



## Population Ecology

# Population Source of Third-Generation Oriental Armyworm in Jilin, China, Determined by Entomology Radar, Trajectory Analysis, and Mitochondrial COI Sequences

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Subject Editor: Rodrigo Mercader

Received 17 October 2021; Editorial decision 20 February 2022.

## Abstract

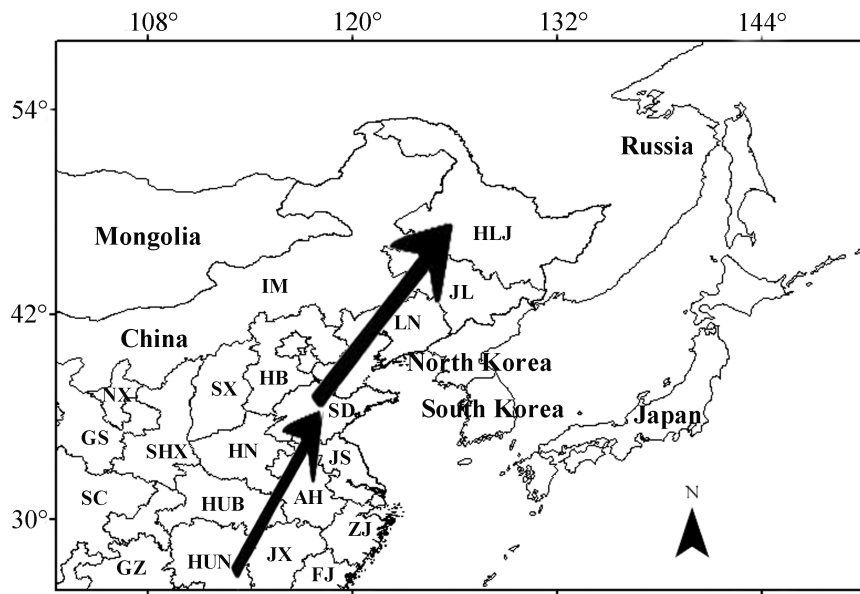
The armyworm, *Mythimna separata* (Walker) (Lepidoptera: Noctuidae), is an important polyphagous pest with a strong migratory ability. Recently, third-generation larvae have become an increasingly serious pest threat in Jilin Province of northeast China. To investigate the population source of this species, scanning entomological radar observations and insect mitochondrial cytochrome oxidase I (COI) genes were used in this study. Five main results were found: (1) The peak period in captured second-generation moths was from mid to late July. The temperature and wind speeds were optimum for the moths to have migrated. Strong southwesterly winds occurred during the peak migration period. (2) Radar observations indicated that most of the moths' migration took place at a height of 600 m, often in a dense layer which formed at heights of 350–800 m. (3) Analyses of adult ovarian development and larval haplotypes showed third-generation larvae were progeny of both locally produced progeny and immigrant moths. (4) Based on our back-tracking and haplotype analyses, immigration led to an outbreak originated in the same source area to the southwest. (5) Emigration of second-generation moths was confirmed by both radar observation and mtDNA analysis. Forward trajectories indicated that the moths were capable of immigrating far from their overwintering range. These results are useful for improving the forecasting systems of this insect pest species.

**Key words:** *Mythimna separata* (Walker), Northeast China, radar, molecular marker, population structure

*Mythimna separata* (Walker) (Lepidoptera: Noctuidae), the oriental armyworm, is an economically important pest of maize, wheat, rice and other staple grain crops in Asia and Australasia (Oku and Kobayashi 1974, Hill and Allan 1986, Mallapur and Kulkarni 1998, Sharma et al. 2002, Jung et al. 2013, Koyama and Matsumura 2019, Li et al. 2021). Its pest status and wide distribution have been attributed to its ability to migrate thousands of kilometres. It is generally believed that *M. separata* populations embark upon four distinct large-scale migration events between southern and northern China every year: two involve moving northward in spring and

early summer and the other two involve moving southward in late summer and autumn (Chen et al. 1995). A possible overwintering boundary is 33–34°N, as south of this the average temperature is above freezing (0°C) in January (Lee and Uhm 1995). As a highly polyphagous insect pest native to China, the larvae of *M. separata* have damaged cereal and grass crops for thousands of years (Zou 1956).

Jilin Province is a major food-producing region of China where the main crops grown include maize, rice, and millet in a highly seasonal climate, whose plant growing season lasts only from April to



**Fig. 1.** *Mythimna separata* migrate from South China to Northeast China by multigenerational migratory flight. IM: Inner Mongolia, HLJ: Heilongjiang, JL: Jilin, LN: Liaoning, HB: Hebei, SD: Shandong, HUB: Hubei, JS: Jiangsu, ZJ: Zhejiang, FJ: Fujian, JX: Jiangxi, HN: Henan, HUN: Hunan, AH: Anhui, GZ: Guizhou, SC: Sichuan, GS: Gansu, NX: Ningxia, SHX: Shaanxi, SX: Shanxi.

October. Because the winters are harsh, *M. separata* is unable to overwinter in Jilin Province. First-generation moths of *M. separata* are known to migrate into Jilin regions in late May and early June (Lin and Zhang 1964) (Fig. 1). The ensuing second-generation of larvae regularly cause substantial damage to crops in June, and historically this generation was solely responsible for most of the crop damage caused by armyworms in Jilin. The subsequent generations of larvae that emerged during July and August were generally considered of only minor economic importance, since they usually occurred in low numbers. Unexpectedly, approximately 375,000 ha of crops were affected by a third generation of larvae in Jilin, in 2012, which led to significant economic losses and much fear and concern among the public (Zhang et al. 2012). Since then, the armyworm has become an increasingly potent pest, with outbreaks confirmed in certain local areas in 2013, 2015, 2017, and 2019. This situation has led to severe losses due to third-generation larvae occurring regularly, imperiling the sought-after high quality and high-value agricultural production in Jilin (Sun et al. 2018). Jiang et al. (2014a,b) have associated these serious infestations of third-generation larvae with climate change and an increased planting area of maize crop in Jilin Province.

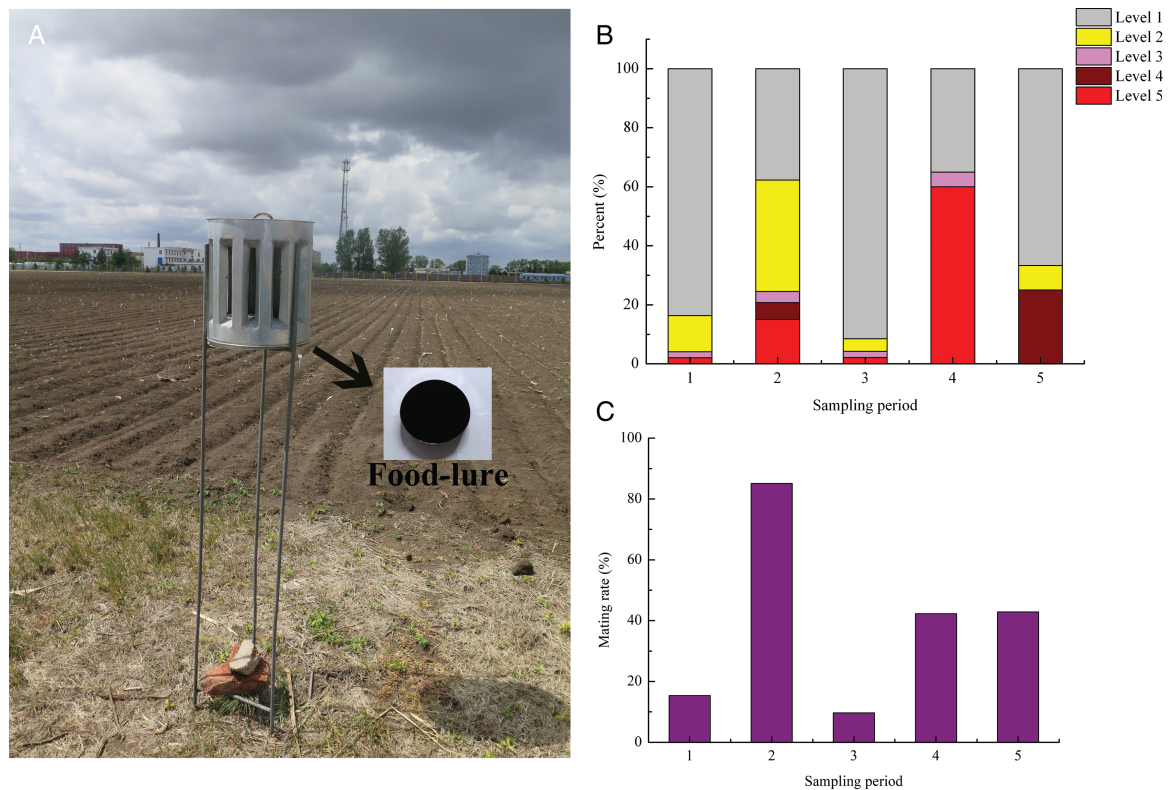
The pest has been extensively studied in Northeast China (Jiang 2018, Zhang et al. 2020). However, due to the previous low occurrence rate, on the question of the population source of third-generation larva has been overlooked in previous studies. A greater spatiotemporal understanding of population structure is essential for predicting the intensity of crop infestations during outbreaking years. Chen et al. (1990, 1995) reported radar observations of *M. separata* migration during summer in 1985 and 1986 and showed the movements were in different directions, which were closely related to the wind direction on different nights. In our previous studies, the migration of second-generation moths was observed by radar in 2015 in Gongzhuling, Jilin Province and possible sources of the third-generation larvae of Gongzhuling were investigated (Sun et al. 2018). However, the study was limited to only a single year and the data were collected at only a single location.

To improve understanding of the pest's population dynamics, long-term observations of *M. separata* in Jilin were carried out. The population structure of this pest was investigated with scanning entomological radar and the cytochrome oxidase I (COI) molecular marker. The aim was to determine the source of the *M. separata* populations in Jilin. These results should lead to an improved forecasting system to predict when damaging pest events are most likely to happen, and allow more effective control strategies against this crop pest to be devised.

## Materials and Methods

### Population Dynamics

*Mythimna separata* moth populations were monitored daily by a network of ground light-traps placed in three regions (Gongzhuling, Meihokou, Taonan) of Jilin Province. The ground lighting units consisted of automatic light-traps (Jiadio Group, Hebi, Henan, China) configured with a 20-W black-light lamp. The lights turned on at sunset and off at sunrise between 2011 and 2019. Data were counted between mid-May and late August each year. Food-lure traps were used to monitor *M. separata* moth populations in Gongzhuling (Fig. 2). The food-lure contained a mixture of liquor, water, brown sugar, vinegar, and trichlorfon (Chen et al. 1989). From the food-lure trap catch, a total of 15 female individuals were randomly selected and dissected every other day in July from 2014 to 2019. Ovarian dissection is an effective method of determining migratory insects' status (Riley et al. 1995, Feng et al. 2004), which has been shown to work well in *M. separata* moths (Feng et al. 2008). Low level of ovarian development and mating rate indicates that adult moths are newly emerged locally or are immigrants. By contrast, high level of ovarian development and mating rate indicated that the moths are breeding locally, though it does not provide information on whether they emerged locally or were immigrants. The level of ovarian development was estimated according to the criteria described below. Stage 1, transparent and light milky-white ovary and no follicular differentiation; Stage 2, presentation of developing eggs; Stage 3,



**Fig. 2.** Ovarian development status of female *Mythimna separata* moths in the food-lure traps during the migration period. (a) The food-lure trap. Twelve rectangular holes (L: 20 cm, W: 1.5 cm) were located around the cylindrical steel trap. (b) Ovarian development status. (c) Mating rates. Data of 2014 and 2016 were uncounted due to low female numbers. 1: 15 July 2015 to 23 July 2015; 2: 24 July 2015 to 1 August 2015; 3: 9 July 2017 to 17 July 2017; 4: 18 July 2017 to 26 July 2017; 5: 18 July 2018 to 26 July 2018.

presentation of mature eggs; Stage 4, some eggs have been laid; Stage 5, the ovary is atrophied, and most eggs have been laid. Mating occurrences were determined by the presence of a spermatophore in the female spermatheca. Field survey reports on the third-generation outbreak regions (Fusong, Jiutai, and Yongji) in 2017 were obtained from Jilin Provincial Agro-Tech Extension Center.

### Radar Observations

Observations of insect flight paths were made using the Jilin Academy of Agricultural Sciences (JAAS) Entomological Radar, China's first entomological radar. It was built in 1982 and updated in cooperation with Wuxi Leyoung Electronics Technology Co., Ltd. (Wuxi, Jiangsu, China). The migration paths of *Loxostege sticticalis* (L.) (Lepidoptera: Crambidae) and *Dendrolimus tabulaeformis* Tsai & Liu (Lepidoptera: Lasiocampidae) were accurately observed by this radar system in the 1980s and 1990s (Cheng et al. 2005), and it has proven effective for directly observing first and second-generation adults of *M. separata* in migration high above the ground (Chen et al. 1989, 1995). Several methods have been used to identify radar observation targets as *M. separata*. (1) Characteristics of the echoes and of the observed flight behaviours, as observed during previous radar studies (Riley et al. 1995, Feng et al. 2008, Drake and Reynolds 2012, Zhang et al. 2013). (2) Catching insects by light-trap, which indicated that *M. separata* was the most abundant macro insect species during the period. There was a positive correlation between radar echoes and *M. separata* catch numbers (Sun et al. 2016). Therefore, *M. separata* was thought to be the dominant species on the radar's plan-position indicator (PPI) display. (3) Detection of noctuid moths, including *M. separata*, by aerial sampling (Chen et al. 1989).

The JAAS radar uses a scanning pencil-beam radar, transmitting at a frequency of  $9,340 \pm 30$  MHz (X-band, wavelength 3.2 cm), pulse length 0.1  $\mu$ s, peak power 10 kW, and pulse repetition frequency 500 Hz. The antenna is a 1.5-m diameter parabolic dish (beam width 1.5°), with an elevation angle variable from  $-2^\circ$  to  $60^\circ$  and a scan rate of 15 revolutions/min; polarization is horizontal. Radar echoes appear on a PPI display which can be adjusted to show maximum ranges of 2, 5, or 10 km. Data acquisition and display system was employed to capture and store the PPI screen image, with migration parameters of the *M. separata* moths being extracted by automated systems (Cheng et al. 2005). The radar was operated using angles of 15°, 30°, and 45° with each angle scanned for 30 s. The repeat interval of the whole process was set to 12 min. All recorded observations were made from sunset through sunrise during July 2017. This study was carried out at Gongzhuling experimental station of JAAS ( $43^\circ 31' 69''$ N,  $124^\circ 49' 28''$ E), located centrally in Jilin Province.

### Analysis of Meteorology and Insect Trajectories

Based on the radar-collected data, the flying height of *M. separata* adults was close to that of the 925 hPa pressure level (approximately 500 m height). Meteorological data for this level, including temperature and u- and v-winds, were therefore obtained from the National Center for Atmospheric Research (NCAR, USA) and National Centers for Environmental Prediction (NCEP, USA). Daily rainfall data were downloaded from the National Meteorological Information Center (China). Field temperature and humidity were recorded using HOBO data loggers in each of the three monitoring regions (Onset Computer Corporation, MA). The geographical

**Table 1.** Details of the samples examined in this study of populations of *Mythimna separata*

Population code	Collecting locality	Geo-coordinates	Sample size	Sampling date	Generation of insects collected
HL-I	Helong	44°02'53"N, 125°12'97"E	30	2017-6	2017-II
TN-I	Taonan	45°19'54"N, 122°50'71"E	14	2017-7	2017-II
SY-I	Songyuan	44°54'31"N, 125°02'05"E	26	2017-6	2017-II
LS-I	Lishu	43°20'51"N, 124°20'50"E	27	2017-6	2017-II
MHK-I	Meihekou	42°38'23"N, 125°50'40"E	30	2017-6	2017-II
GZL-I	Gongzhuling	43°31'69"N, 124°49'28"E	30	2017-6	2017-II
HLH-II	Halahe	44°35'57"N, 125°07'99"E	30	2017-8	2017-III
NA-II	Nongan	44°19'23"N, 125°07'44"E	30	2017-8	2017-III
LS-II	Lishu	43°20'51"N, 124°20'50"E	30	2017-7	2017-III
CL-II	Changling	44°13'33"N, 124°27'50"E	30	2017-8	2017-III
GZL-II	Gongzhuling	43°31'69"N, 124°49'28"E	30	2017-8	2017-III
WLJ-II	Jilin	44°03'99"N, 126°28'66"E	30	2017-8	2017-III
KQF-II	Yongji	43°38'01"N, 126°24'61"E	30	2017-8	2017-III
KQS-II	Yongji	43°40'01"N, 126°22'62"E	30	2017-8	2017-III
JT-II	Jiutai	44°13'14"N, 126°05'50"E	30	2017-8	2017-III
DH-II	Dunhua	43°15'99"N, 128°07'24"E	30	2017-8	2017-III
FSF-II	Fusong	42°23'97"N, 127°06'08"E	30	2017-8	2017-III
FSS-II	Fusong	42°21'21"N, 127°06'16"E	30	2017-8	2017-III
MHK-II	Meihekou	42°38'23"N, 125°50'40"E	29	2017-8	2017-III

destination and source area of the migrating moths were estimated via constructed trajectories carried out by the Hybrid Single Particle Lagrangian Integrated Trajectory (HYSPPLIT) facility (Stein et al. 2015, Rolph et al. 2017). HYSPPLIT is a professional model for computing simple air parcel trajectories that has previously been employed to simulate insect migratory trajectories (e.g., Westbrook et al. 2019, Wainwright et al. 2020) and specifically to investigate likely source and destination regions (Rosenberg and Magor 1983, Dingle 1996, Chapman et al. 2010, Zhang et al. 2018). The trajectory simulations were based on the following assumptions: (1) the moths flew approximately downwind (Chen et al. 1989); (2) they remained at the altitude of the 925 hPa level; (3) wind speed was assumed to be the moths' flight speed, flights lasted 8 hr (from 19:00 to 03:00, Beijing local time), and; (4) flights continued for up to five successive nights.

### MtDNA Analysis

**Sampling:** A total of 19 populations, including 6 populations of second-generation (I) and 13 populations of third generation (II) *M. separata* were sampled in maize fields across Jilin Province between June and August in 2017 (Table 1). In all, 546 individuals were captured, all as larvae except at Taonan (where 7 adults and 7 larvae were obtained). Three of the samples (Fusong, Jiutai, and Yongji) were from third-generation outbreak populations. Specimens were immediately killed and preserved in absolute ethanol for their subsequent DNA extractions.

**MtDNA Extraction:** Genomic DNA was extracted from the whole insect body, by using a genomic DNA purification kit (Dingguo Co., Ltd, Beijing, China). A 676-bp COI fragment of mitochondria was amplified using primer pairs (F: TTCGAGCTGAATTAGGAACCC, R: CTTTCTTGGGAAATAATGTGAGAAA). This fragment contains a portion of the fast-evolving COI region, which is known to be suitable for low-level phylogenetic investigations and is applicable to population genetic studies (Lunt et al. 1996).

**PCR:** PCR amplifications were carried out with 25 µl of the 2×Taq PCR mix (Dingguo Co., Ltd, Beijing, China), 1 µl of each primer (10 µM), 1 µl of DNA template in a 50-µl reaction mixture topped with molecular grade water. The thermal cycling conditions consisted of initial denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 30 s, extension

at 72°C for 40 s, followed by a final extension at 72°C for 10 min. These amplifications were conducted in a PCR-Cycler ABI 9700, and the purified products were sequenced on an ABI 3730 automatic sequencer (Applied Biosystems, Foster City, CA).

### Statistical Analyses

Sampling date denotes the evening of that day through to sunrise of the next day. Meteorological data were processed and checked in 'Grads' software (Kalnay et al. 1996). ArcGIS v10.2 was used to construct the trajectory images. Monitoring data computations were carried out in Excel 2003. Figures were drawn in Origin v8.5 software. The COI gene partial sequences were edited using the Chromas tool. Sequences were identified by comparing them with already submitted sequences obtained from the NCBI database. All the sequences alignments and assembly were done by DNAMAN software. Mega v4.0 (Tamura et al. 2007) was used to analyze the nucleotide composition, the variable sites, transition, and transversion, for which distances for haplotypes were calculated according to the K-2-P model. The neighbor-joining (NJ) phylogenetic tree was also built in this software. To depict geographical associations of the *M. separata* populations, a haplotype network was created using Network v4.6.1.1 based on the median-joining method (Bandelt et al. 1999).

## Results

### Population Dynamics

The *M. separata* occurrence period can be divided into two main parts: the period of spring immigration of moths (first-generation moths) and the period of second-generation moths (Fig. 3). Moths of the spring migration were regularly captured from early May to late June, their abundance peaking from early to mid June. The second-generation moths were regularly captured from early July to mid August, peaking from mid to late July. In 2017, the second-generation moths appeared earlier than in other years, showing a peak from early to mid July. Compared with the first-generation moths, the numbers of second-generation moths were relatively high in 2012, 2015, and 2017.

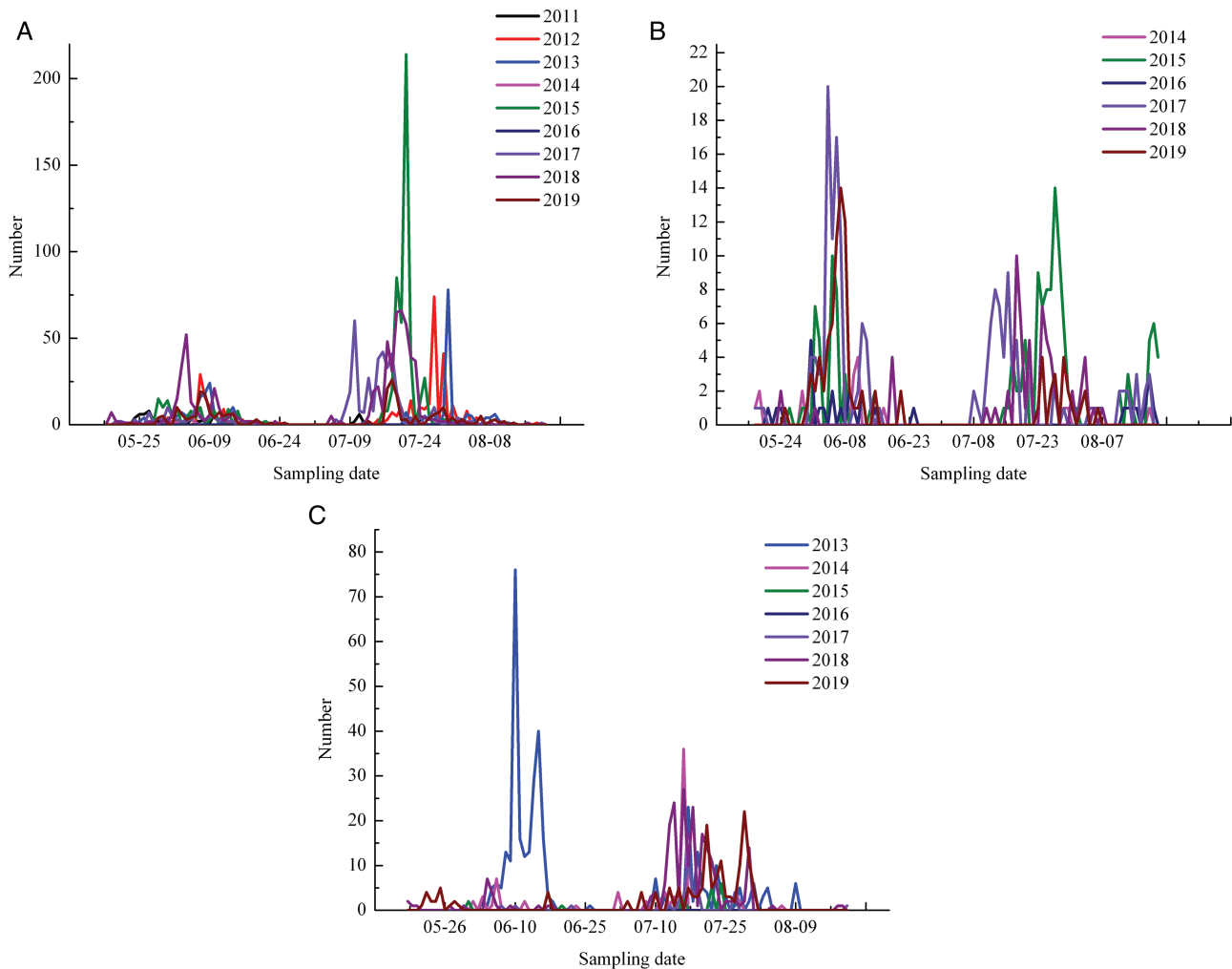


Fig. 3. Daily variation in the number of *Mythimna separata* moths in the ground light-traps between 2011 and 2019. (a) Gongzhuling, (b) Meihokou, (c) Taonan.

Results of ovarian dissection showed that the moth population was a mixture of locally emerged moths and immigrants in July (Fig. 2). This finding was confirmed by the following evidence. Analysis was divided into earlier period (15 July 2015 to 23 July 2015, 9 July 2017 to 17 July 2017) and later period (24 July 2015 to 1 August 2015, 18 July 2017 to 26 July 2017). For the earlier period, the female moths had evidently undergone a low level of ovarian development and experienced a low mating rate (Fig. 2), indicating that this population consisted of new locally-emerged moths, recent immigrants, or a mix of the two. By contrast, female moths caught in the later period were in various stages with a high mating frequency (Fig. 2). They were therefore a mix of newly emerged moths and older moths that had commenced local breeding; as with the earlier population, the ovarian data does not reveal whether these moths had emerged locally or were in immigrants.

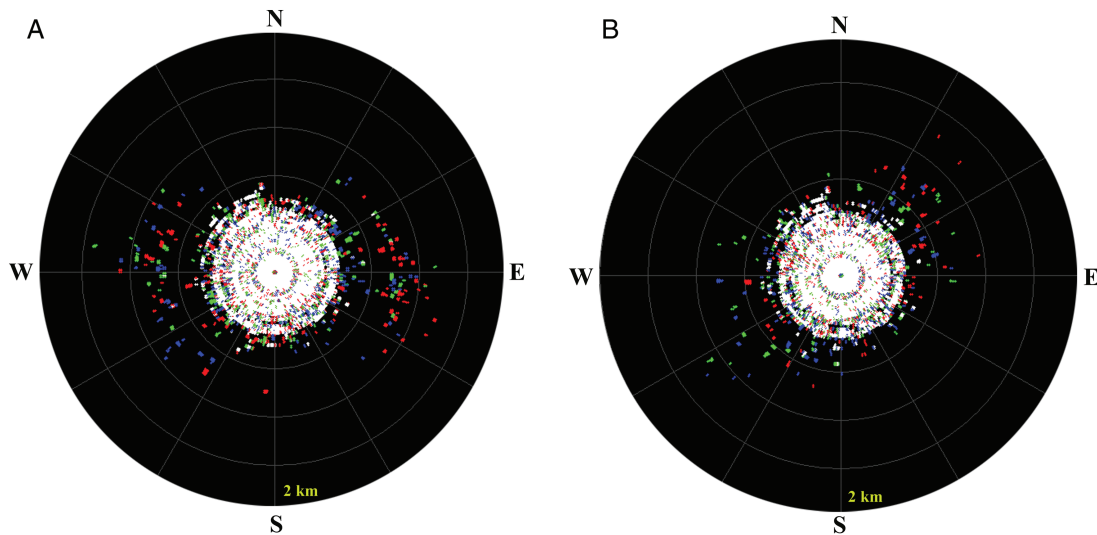
#### Analysis of Meteorology

According to the meteorological data, the temperature at 925 hPa ranged from 20–30°C, well above the threshold temperature required for the moths' take off (8°C) (Zhang and Li 1985). During the peak periods, wind speeds ranged between 1 and 20 m/s, averaging 9.9 m/s, which is more than double the moths' flight speed of 4 m/s (Chen et al. 1995); hence, the tracked moths will have

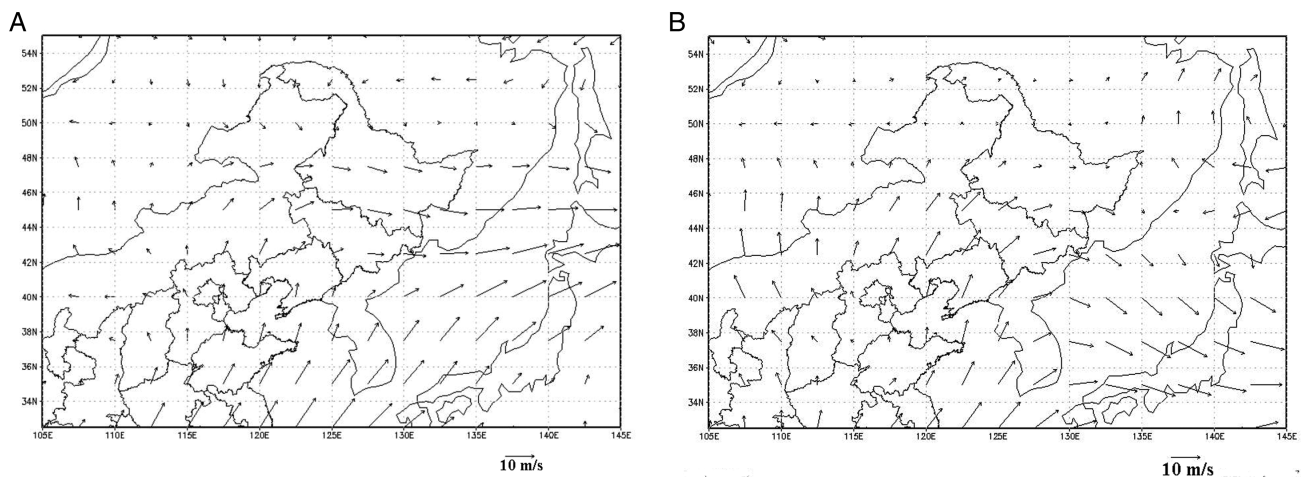
moved approximately downwind. The winds come from a variety of directions, and southwesterly winds account for 75% of the total. Significant amounts of rainfall occurred on 9–10, 13–16, and 19–21 July, and these heavy rains suppressed moth emigrations.

#### Radar Observation and Insect Trajectory

Most of the targets were concentrated at a height of 600 m above ground level, with an 800 m maximum height, reached on some nights. The moths migrated frequently, and observation of the radar's PPI display indicated that they were often concentrated in a high-density layer. These layers were found mainly at heights of 350 m to 800 m. Winds were predominantly southwesterly during this period. Northward immigrations were detected twice during the migration period in 2017. On July 14, few echoes were detected in the early observations on the PPI display. A layer between heights of 450 and 800 m was detected at 4:34 hr on 15 July (Fig. 4a), indicating that migrants moved northwards. Wind direction was southwesterly (Fig. 5a). Afterward, the layer disappeared, indicating the end of migration. The number of moths caught in the light trap was high that night, which indicated an immigrant population. The second observation was similar to the first one. A layer was observed between heights of 400 and 700 m at 4:27 hr on 17 July 2017 (Fig. 4b). These migrants were also moving northwards, riding southwesterly winds (Fig. 5b). The ceased observation indicated the end of migration. The



**Fig. 4.** Photograph of radar PPI display strong layer concentration of *Mythimna separata* migration. (a) The layer extends between altitudes of 450–800 m at 4:34 hr on 15 July, 2017. (b) The layer extends between altitudes of 400–700 m at 4:27 hr on 17 July, 2017. The elevation angle was 30°. The distance to the edge of the display was 2 km. The image shows three successive scans of the antenna, with each scan recorded in a different color. A single color, therefore, represents a moving echo. If a target is detected in the same place on all three scans, the colors will superimpose and appear white. The mass of white echo around the center is due to immobile surface structures.

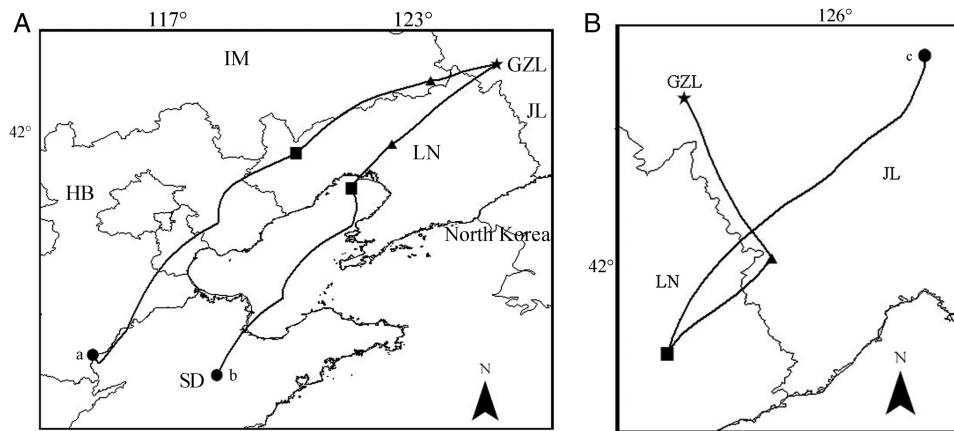


**Fig. 5.** Wind fields during the migration of *Mythimna separata*. (a) Wind field on the 925 hpa level at 02:00 15 July, 2017, (b) Wind field on the 925 hpa level at 02:00 17 July, 2017.

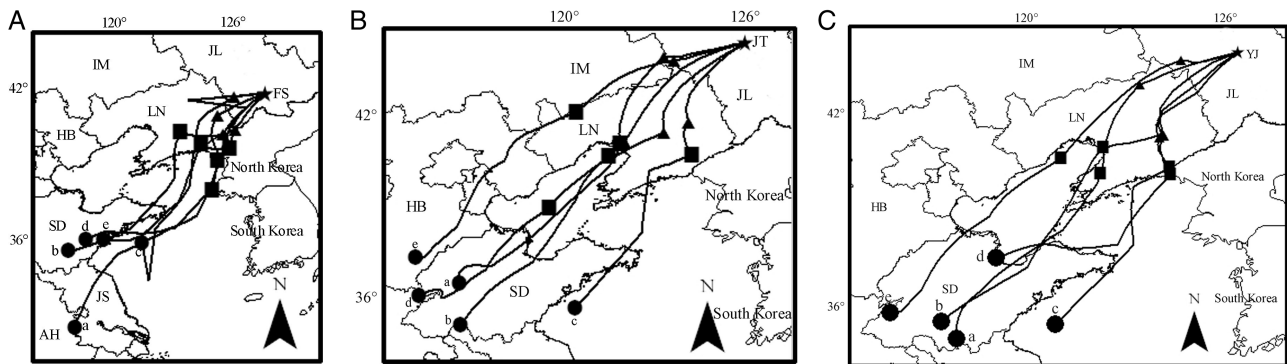
number of moths caught was again relatively high that night, and the population was assumed to be immigrants. These immigrants had two possible destinies: to terminate their migration and be the source of a third-generation population in Jilin; or to continue their journey, departing on the next suitable night. Possible source areas were calculated by trajectory analysis following the immigrant moths' observations. The endpoints of backward trajectories of one or two nights were mainly in Liaoning Province, and the endpoints of the five-night trajectories were as far as Shandong Province (Fig. 6A).

Based on analysis of immigrants, we estimated the source areas for *M. separata* populations that reached the outbreak region. In 2017, some areas of Jilin Province experienced heavy rains. Afterward, third-generation larvae caused significant yield losses. Although damage by third-generation larvae in outbreak regions (Fusong, Jiutai, Yongji) was serious, damage by second-generation larvae there was light. Therefore, we infer that heavy rain caused immigrants to land and prevented locally emerged adult moths and

immigrants from taking off at dusk. Then, the retained moths laid eggs, and a subsequent generation of larvae arose. The migration period of the immigrant moths was analyzed. The oviposition date was inferred from the observed local stage using the effective accumulated temperature. The result showed that the dates when the ovipositing moths arrived coincided with the earlier period (9 July 2017 to 17 July 2017) of the second-generation moths. In combination with the date of heavy rainfall in the outbreaking areas, immigrant moths from 10 to 14 July 2017 were considered to be a major component of immigration. We calculated backward trajectories starting on these dates. Based on simulation periods from 1 to 5 d, the source regions were in northeast, central, and southern China (Fig. 7). The endpoints of backward trajectories with one or two nights were mainly in Liaoning Province, and the endpoints of five-night trajectories were as far away as Anhui and Shandong Provinces. However, migration for 1 or 2 d seems much more likely than a 5-d flight.



**Fig. 6.** Trajectory analyses for the nights when the radar showed immigration and emigration of the moths. (A): Backward trajectories for immigration on July 15 and 17, 2017. (B): Forward trajectory for emigration on July 27, 2017. a: 7–15, b: 7–17, c: 7–27. \* Starting point ▲ First night endpoint ■ Second night endpoint ● Fifth night endpoint.



**Fig. 7.** Source regions of second-generation *Mythimna separata* moths led to the outbreak in 2017. (A) The backward trajectories from July 10 to 14 in FS (Fusong). (B) The backward trajectories from July 10 to 14 in JT (Jiutai). (C) The backward trajectories from July 10 to 14 in YJ (Yongji). a: 7–10, b: 7–11, c: 7–12, d: 7–13, e: 7–14. \* Starting point ▲ First night endpoint ■ Second night endpoint ● Fifth night endpoint.

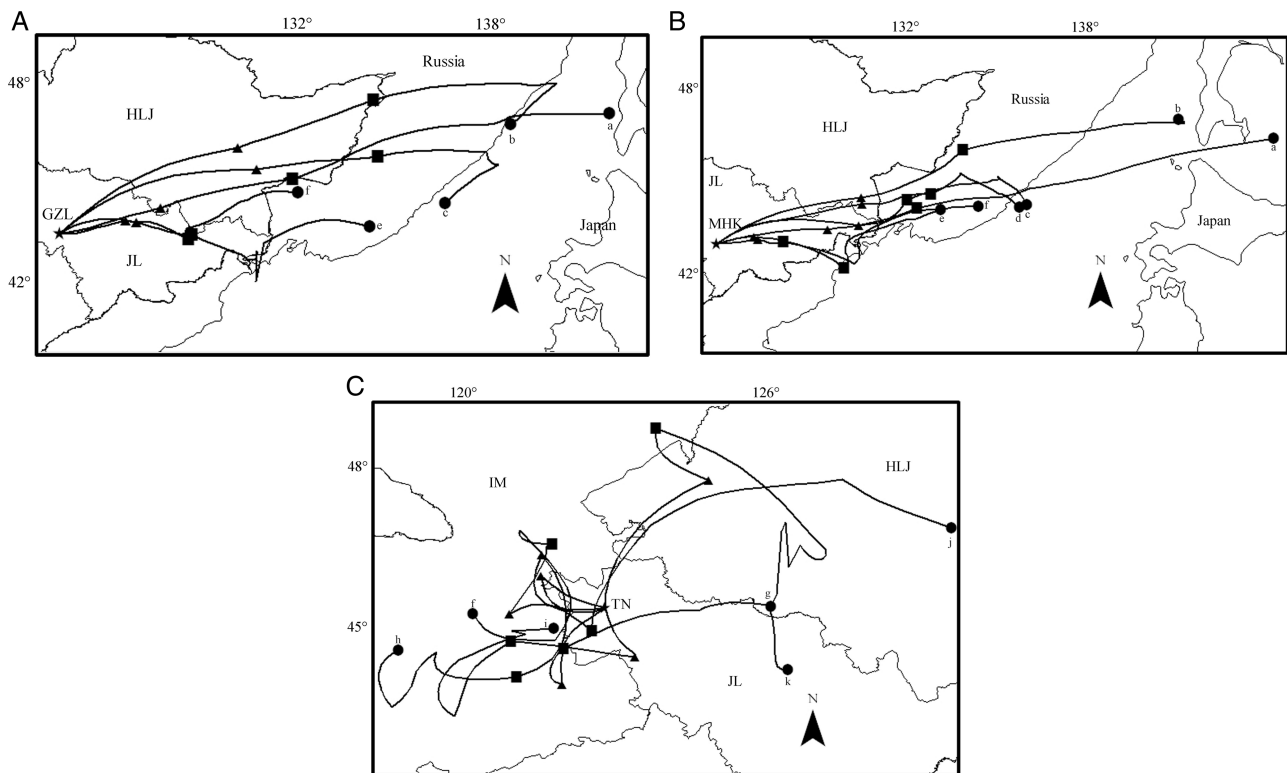
The radar revealed that *M. separata* moths begin to take flight just after dusk. The earliest layer that formed was detected at 19:52 hr, and identified as an ‘emigration population’. The layer was observed between the heights of 350 and 550 m on 27 July 2017. The possible destination region was analyzed following the observation. The forward trajectory of one or two nights reached Liaoning Province, and the forward trajectory with a five-night trajectory reached the central part of Jilin Province (Fig. 6B). The plotted forward trajectories indicated that nonretained moths originating in Gongzhuling could migrate into Jilin and Liaoning Provinces and even into Russia; moths from Meihekou could migrate into Liaoning Province as well as Russia; moths from Taonan could migrate most widely, into Russia, Inner Mongolia, and both Jilin and Heilongjiang Provinces. The trajectories for the number of moths were high (Gongzhuling, July 10–12 and 14–15; Meihekou, July 10–15; and Taonan, July 15–20) as shown in Fig. 8. The forward trajectories with one or two nights were primarily in northern China, whereas the forward trajectories with five nights could reach as far as Russia. The destinations were locations that are very cold winter, where no host plants for the pest are available. This analysis showed that these moths eventually landed in regions where they could not overwinter.

### Mitochondrial DNA Analysis

A 605-bp fragment of the Mitochondrial DNA (MtDNA) COI was successfully amplified and edited in all samples. No insertions or

deletions were observed, and its alignment showed the COI fragment contained 562 conserved sites and 43 variable sites; this included 22 parsimonious informative sites and 21 single variable sites. The average nucleotide sequence was as follows: 29.1% of A, 42.7% of T, 14.5% of C, and 13.7% of G. This high A+T content (71.8%) agrees with values for other insects. Of the 44 base substitutions present, a total of 41 were transition and 3 were transversion types, with most of the former being C–T, and the overall transition/transversion bias was  $R = 26.7$ .

The variable sites were distinguished by 69 different haplotypes (H1, H3–H6, H8, H10–12, H16, H18–76), whose corresponding sequences were submitted to GenBank database (under accession nos. MH 900379, 900381–900384, 900386, 900388–900390, 900394, 900396–900454). The average genetic distance of these different haplotypes was 0.005 (range: 0.002–0.013). The dominant haplotype, H1, accounted for 50.91% of all the sampled specimens, and it was widespread in each population. The second most common haplotype was H3 (18.86%), and it was also found in all 19 populations. The third most common, H8 (6.41%), was found in 14 of the 19 populations. Twenty-two shared haplotypes were found, while the remaining 47 haplotypes were unique. Twenty-nine haplotypes were present in the second generation, of which 10 were shared, leaving 19 unique haplotypes identified. Fifty-seven haplotypes were present in the third generation, of which 19 haplotypes were shared and 38 unique. Ten haplotypes were shared by the two generations. As the first generation was sampled as larvae, this indicates that



**Fig. 8.** Destination regions during the peak migration period of second-generation moths. (A) Forward trajectories from July 10 to 12 and 14 to 15 starting at GZL (Gongzhuling). (B) Forward trajectories from July 10 to 15 from MHK (Meihekou). (C) Forward trajectories from July 15 to 20 from TN (Taonan). a: 7–10, b: 7–11, c: 7–12, d: 7–13, e: 7–14, f: 7–15, g: 7–16, h: 7–17, i: 7–18, j: 7–19, k: 7–20. \* Starting point ▲ First night endpoint ■ Second night endpoint ● Fifth night endpoint.

locally emerged moths had bred locally. The unique haplotypes were detected in both generations, suggesting emigration and immigration had both occurred.

The phylogenetic network was complicated and no geographical pattern could be detected from it (Fig. 9). Valid data are briefly discussed below. Samples collected in the same location between two generations had the same haplotype (H12); likewise, some samples collected in the outbreak regions shared the same haplotypes (H36, H57). From the built NJ tree, no significant geographical clusters were found that corresponded to the sampling locations. Nonetheless, these results did indicate that moths from the same locations had closely related haplotypes; for example, the clade formed by H12 and H32, with a high support value (53%), came from the GZL-I population, and the clade formed by H72 and H75 (77%) came from the MHK-II population. Closely related haplotypes were present in locations nearer to each other; for example, the clade containing H37 (HLH-II) and H41 (NA-II) had a high bootstrap value (53%). Furthermore, the cluster consisting of H6 (KQS-II), H65 (FSF-II), and H54 (KQF-II), as well as that consisting of H35 (JT-II) and H62 (FSF-II), all originated from outbreaking populations (Fig. 10). Collectively, same (H36 and H57) and closely related (H6, H65, and H54; H35 and H62) haplotypes were obtained from the third-generation outbreaking populations, indicating a common region of origin.

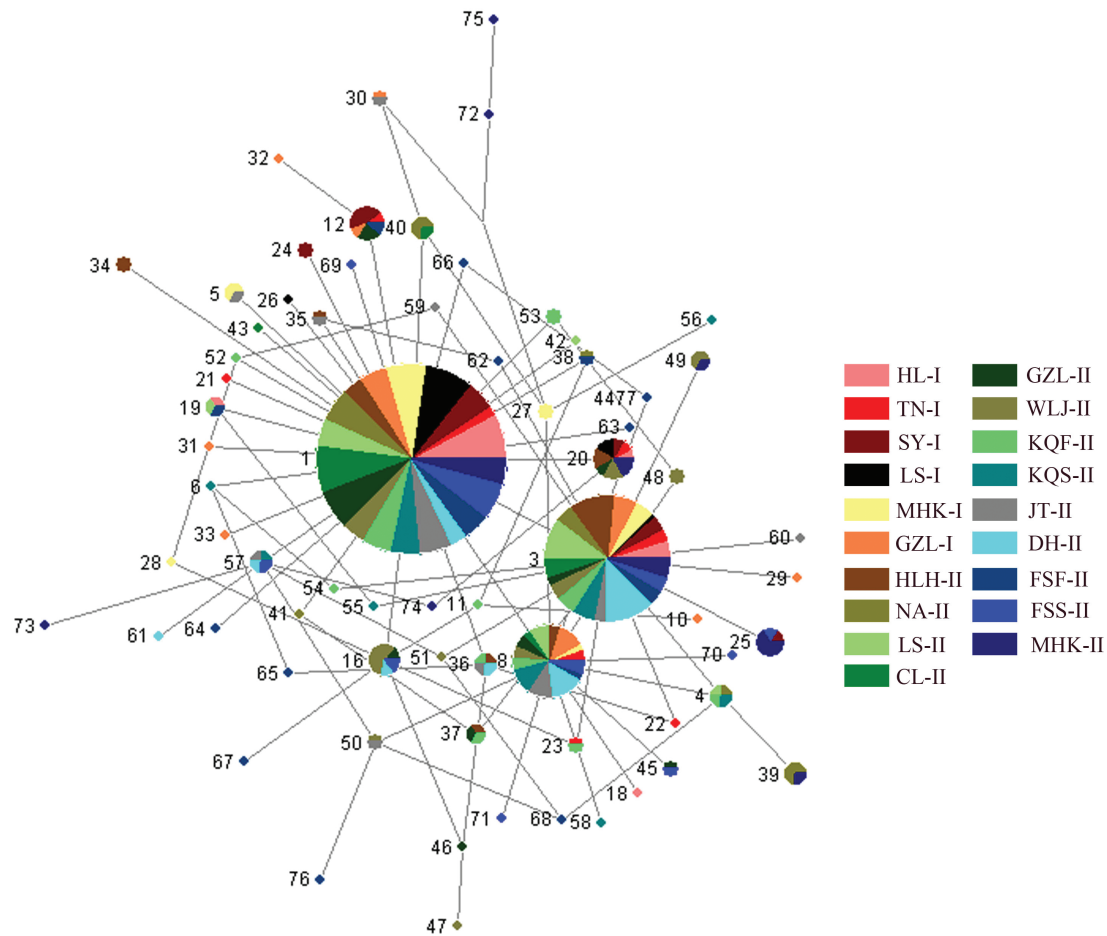
## Discussion

Scanning entomological radars have been used extensively in investigating the region of origin of migratory insects (Reynolds et al. 2009, Jeffries et al. 2013). In the past decade, molecular

markers have become a commonly relied-upon approach to directly measure genetic differentiation in migratory animals, especially in an evolutionary context (Regalado et al. 2017, Choi et al. 2020). MtDNA has proven itself particularly useful as a genetic marker in phylogeographic research of insects (Sun et al. 2015, Nagoshi et al. 2020). An innovative aspect of the research reported here was its combination of two established methods, those of radar observation and mtDNA analyses, for studying the population source of a moth.

The period of first-generation adults in Jilin was clarified. This period coincided with the emergence of first-generation adults across China (Jiang et al. 2011). Previous research indicated that Jilin's spring (May and June) moths are immigrants from eastern China, from areas such as Shandong, Jiangsu, and Anhui Provinces (Chen et al. 1989, 1995; Sun et al. 2019). The second generation adult period (July and August) also coincided with the emergence of second-generation adults across other areas of China (Jiang et al. 2011). This research was aimed at determining the composition of the second-generation moths in Jilin, and the population source of the third-generation larvae. Many researchers believed that these larvae arise from moths immigrating from northern regions (e.g., Heilongjiang) as part of a southward 'return movement' (Chen et al. 1995). However, in our study, the results of ovarian development and mating rate indicated that third-generation larvae originated from oviposition by locally emerged moths or immigrants from the southwest, or both. This finding indicated the existing possibilities of locally merged moths bred locally. Our molecular data supported the interpretation: the presence of haplotypes that were shared between the two generations at the same sampling locations indicated that retention may occur in those populations. Therefore, we propose that locally emerged moths are an important component of the





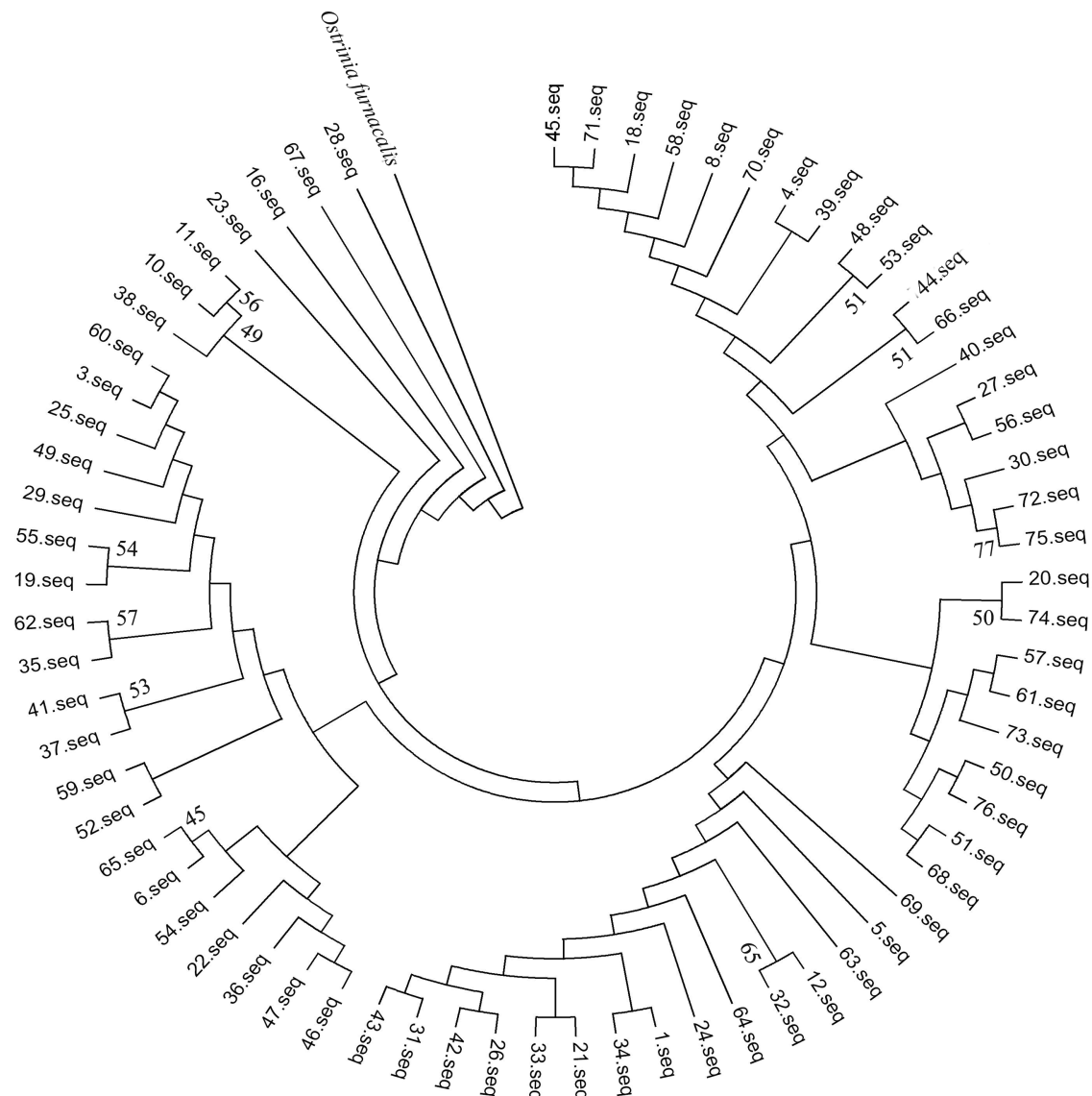
**Fig. 9.** Haplotype network for *Mythimna separata* haplotypes based on cytochrome c oxidase subunit (COI) fragment. Each circle represents a different haplotype, and each color corresponds to a population refer to Table 1 for abbreviations. Circle areas indicate the haplotype frequencies.

second-generation breeding population. These results are similar to those found in Gongzhuling in 2015 (Sun et al. 2018). This phenomenon was also analyzed by Zhang et al. (2012), who found that local emergence was the major contributor to the seminal moth outbreak that occurred in 2012. Furthermore, among research conducted in neighboring countries, similar results were obtained by South Korean researchers who showed that moths appearing in July were likely locally bred population (Lee and Uhm 1995).

The retention of locally emerged adult moths appears to have been an important factor leading to the outbreaks of this pest in Jilin Province. Two factors combined to induce these serious infestations of third-generation larvae. First, climate change affected the migration and retention of *M. separata* moths. Second, since 1995 maize has become the host plant most seriously damaged by *M. separata*, and the planting area of the Jilin maize crop has increased in recent years (Jiang et al. 2014a,b).

In addition to the locally bred population, immigration was also detected, as confirmed by three factors. First, *M. separata* movements on southwesterly winds were directly observed by radar after midnight, which suggests they had been migrating for several hours, following a dusk take-off, and were therefore immigrating populations. Second, the data of ovarian development showed that third-generation larvae originated from oviposition by locally emerged moths or immigrants. This finding indicated the existing possibilities of immigrant moths. Third, our mtDNA analysis demonstrates that the same or closely related haplotypes are shared among the populations of

outbreking areas, implying a common region of origin. Our backtracking analyses showed that the immigrants that led to outbreaks originated from the same source area to the southwest. Based on the analyses, it was postulated that there might have been a shorter trajectory originating in Liaoning Province, and a longer one originating in central and southern China. The finding of shorter movement is similar to that in our earlier work (Sun et al. 2018), in which the source of third-generation larvae in Gongzhuling in 2015 was associated with the second-generation moths of Liaoning Province. There is also earlier evidence for second-generation moths being capable of long-distance migration on southwesterly winds (Chen 1990, Chen et al. 1995). This phenomenon has already been discussed by Jiang et al. (2011), who showed that adults emerging in northwestern regions (Gansu Province) migrate in a northeasterly direction to the region with the third-generation outbreak. An old mark/recapture record showed that moths could fly 1,500 km from Gansu to Liaoning Province (Chen et al. 1995). The prevailing winds over Jilin Province in July are southwesterly and are relatively strong. These winds take the second-generation moths from southwestern areas to Jilin. These moths constitute the majority of the immigrants. As previously mentioned, although some moths could fly for five nights, many others will fly for short periods (1 night or 2 nights only). Source areas calculated from shorter flights were more possible following the analysis. However, in other years, owing to the varied wind directions at that time of year, populations may move short distances from northern regions (e.g., Heilongjiang Province) (Hu et al. 2014).



**Fig. 10.** Neighbor-joining circle tree showing the phylogenetic relations among all the haplotypes. The outgroup taxon was *Ostrinia furnacalis* (Guenée, 1854) (Lepidoptera: Pyralidae). Bootstrapping for NJ tree was done using 1000 replicates. Bootstrap values below 40 are not shown.

While the immigrants flew into Jilin Province, there were also locally bred populations that emigrated from their sites of emergence. This was demonstrated by the following factors. First, flights early in the night were observed by radar, indicating emigration; and second, the molecular sampling was numerous and widespread in this study. Consequently, 69 mtDNA haplotypes were detected in 546 individuals. These haplotypes could represent the genetic *M. separata* background in Jilin Province. As a conserved gene, mtDNA COI is universally used in evolutionary analyses (Lunt et al. 1996). Similar-origin insects were expected to have similar genetic backgrounds. However, only 10 haplotypes were shared by the two generations, whereas the remaining 59 haplotypes were unique. The significantly different haplotypes between the two generations may show different genetic backgrounds. Considering the characteristic behavior of the moth's migration phenomenon, the haplotype differentiation indicated the occurrence of both emigration and immigration. Our genetic results showed that unique mtDNA haplotypes were present in the second-generation *M. separata* populations. These haplotypes were not sampled in the following generation, suggesting that some emigration occurred.

It has long been debated whether the return migration of *M. separata* from northeast China actually occurs. Many Chinese entomologists believe that the moths should make a return flight to their summer habitats in northern China (i.e., Shandong and Hebei Provinces) and produce the next (third) generation of moths that appear in fall and migrate to lower latitudes to overwinter. There is evidence supporting this hypothesis (Feng et al. 2008). However, several authors argued that the prevailing winds in late summer seem to lead the moths astray, to Far East Asia and northern mountainous regions (Pan et al. 2014), leaving them victims of 'Pied Piper' effects (Pedgley et al. 1995). Studies carried out by Japanese researchers also support this 'Pied Piper' hypothesis as they found *M. separata* having a summer generation in Japan consisting of locally produced moths and immigrants from Jilin Province (Hirai 1995). The results of our study are clearly consistent with the 'Pied Piper' hypothesis. However, moths of the third-generation *M. separata* in Jilin emerged in September, and catch numbers that were markedly greater than for previous generations. These moths were identified as 'locally emerged moths that are about to emigrate.' These findings will be the topic of a later paper. Future

investigation is needed to shed light on different possible origins of the second and third generations and of where, if anywhere, they migrate to. Understanding the population dynamics and migratory movements of the third-generation moths is important for devising effective region-wide pest management procedures for this important pest.

## Acknowledgments

Funding was provided by Jilin Province Science and Technology Development Plan (20210202120NC), Basic Research Funds of Jilin Academy of Agricultural Sciences (KYJF2021ZR015), the Agricultural Science and Technology Innovation Program of Jilin Province (CXGC2021TD001), and China Agriculture Research System (CARS-02). We are grateful to X. G. Li, X. Q. Wang, J. Zhang, and S. Wen from the Tonghua Academy of Agricultural Sciences; and to C. H. Xu and F. M. Shi from the Jilin Academy of Agricultural Sciences Taonan experimental farm for their field work done. We also thank Z. J. Cheng, X. B. Li, and Q. Zhang for field sampling assistance in Gongzhuling.

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