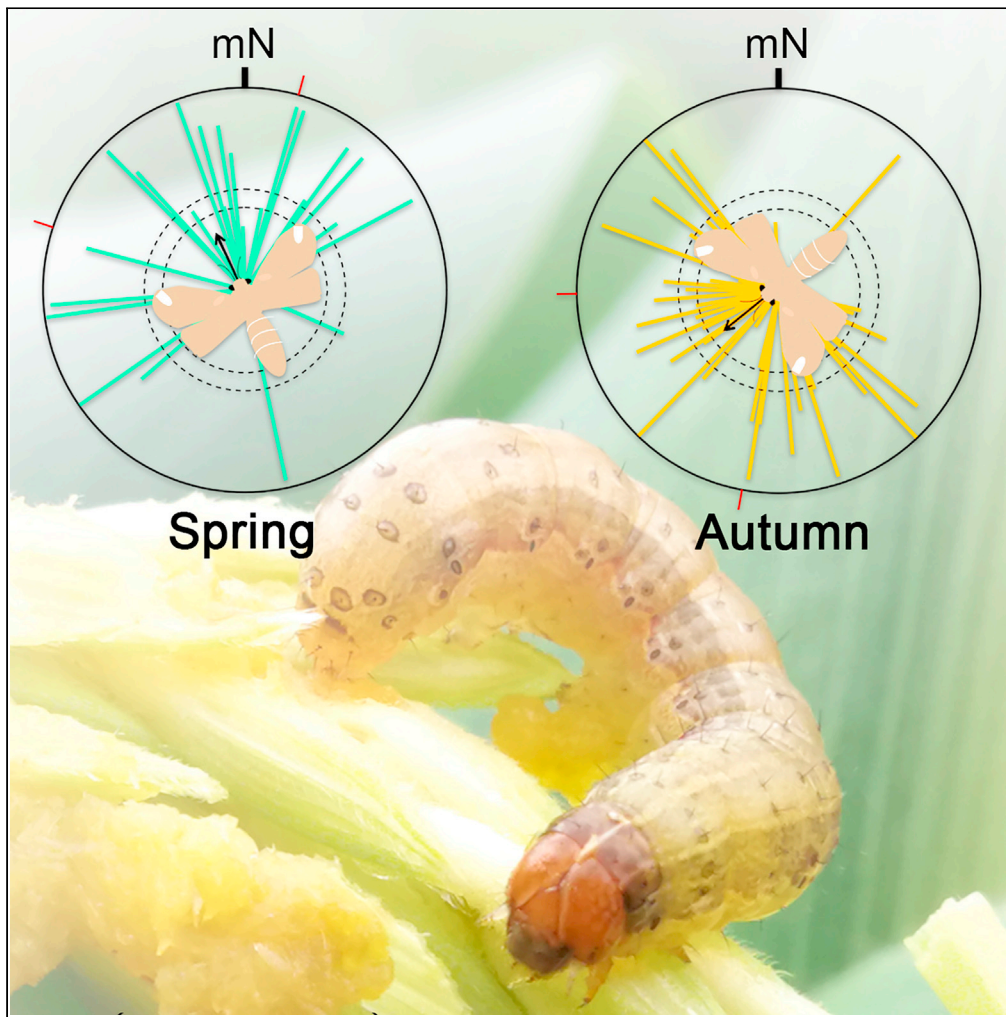


## Article

## Adaptive migratory orientation of an invasive pest on a new continent



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**Highlights**

Fall armyworm exhibits locally adaptive migratory orientation on a new continent

Moths in China preferentially head northwards in spring and southwards in autumn

The driver of the seasonal switch in orientation is changing photoperiod

Chen et al., iScience ■ ■ , 108281

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[https://doi.org/10.1016/](https://doi.org/10.1016/j.isci.2023.108281)

[j.isci.2023.108281](https://doi.org/10.1016/j.isci.2023.108281)

Article

# Adaptive migratory orientation of an invasive pest on a new continent

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## SUMMARY

Many species of insects undertake long-range, seasonally reversed migrations, displaying sophisticated orientation behaviors to optimize their migratory trajectories. However, when invasive insects arrive in new biogeographical regions, it is unclear if migrants retain (or how quickly they regain) ancestral migratory traits, such as seasonally preferred flight headings. Here we present behavioral evidence that an invasive migratory pest, the fall armyworm moth (*Spodoptera frugiperda*), a native of the Americas, exhibited locally adaptive migratory orientation less than three years after arriving on a new continent. Specimens collected from China showed flight orientations directed north-northwest in spring and southwest in autumn, and this would promote seasonal forward and return migrations in East Asia. We also show that the driver of the seasonal switch in orientation direction is photoperiod. Our results thus provide a clear example of an invasive insect that has rapidly exhibited adaptive migratory behaviors, either inherited or newly evolved, in a completely alien environment.

## INTRODUCTION

Each year, numerous insect species undertake seasonal bidirectional migrations, flying hundreds of meters above the Earth's surface by day and night, to reach new habitats, which may be located hundreds or even thousands of kilometres away.<sup>1–4</sup> Previous studies suggest that many of the larger (above ~20 mg) high-flying migrants rely on a suite of behaviors, including maintaining preferred headings, and selecting suitable tailwinds and flight altitudes, to promote optimal migration trajectories,<sup>5,6</sup> and even to correct for crosswind drift.<sup>7–9</sup> However, when invasive insect migrants arrive in new biogeographical regions, it is unclear whether, and/or how quickly, they retain, lose, or regain their ancestral behavioral traits,<sup>10</sup> such as adaptive seasonal flight headings.

The fall armyworm (*Spodoptera frugiperda*) is a migratory pest moth native to the Americas, where it can live year-round in tropical and subtropical regions.<sup>11–15</sup> In North America, *S. frugiperda* moths undergo windborne seasonal migrations, expanding northwards into temperate North America during the spring to take advantage of vast areas of maize crops, and returning south to the subtropics (extreme south Texas and south Florida) during autumn, on favorable tailwinds.<sup>14,15</sup> In addition to selecting favorable winds, it is highly likely that native populations of *S. frugiperda* migrants adopt beneficial headings during flight to increase distance and directionality (as has been observed in several species of migrant moths in Europe<sup>2,8,9</sup>), although these behavioral adaptations are yet to be documented in its native range. Since its first reported sighting in West Africa in 2016,<sup>16</sup> *S. frugiperda* rapidly expanded its invasive range through most of Sub-Saharan Africa, the Middle East, much of Asia and most recently reaching Oceania, where it now constitutes a major threat to maize production throughout its vast invasive range.<sup>17–19</sup> This rapid spread through a wide variety of biogeographical regions,<sup>20</sup> provides an opportunity to investigate whether any migratory behaviors (including seasonally preferred flight headings) of the new populations are adapted to these new regions within just a few years of arriving. In this study, we document the seasonal pattern of migratory orientation in the recently established migratory population in East China.

After rapidly spreading through Africa, *S. frugiperda* jumped to India by early 2018, quickly followed by spread into Southeast Asia,<sup>20,21</sup> and reached Yunnan in Southwest China by December 2018.<sup>22,23</sup> Trap catches and atmospheric trajectory simulations indicate that the newly established East Asian population uses favorable tailwinds to expand from year-round breeding areas in subtropical South China and Southeast Asia throughout East Asia each spring and summer, as far north as the Bohai Gulf of North China, the Korean Peninsula and Japan,

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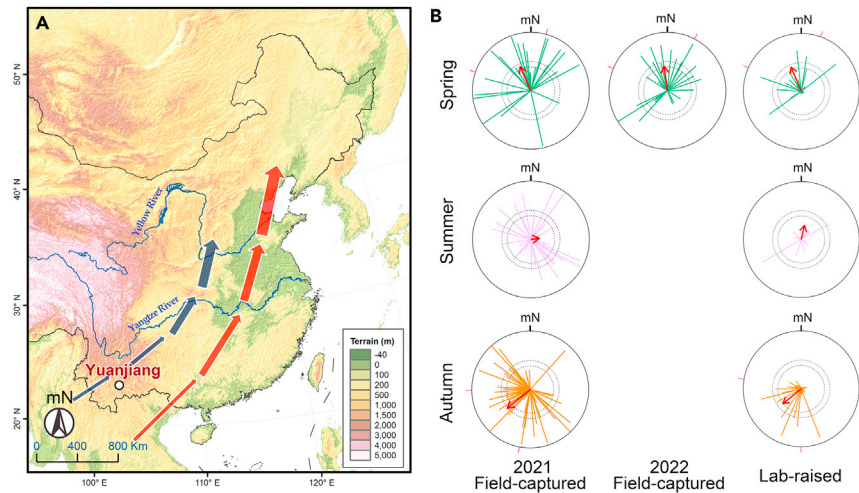
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<https://doi.org/10.1016/j.isci.2023.108281>



**Figure 1. Orientation behavior of field-captured and lab-raised *S. frugiperda* moths in Southwest China**

(A) The spring migratory routes of *S. frugiperda* in East Asia. East Asia is a large area of mostly relatively flat land with few natural barriers to insect migration. Transported by favorable winds associated with the East Asian monsoon, the invasive *S. frugiperda* moth, as well as many native migrant insects, makes regular, seasonal round-trip migrations within this region. Blue and red arrows approximate the western and eastern migratory routes from Southeast Asia to East Asia, respectively.<sup>10</sup>

(B) Circular distributions of moth flight headings in the flight simulator. The directedness (length) of the individual moth orientation vector is given by its  $R$  value (between 0 and 1, where the outer radius of each plot corresponds to  $r = 1$ ). Green vectors represent the spring generation, pink vectors represent the summer generation, and orange vectors represent the autumn generation. Each red mean vector (MV) results from weighting the mean directions and mean directedness (vector lengths) of all individual moths (Moore's modified Rayleigh test; see STAR methods). Directedness of the MV is given by the  $R^*$  value. Dashed circles indicate the required  $R^*$  value for statistical significance:  $p < 0.05$  and  $p < 0.01$ , respectively, for increasing radius. The outer radius of each plot corresponds to  $R^* = 2.5$ . Red radial dashes indicate 95% confidence intervals; mN = magnetic north. For the field-captured group, mature larvae (5<sup>th</sup> or 6<sup>th</sup> instar) or pupae were collected from maize fields in April, July and October 2021 and April 2022 at Yuanjiang county and were kept indoors under natural ambient temperature and photoperiod conditions until moths were ready to test. See also Tables S2–S4 for full results.

returning south during the autumn.<sup>10,19,21</sup> However, whether this population displays seasonally beneficial headings during migratory flights, and the environmental cues that are involved in reversing any seasonally preferred migratory headings, are until now unknown.

Previous studies have revealed that timing is integral to seasonal insect migrations, and the onset of migratory behavior is tightly linked to the change in season.<sup>1,24</sup> This means that migratory insects use an endogenous clock to track the seasonal variations in environmental cues, among which photoperiod would be a prime option.<sup>25</sup> In the present study, in order to investigate the orientation behavior of *S. frugiperda* moths, we explored seasonal preferences in flight headings outdoors in Yunnan with an insect flight simulator,<sup>6,26,27</sup> revealing that the newly established East Asian *S. frugiperda* population can indeed orient adaptively on their new continent. We then assessed the role of photoperiod in determining heading direction, by performing the flight orientation indoors under simulated seasonal photoperiods. These studies indicated that photoperiod change drives the reversal of the seasonal migratory heading direction. In summary, our results thus provide a unique example of an invasive species that has either retained key aspects of its migration behavior during a rapid spread through several new biogeographical realms (from the Neotropical region, via the Afrotropical and Indo-Malayan regions, to the Eastern Palearctic region<sup>20,21</sup>), or has rapidly evolved them anew in a radically new migration “arena” on a different continent.

## RESULTS

### The natural migratory orientation of *S. frugiperda* moths

To determine whether the East Asian *S. frugiperda* population can orient adaptively on its new continent, we studied a field population from Yuanjiang, southern Yunnan, Southwest China (Figure 1A) in spring (April), summer (July), and autumn (October) 2021, and again in spring (April) 2022. Two-day-old moths were tethered and flown outdoors for 5 min in a flight simulator where the insects cannot detect the wind because of the presence of a clear lid on the simulator barrel, under natural night skies (Figure S1A).<sup>6,25,26</sup> We found that the spring 2021 generation preferentially selected headings with a group mean close to north-northwest ( $n = 25$ , mean vector [MV] =  $335^\circ$ , 95%CI =  $287^\circ$ – $15^\circ$ ,  $R^* = 1.123$ ,  $p < 0.05$ ; Figure 1B), whereas the autumn generation orientated southwest ( $n = 52$ , MV =  $231^\circ$ , 95%CI =  $190^\circ$ – $269^\circ$ ,  $R^* = 1.27$ ,  $p < 0.01$ ; Figure 1B). The spring and autumn orientation directions were significantly different (Watson-Wheeler test:  $W = 14.38$ ,  $p = 0.0008$ ). In contrast, the summer generation did not show a group orientation ( $n = 31$ ,  $R^* = 0.365$ ,  $p > 0.5$ ; Figure 1B), which is consistent with radar studies of high-flying insects over the UK.<sup>2,28</sup> We repeated this experiment in spring 2022 ( $n = 26$ , MV =  $352^\circ$ , 95%CI =  $292^\circ$ – $27^\circ$ ,  $R^* = 1.05$ ,  $p < 0.05$ ; Figure 1B); the direction in spring 2022 was closer to north but not significantly different from that measured in spring 2021 ( $W = 1.83$ ,  $p = 0.44$ ; note that the 95% C.I. overlaps with northwest as well as north in both years).

## Effects of photoperiod on the migratory orientation directions of *S. frugiperda* moths

To assess the role of photoperiod, we tested whether lab-raised moths can maintain their seasonally preferred orientations when flown under lab conditions, including artificial light sources, regulated photoperiod regimes, and controlled climate conditions. Lab-raised populations were reared from egg to adult in climate chambers with the photoperiod set to one of three seasonal treatments: (1) summer conditions (constant photoperiod of 14L:10D), (2) spring conditions (starting photoperiod of 12L:12D, gradually lengthened to 13L:11D), and (3) autumn conditions (starting photoperiod of 13L:11D, gradually shortened to 12L:12D). In the spring and autumn treatments, daily photoperiods were changed by 2 min per day to mimic natural seasonal changes (Table S1). Moth orientation behavior was again tested in a flight simulator, and since visual cues are essential for insect orientation,<sup>6</sup> moving ventral optic flow (a satellite image of the Yuanjiang countryside) and a local starry night sky (generated using the software *Stellarium*) were projected via two projectors (see details in Figures S1A–S1D).<sup>6,29</sup> We found that spring treatment moths generally headed toward the north-northwest ( $n = 40$ ,  $MV = 337^\circ$ ,  $95\%CI = 294^\circ\text{--}20^\circ$ ,  $R^* = 1.311$ ,  $p < 0.025$ ), while autumn treatment moths headed southwest ( $n = 45$ ,  $MV = 231^\circ$ ,  $95\%CI = 180^\circ\text{--}279^\circ$ ,  $R^* = 1.012$ ,  $p < 0.05$ ; Figure 1B, see also detail in Table S2). All summer treatments of lab-raised moths showed a random heading direction, but the orientation directions of spring and autumn treatment moths were significantly different ( $W = 6.99$ ,  $p = 0.03$ ; Figure 1B, see also detail in Table S4). Moreover, the common orientation directions of these laboratory-raised moths were indistinguishable from those of the field-captured migrants in Yuanjiang from the same season ( $p > 0.1$  in both spring and autumn conditions; Figure 1B, see also detail in Table S3), thus opening avenues to explore their orientation mechanisms under controlled laboratory conditions.

## DISCUSSION

*Spodoptera frugiperda* is a highly destructive invasive migratory pest that inflicts substantial damage to various crops, particularly maize.<sup>21</sup> Although the migration routes both in North America and East Asia have been described,<sup>10,14,15</sup> whether this moth displays seasonally beneficial orientation behavior in its native range is unknown, although we strongly suspect it based on our knowledge of similar migratory moths elsewhere.<sup>1–3,5</sup> Our results quantitatively demonstrate the seasonal north-south migratory flight headings of *S. frugiperda* populations in East Asia, in the absence of any influence of the wind. The MV direction of *S. frugiperda* moths deviates slightly from the predicted heading (depicted in Figure 1A), which can be attributed to the trajectory simulation used to construct the migration pathway. This simulation primarily assumes a downwind direction but does not account for the seasonal orientation ability of the moths.<sup>10,30</sup> Nevertheless, the orientation of these moths in our flight simulator is still highly advantageous and would allow them to harness the prevailing winds of the annual East Asian summer monsoon,<sup>5,6,9</sup> which blow generally northward during spring and southward during autumn.<sup>10,21</sup> This orientation pattern is potentially a consequence of rapid and ongoing adaptation, or a reflection of a retained, ancestral orientation from the native range, which will accelerate their swift expansion in East Asia, especially in areas with abundant maize along their migratory path. To our knowledge, this is the first demonstration of seasonally beneficial migratory orientations in *S. frugiperda* populations anywhere in their global range.<sup>21,31</sup>

Animals, including migratory insects, possess an endogenous clock that enables them to perceive the time of day and seasonal variations in environmental cues to coordinate the timing of their behaviors, such as migration. Photoperiod is a critical cue for both physiological and behavioral timing in insects, as demonstrated by extensive research on *Drosophila* and monarch butterflies. This includes the involvement of key genes such as *Eyes absent*, *Pigment-dispersing factor*, *ninaB1*, and core circadian clock genes.<sup>32–35</sup> Although photoperiodic research on *S. frugiperda* is limited,<sup>36,37</sup> our discovery of a photoperiod-driven seasonal switch in orientation direction provides an important nocturnal migratory model for investigating the mechanism underlying the photoperiod-induced migratory switch, which may differ from those observed in diurnal migratory monarch butterflies.<sup>32,33</sup>

The recent population trajectory of East Asian *S. frugiperda* was likely a rapid spread through Africa and India to Southeast Asia.<sup>20,25,38</sup> Although the behavioral testing of *S. frugiperda*'s seasonal migratory orientations in these regions is lacking, a north-south orientation will likely not be beneficial in parts of this new range, such as Sub-Saharan Africa. Thus, our evidence suggests that the preferred migration direction of the East Asian population was either retained from their North American ancestors (despite multiple generations in areas where it might not be adaptive), or has evolved anew upon reaching East Asia (within just a few years). The invasive population of *S. frugiperda* is thus an ideal model system to explore how migratory insects retain, discard or re-evolve beneficial migratory traits during an extremely rapid global expansion. Interestingly, compared to monarch butterflies and Bogong moths (*Agrotis infusa*), the orientation of *S. frugiperda* moths is notably less directed, with shorter  $R^*$  values.<sup>6,24</sup> This may imply that the East Asian *S. frugiperda* population is still adapting to their new migration arena. The photoperiod-induced reversal of seasonal flight orientation in *S. frugiperda* moths unveiled here offers critical parameter inputs for future simulations of their migration throughout their newly invaded global range.

## Limitations of the study

Much still needs to be addressed regarding how these moths retained or re-evolved their migration phenotypes genetically and behaviorally during the invasion and subsequent spread to new biogeographical realms. Likewise, the orientation mechanisms that *S. frugiperda* use to steer their migration, which likely relies on celestial visual cues, and possibly magnetic ones,<sup>6</sup> are similarly unknown, and these open questions provide exciting avenues for future research.

## STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- [KEY RESOURCES TABLE](#)
- [RESOURCE AVAILABILITY](#)
  - Lead contact
  - Materials availability
  - Data and code availability
- [EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS](#)
  - Collection and feeding of fall armyworms
  - Treatments of lab-raised population
- [METHOD DETAILS](#)
  - Objectives of the study
  - Attachment of tethering stalks on moths
  - Behavioral apparatus
  - Experimental procedures
- [QUANTIFICATION AND STATISTICAL ANALYSIS](#)

## SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.isci.2023.108281>.

## ACKNOWLEDGMENTS

We express our gratitude to Dr. Aidong Chen from Yunnan Academy of Agricultural Science and Mr. Daihong Yu from the Plant Protection Station of Yuanjiang County for providing experimental field and other logistic support. This work was supported by the National Key Research and Development Program of China (2021YFD1400700) to G.H. Rothamsted Research receives grant-aided support from the Biotechnology and Biological Sciences Research Council UK.

## AUTHOR CONTRIBUTIONS

Conceptualization, G.H. and H.C.; methodology, H.C., G.W., J.L., Y.M., and D.D.; formal analysis, H.C. and G.W.; investigation, H.C., G.W., J.L., and Y.M.; writing – original draft, H.C. and G.W.; writing – review & editing, H.C., G.W., D.R.R., E.J.W., and J.W.C.; funding acquisition, G.H.; supervision, G.H.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

## INCLUSION AND DIVERSITY

We support inclusive, diverse, and equitable conduct of research.

Received: March 8, 2023

Revised: October 12, 2023

Accepted: October 18, 2023

Published: November 20, 2023

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## STAR★METHODS

### KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Raw data	This paper	Mendeley Data: <a href="https://doi.org/10.17632/6jkvpybswd.1">https://doi.org/10.17632/6jkvpybswd.1</a>
Experimental models: Organisms/strains		
Fall armyworm ( <i>Spodoptera frugiperda</i> )	Wild caught	N/A
Software and algorithms		
Data Acquisition ( <i>Custom single-chip</i> )	This paper; Hui Chen, Nanjing Agricultural University, China	N/A
Custom software ( <i>Flash Flight Simulator Data Acquisition System</i> ) for recording and saving data files generated in Data Acquisition	This paper; Hui Chen, Nanjing Agricultural University, China	Mendeley Data: <a href="https://doi.org/10.17632/6jkvpybswd.1">https://doi.org/10.17632/6jkvpybswd.1</a>
Custom software ( <i>Mapflows</i> ) for generating optic flow	This paper; Hui Chen, Nanjing Agricultural University, China	Mendeley Data: <a href="https://doi.org/10.17632/6jkvpybswd.1">https://doi.org/10.17632/6jkvpybswd.1</a>
Custom R-script was used for Moore's Modified Rayleigh test	Richard Massy, University of Exeter, UK	<a href="https://doi.org/10.1098/rspb.2021.1805">https://doi.org/10.1098/rspb.2021.1805</a>

## RESOURCE AVAILABILITY

### Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Gao Hu ([hugao@njau.edu.cn](mailto:hugao@njau.edu.cn)).

### Materials availability

This study did not generate new unique reagents.

### Data and code availability

- The authors declare that the data supporting the findings of this study are available within the paper and its Supplemental Information. Individual data files used to generate the figures in the paper have been deposited in Mendeley Data (<https://doi.org/10.17632/6jkvpybswd.1>).
- Custom-written Visual Basic software *Flash Flight Simulator Data Acquisition System* and *Mapflows* have been deposited in Mendeley Data (<https://doi.org/10.17632/6jkvpybswd.1>).
- *Custom single-chip* used for data acquisition is available from the corresponding authors upon reasonable request ([hugao@njau.edu.cn](mailto:hugao@njau.edu.cn), [hui.chen@njau.edu.cn](mailto:hui.chen@njau.edu.cn)).
- Any additional information required to reanalyze the data reported in this work paper is available from the [lead contact](#) upon request.

## EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

### Collection and feeding of fall armyworms

#### Field population

Mature larvae (5<sup>th</sup> or 6<sup>th</sup> instar) or pupae of fall armyworm moths were collected from maize fields (Yuanjiang, Yunnan, China (elevation 423 m): Google Maps coordinates: 23.604°N, 101.977°E). Each of these larvae was kept in a separate plastic tube, placed in a rearing room in the Yuanjiang County Plant Protection Station, and fed with maize leaves until pupation. The rearing room was well-ventilated with no electronic equipment running, which ensured a temperature cycle closely aligned with the natural cycle outdoors. The room also received ample natural light to provide larvae with natural lighting and photoperiod conditions. Pupae were put on a layer of dampened cotton until emergence. The newly emerged adults were transferred to 250 mL plastic cups with a layer of cotton dipped in 10% honey solution for feeding before testing their orientation behavior. The spring, summer and autumn populations were collected in April, July and October, respectively.

### Lab-raised population

The lab-raised population in Yuanjiang was originally collected from a maize field at the Yuanjiang experimental site (Google Maps coordinates: 23.604°N, 101.977°E) in March 2021 and maintained with maize leaves. The moths tested under constant temperature conditions (Lab-CT) were 8<sup>th</sup> generation. These lab-raised populations were reared in artificial climate chambers under a 14-h light: 10-h dark (LD) cycle at  $27 \pm 1^\circ\text{C}$  and  $60\% \pm 5\%$  relative humidity.

### Treatments of lab-raised population

In the assay for lab-raised populations under constant temperature conditions, we set several treatments with a programmed series of photoperiod conditions to mimic seasonal changes. The rearing temperature in all treatments was  $27 \pm 1^\circ\text{C}$  with a relative humidity of  $60\% \pm 5\%$ . The initial photoperiod for spring treatments was set to 12L:12D when eggs hatched, and then the light period was programmed to be extended by 2 min per day. The final photoperiod, one month later when the moths emerged and were tested, was about 13L:11D. The initial photoperiod for the autumn treatments was set to 13L:11D when the eggs hatched, and then the light period was shortened by 2 min per day. The final photoperiod was about 12L:12D one month later, when moths emerged and were tested. The photoperiod for the summer treatments was set to 14L:10D throughout the whole experiment (Table S1).

## METHOD DETAILS

### Objectives of the study

The objectives of the study were (1) to determine whether an invasive species, the migratory fall armyworm moth, acquired or showed a seasonally beneficial orientation behavior when they arrived on a new continent, and if so (2) to determine if the photoperiod cues from the new continent might be involved in steering seasonally beneficial orientation behavior. To achieve these objectives, wild fall armyworms were captured during different seasons (spring/summer/autumn) in Yunnan, China, and tethered within a flight simulator. Moreover, lab-raised moths were reared in controlled climate chambers with manipulated photoperiods to mimic natural seasonal variations. They were tethered within a flight simulator that incorporated both moving ventral optic flow and a simulated starry night sky.

### Attachment of tethering stalks on moths

Before attaching the tethering stalks, moths (2-day-old) were initially kept in plastic cups and were sedated at  $4^\circ\text{C}$  for a minimum of 2 min before tethering. Moths were then transferred to an operating platform to remove the scales at the conjunction of the dorsal thorax and the abdomen. A thin vertical copper (non-magnetic) stalk of diameter 0.75 mm and length of about 2 cm (Figure S1C-7) was bent at its end to create a fork-like protuberance which was used to stick the stalk to the conjunction of the moth's thorax and abdomen (using Pattex PSK12CT-2 glue). This method for attaching stalks was adopted in all experiments.

### Behavioral apparatus

The Flash Flight Simulator system was modified by Hui Chen from the earlier designs of the Mouritsen-Frost flight simulator (Figures S1A and S1B). This flight simulator was used in our field and laboratory experiments in Yuanjiang (101.98°E, 23.60°N). The moths can rotate freely in the horizontal plane within the simulator and their orientation can be measured with an angular resolution of  $0.9^\circ$ . The instantaneous heading directions (relative to *magnetic north*) for individual moths were recorded as angles by the encoder system and saved as text files on the computer using the recording software *Flash Flight Simulator Data Acquisition System* (created by Hui Chen). In our experiment, the encoder (made from non-magnetic materials) sampled 5 heading directions per second, and the software featured a graphical interface that reconstructed the flight path by constantly monitoring the current heading direction and the flight status of the moth. The shaft connecting the encoder to the tethered moth (Figure S1A-5) was a carbon fiber rod of length 15 cm and diameter 1 mm, and a carbon fiber tube surrounding the rod was used to protect it (Figure S1A-4). The behavioral arena used for testing insects was a circular cylinder of opaque PVC with a diameter of 400 mm, a height of 400 mm and a thickness of 5 mm (Figure S1A-6). As noctuid moths are sensitive to surrounding visual landmarks, the interior of the arena wall was covered with black felt to avoid distracting cues.

### Experimental procedures

#### Flight orientation assay in the field

Flight orientation assays were conducted with the Flash Flight Simulator system both in the field and inside the laboratory in Yuanjiang. For the field experiment, any obstructions visible above the arena, as well as other artificial visual cues, were minimized or eliminated. Other factors considered are the following (see<sup>29</sup>): 1) Moon Phase: Since moonlight can introduce an intensity gradient on the arena wall and provide unwanted orientation cues, we avoided doing experiments on nights with a full moon or with strong moonlight. Moreover, access of direct moonlight to the arena was blocked using a beach umbrella; 2) Weather: temperature, prevailing winds and dew point temperature were monitored by a nearby meteorological station (Yuanjiang: ONSET, HOBO-U30, USA). To avoid test results being affected by wind, we used a UV-transmissive and transparent plexiglass lid on the barrel of the flight simulator to shield the experimental arena from wind influence. Experiments were not conducted when there was high humidity (relative humidity > 85%) or rain to avoid the unstable performance of moths<sup>29</sup>; 3) Stray light: during the experiment, the computer screen (and other devices) were visually shielded behind a shading cloth.



Before darkness, each moth was transferred to its own clear UV-transmissive plastic container (not airtight), given 10% honey solution on cotton wool, and stored in a cool outdoor location with a natural photoperiod. Shortly before sunset, containers holding moths were placed on an elevated location (such as a rock) and provided with a clear view of the western sky and the setting sun (and the skylight polarization pattern). After sunset, moths could also see the stars (and the celestial rotation).

Moths chosen for analysis in our experiment were required to satisfy three *ante hoc* criteria<sup>29</sup>: (1) the tethering stalk was perfectly vertical, (2) wing-flapping was vigorous, and its amplitude was large and equal for both wings (indicating that the contact glue had not interfered with the wings), and (3) the moth flew continuously for at least 5 min. If the moth stopped flying, the arena was gently tapped to stimulate the moth to continue flying. If a moth stopped flying twice during testing, it was rejected, and the recording was aborted. Similarly, moths flying in a continuous spiral were rejected as well. Dim red lighting was used while the moths were handled in their dark-adapted state.

### *Flight orientation assay in the laboratory*

The flight orientation assay in the laboratory was also conducted in Yuanjiang in April 2022. Some modifications were made to the flight simulator (similar to that used in the Bogong moth experiments,<sup>6,29</sup> see [Figure S1D](#)): 1) An optic flow stimulus was added beneath the moths on the floor of the flight simulator in order to produce continuous and stable flight and to provide the moth with the appearance of moving forward over the terrain. A projector (Philips, PicoPix MAX PPX630, USA), a mirror placed at 45° and one layer of white opaque diffuser paper (Lee Filters 251 one-quarter-white diffuser), were used to produce a very dim bitmap image of the Earth's surface near Yuanjiang (at approximately 800 m altitude, from Google Maps) that was back-projected onto a tracing paper screen placed below the arena. Custom-written software (MapFlow: Hui Chen, Nanjing Agricultural University, China) controlled the direction of movement of the image (but not its orientation), and its output was coupled to the flight simulator via a feedback loop. This feedback ensured that the resultant ventral flow-field image always moved backwards relative to the moth's heading (i.e., backward from nose to tail), creating a visual sensation of forward movement irrespective of which direction the moth flew, instantaneously turning with the moth as it changed flight direction.

A simulated starry sky visible in China at the experimental site was also created over our experimental apparatus on the date and time of our indoor experiments. We created screenshots by using Stellarium (a free planetarium software) and then modified them to create stimulus images that were circular in shape (300 dpi, JPG files, using Adobe Photoshop 2020). These circular images were then projected onto a screen consisting of a UV-transmissive Plexiglass lid placed on top of the arena and covered in a sheet of Lee Filters 250 half-white diffuser. An adjustable UV-LED lamp was combined with the projector to ensure a full light spectrum. The light intensity of the UV-LEDs could be controlled by software and neutral density filters, thus allowing the intensity of UV illumination to be adjusted to natural nocturnal levels.<sup>29</sup> All laboratory assays were conducted at  $27 \pm 1^\circ\text{C}$  and  $60\% \pm 5\%$  relative humidity.

## QUANTIFICATION AND STATISTICAL ANALYSIS

All statistical analysis and graphing were performed in R (version 4.1.3, <https://www.r-project.org/> (accessed on 10 March 2022)). A custom R-script was used for MMRT (including the bootstrap confidence intervals),<sup>39</sup> which is becoming widely used in studies of orientation behavior. The advantage of the MMRT is that it accounts for both the mean directions and directedness (vector lengths) of individuals. This non-parametric test is an analog of the Rayleigh test and is used to determine whether there is a significant orientation tendency ( $R^*$ , an arbitrarily scaled alternative to  $r$  in circular regression) and corresponding rank-weighted mean direction ( $MV$ ) of a group of individuals. Thus, each green, orange or pink vector (see [Figure 1B](#)) in the circular plots shows the mean orientation direction  $\theta$  of a single moth's individual recorded flight path as well as its  $R$  value (i.e., length, or directedness, of the flight path vector, ranging from 0 (random distribution) to a maximum of 1 at the edge of the circle), which is calculated in R (by Packages: Circular). MMRT uses these individual vectors (i.e., flight trajectories) to determine an average  $MV$  for the entire migratory group. In addition, a Watson-Wheeler test was used to confirm that the different distributions (e.g., season: spring/summer/autumn) of flight directions in a population of moths were significantly different from each other. Finally, it should be noted that  $R^*$  depends on sample size  $N$  (ref. 39). For example, for  $N = 25$  (field experiments in Yuanjiang, spring), 31 (field experiments in Yuanjiang, summer) and 52 (field experiments in Yuanjiang, autumn), the  $R^*$  value for 95% likelihood ( $p = 0.05$ ) is 1.020, 1.016 and 1.009, respectively. For 99% likelihood ( $p = 0.01$ ), it is 1.246, 1.245 and 1.242, respectively. For ease of comparison, we normalized the lengths of all  $MVs$  and their significance levels (dashed circles) within the circular plots ([Figure 1B](#)) by allowing all plots to have an outer radius corresponding to  $R^* = 2.5$ .