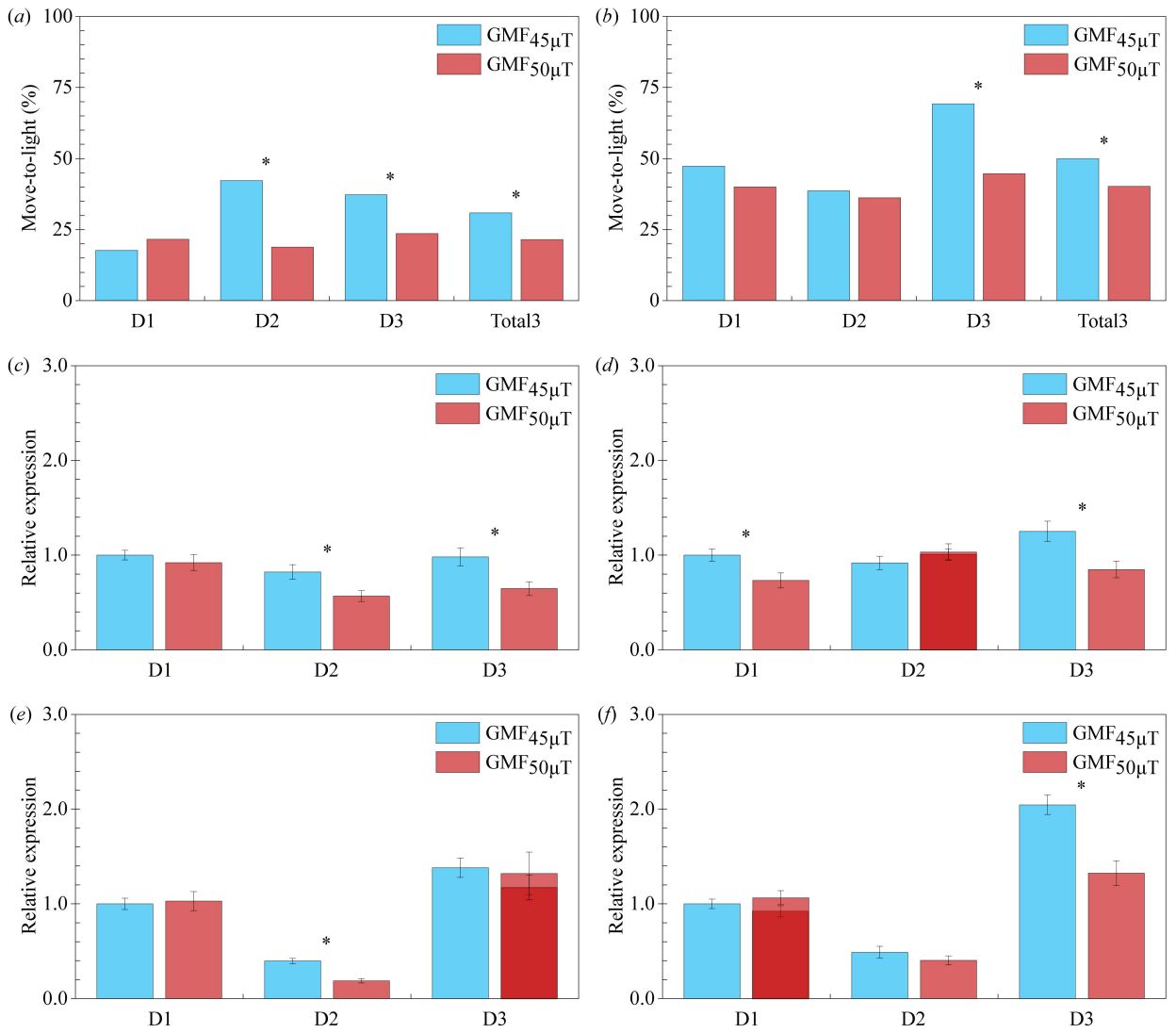
[40] Mazzotta, G., Rossi, A., Leonardi, E., Mason, M., Bertolucci, C., Caccin, L., Spolaore, B., Martin, A.J., Schlichting, M., Grebler, R., et al. 2013 Fly cryptochrome and the visual system. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 6163-6168. (doi:10.1073/pnas.1212317110).

[41] Kutta, R.J., Archipowa, N., Johannissen, L.O., Jones, A.R. & Scrutton, N.S. 2017 Vertebrate cryptochromes are vestigial flavoproteins. *Sci Rep* **7**, 44906. (doi:10.1038/srep44906).

[42] Bazalova, O., Kvicalova, M., Valkova, T., Slaby, P., Bartos, P., Netusil, R., Tomanova, K., Braeunig, P., Lee, H.J., Sauman, I., et al. 2016 Cryptochrome 2 mediates directional magnetoreception in cockroaches. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 1660-1665. (doi:10.1073/pnas.1518622113).

[43] Merlin, C. & Liedvogel, M. 2019 The genetics and epigenetics of animal migration and orientation: birds, butterflies and beyond. *J. Exp. Biol.* **222**. (doi:10.1242/jeb.191890).


(a)
Macropterous ratio (%)
100
75
50
25
0
GMF45μT
GMF50μT
*
*
Female
Male
(b)
Flight distance
0.8
0.6
0.4
0.2
0.0
GMF45μT
GMF50μT
*
*
Female
Male
(c)
Trehalose level (mg/g)
9.0
6.0
3.0
0.0
GMF45μT
GMF50μT
*
Female
Male
(d)
Triglyceride level (mmol/gprot)
1.5
1.0
0.5
0.0
GMF45μT
GMF50μT
*
Female
Male


(a)
Move-to-light (%)
100
75
50
25
0
GMF45μT
GMF50μT
*
*
*
D1
D2
D3
Total3
(b)
Move-to-light (%)
100
75
50
25
0
GMF45μT
GMF50μT
*
*
D1
D2
D3
Total3
(c)
Relative expression
3.0
2.0
1.0
0.0
GMF45μT
GMF50μT
*
*
D1
D2
D3
(d)
Relative expression
3.0
2.0
1.0
0.0
GMF45μT
GMF50μT
*
*
D1
D2
D3
(e)
Relative expression
3.0
2.0
1.0
0.0
GMF45μT
GMF50μT
*
D1
D2
D3
(f)
Relative expression
3.0
2.0
1.0
0.0
GMF45μT
GMF50μT
*
D1
D2
D3