Destinations of third-generation *Mythimna separata* (Lepidoptera: Noctuidae) moths in Jilin and its effects on population genetic diversity

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Abstract
Third-generation oriental armyworm larvae, *Mythimna separata* (Walker) (Lepidoptera: Noctuidae), have caused considerable economic losses in Jilin. Understanding the occurrence, regularity and return movement of third-generation adults is crucial for pest forecasting. The study was conducted using systemic monitoring, radar observations and molecular markers from 2015 to 2019. The main results are as follows: (1) third-generation moths were regularly captured from late August to late September. These moths have been attributed to local emergence and were identified as an “emigration population.” Wind speed and temperature were advantageous for moth migration, and advantageous northeasterly winds account for 9.6% of the total. Radar studies determined that the migrant flying height was mainly below 600 m. Moths migrated over a range of directions, including their return movements. Forward trajectories predicted that the destination regions of the Korean Peninsula, the Russian Far East, and northern China were unfavorable for survival. (2) A total of 29 haplotypes were detected. There were unique haplotypes between two sampling years. The haplotype analysis indicated similar content in the two sampling years. The haplotype diversity indices appear to have been maintained. Overall, our findings indicate that the moths did not make a successful return flight, but the genetic diversity was unaffected.

Keywords Oriental armyworm · Return migration · Entomological radar · Molecular marker · Population structure

Introduction
The oriental armyworm, *Mythimna separata* (Walker) (Lepidoptera: Noctuidae), is a widely distributed pest in Asia and Australasia (Hill and Allan 1986; Jiang 2018; Koyama and Matsumura 2019; Lee and Uhm 1995; Li et al. 2021; Mallapur and Kulkarni 1998; Sharma et al. 2002). This polyphagous species leads to infestation of maize, wheat and other cereal crops and causes greatly reduced crop yields in some years. As a well-studied migrating pest, overwintering generation adults of *M. separata* migrate to first-generation outbreak regions (33°N–36°N, including Shandong, Henan, Anhui, and Jiangsu Provinces) between March and mid-April. The subsequent generations undergo the partial lifecycle (egg, larvae, and pupa) here, after which they produce the first generation of moths. Consequently, these moths make annual seasonal migration flights to growing regions with spring crops in Japan, the Korean Peninsula, and Northeast China from May to June (Hirai 1995; Jung et al. 2013; Oku and Kobayashi 1974). Jilin Province is an important crop-producing region in China and is located in the middle of the Northeast Plain. The main crops include maize, rice, millet and sorghum. The climate is highly seasonal and has a growing season that lasts from April to October. Second-generation armyworm invasions between June and July were previously considered to cause most damage (Chen et al. 1995). However, a major third-generation armyworm larval plague was reported in northern China in 2012 and led to invasions over an area of 375 000 ha in Jilin (Zeng et al. 2013). The pest also caused yield losses in 2013, 2015, 2017 and 2019 with high larval densities in some crops. Third-generation armyworms have become a significant economic pest in the region since 2012 (Sun et al. 2019a).

After the pests finish the larval and pupal stages, they initiate third-generation moths in northeastern China in
autumn. Where should these *M. separata* adults go? Due to the previous low occurrence rate, research on the moths’ population dynamics has been overlooked by previous workers. In our previous study, moths’ migration was observed by radar in 2015 in Gongzhuling, Jilin Province (Sun et al. 2018). The research also showed the following: the catch numbers of third-generation moths by light traps were 10.3 times those of first-generation moths and the average number of third-generation moths was 6.3 times greater than second-generation moths. Trajectory analysis indicated that landing areas for most moths were restricted to either the north of Gongzhuling or North Korea, where is impossible to overwintering in 2015.

However, the previous study was limited to only a single year of data collected at one location. Is the moths’ wrong-way migration normal? If that was so, it remains unclear whether the genetic diversity of the species has reduced due to unsuccessful migrations. Therefore, a better understanding of the scientific issues and an intensive and comprehensive study is lacking. The objective of this study is key to proper understanding of the species’ population dynamics and return movement. The research utilized data from scanning entomology radar, field surveys, trajectory simulations and weather analysis. The study also used mitochondrial DNA (mtDNA) markers with the aim of characterizing the genetic differentiation between two sampling years. The molecular study examined if migratory genes could be maintained at the mtDNA level. Combining multiple approaches is valuable in clarifying the ecology of migration, which is difficult to observe directly. These results should lead to an improved understanding of the pest species’ behavior and provide an important basis for effective management strategies.

**Materials and methods**

**Systemic monitoring**

To investigate the population dynamics of *M. separata* moths, light and food-lure traps were effectively used in Jilin Province (Sun et al. 2019b). In this study, light traps network conducted daily monitoring of the *M. separata* moth populations throughout Gongzhuling, Meihekou, and Taonan of Jilin Province, China. The light was automatic and configured with a 20 W blacklight lamp (Jiaduo Group, Henan, Hebi, China). The lights were turned on and off at 19:00 in the evening 05:00 in the morning, respectively, every day from 2015 to 2019. Data were collected between early August and October annually. Due to disadvantages of specimens ovarian dissecting catching in the light trap, we employed two food-lure traps to lure the moths for dissection. The food-lure trap consists of a covering cylinder, base plate and lure dish. The covering cylinder (R: 17.5 cm, H: 35 cm) is made of iron with 12 rectangular holes (L: 20 cm, W: 1.5 cm), and the bottom of the cylinder is open and placed in the base plate (R: 16 cm, H: 3 cm). The lure dish (R: 12.5 cm, H: 3 cm) is located inside the base plate. The food lure consisted of a trichlorfon, water, vinegar, brown sugar, and liquor mixture. Fifteen female individuals from the food-lure traps were randomly selected and dissected every other day in Gongzhuling between 2015 and 2019. If the number of samples was < 15, all of them were dissected. The ovarian development criteria were categorized into five levels according to Feng et al. (2008), as described in Table 1. Mating occurrences were determined by the spermatophore in the female spermatheca.

**Radar observation**

We used Jilin Academy of Agricultural Sciences (JAAS) radar to directly observe the movements of third-generation moths during the migration period. The current study was conducted from May to October 2015–2019 on the JAAS experimental farm at Gongzhuling (43°31’69”N, 124°49’28”E) which is located in the central agricultural plain of Jilin Province. Several methods were used to identify radar echoes as *M. separata* moth. (i) Parameter of moths’ flight behavior obtained from previous radar studies (Feng et al. 2008; Riley et al. 1995; Zhang et al. 2013). (ii) Catching the insects by the light trap showed that *M. separata* moth was the most numerous macro-insect species during the period of the study. A positive correlation between the population of *M. separata* and radar echoes was found.

| Development level | Characteristics of ovary                               | Status                                               |
|-------------------|--------------------------------------------------------|------------------------------------------------------|
| 1                 | Transparent and light milky-white ovary and no follicular differentiation | Locally emerged moths                                |
| 2                 | Presentation of developing eggs                        | Locally emerged or immigrants moths                  |
| 3                 | Presentation of mature eggs                            | Locally emerged or immigrants moths that are breeding locally |
| 4                 | Some eggs have been laid                               | Locally emerged or immigrants moths that had bred locally |
| 5                 | The ovary is atrophied, and most eggs were laid        | Locally emerged or immigrants moths that had finished breeding |
Magor 1983; Zhang et al. 2018). The analyses were based on regions (Chapman et al. 2010; Dingle 1996; Rosenberg and Westbrook et al. 2019). The analysis had been used successfully to specifically investigate likely source and destination of an insect’s migratory trajectory (Wainwright et al. 2020; Stein et al. 2015). HYSPLIT is a professional model for computing simple air parcel trajectories, and it can simulate integrated trajectory (HYSPLIT) (Rolph et al. 2017; Nagoshi et al. 2020; Sun et al. 2022). This study brought new findings in the estimation of migration distances of the third-generation moths, by comparison. Two hundred and thirty seven larvae were hand collected in maize fields and then preserved immediately in absolute ethanol for genomic DNA extraction. The cyt b oxidase subunit (COI) gene is well characterized and widely used in intraspecific variation analysis (Nagoshi et al. 2020; Sun et al. 2015; Tan et al. 2013; Zhang and Hewitt 1997). For amplification of a 676 bp fragment COI gene, a pair of primers, namely, 5′-TTGAGCTGAATTAGGAAACC-3′ for the forward direction and 5′-CTTCTTGGAATAATGTGAAAAA-3′ for the reverse direction, were used. The fragment contains regions which are suitable for low-level phylogenetic investigations (Lunt et al. 1996). DNA was extracted using a genomic DNA purification kit (Dingguo Co., Ltd, Beijing, China).

A forward trajectory model was used to predict the destination of emigrations using Hybrid Single Particle Lagrangian Integrated Trajectory (HYSPLIT) (Rolph et al. 2017; Stein et al. 2015). HYSPLIT is a professional model for computing simple air parcel trajectories, and it can simulate insect’s migratory trajectory (Wainwright et al. 2020; Westbrook et al. 2019). The analysis had been used successfully to specifically investigate likely source and destination regions (Chapman et al. 2010; Dingle 1996; Rosenberg and Magor 1983; Zhang et al. 2018). The analyses were based on the following assumptions: (i) Moth orientations are downwind (Chen et al. 1989); (ii) The heights used were determined from the radar observation; (iii) The moths’ flight speed was set as the wind speed, with an 8 h flight duration (19:00–3:00); (iv) Five consecutive nights were analyzed. The endpoint of the 5-day trajectory, which reached over the land, was assumed to be the destination. If the trajectory ended at the sea surface, the trajectory was extended by 4 h. If the extended trajectory reached over the land, the region was recorded as the destination. Otherwise, the trajectory of the 5 days was considered invalid, and the last endpoint of the day which reached over the land was recorded as the destination. The selection of the trajectory period analysis was confirmed using the light trap occurrence dates in the three regions. The dates when no moths were caught were not evaluated. Several typical trajectories were selected to display the moths’ flight pathways. The trajectory images were constructed by ArcGIS 10.2.

**Molecular markers**

A total of ten geographical populations were obtained, including four populations from the third generation in 2016 and six populations from the second generation in 2017 (Table 2). To determine the population source of the third-generation M. separata in Jilin Province, the 2017 samples combined with other molecular data were used in our previous study (Sun et al. 2022). This study brought new findings in the estimation of migration destinations of the third-generation moths, by comparison. Two hundred and thirty seven larvae were hand collected in maize fields and then preserved immediately in absolute ethanol for genomic DNA extraction. The cytochrome c oxidase subunit (COI) gene is well characterized and widely used in intraspecific variation analysis (Nagoshi et al. 2020; Sun et al. 2015; Tan et al. 2013; Zhang and Hewitt 1997). For amplification of a 676 bp fragment COI gene, a pair of primers, namely, 5′-TTGAGCTGAATTAGGAAACC-3′ for the forward direction and 5′-CTTCTTGGAATAATGTGAAAAA-3′ for the reverse direction, were used. The fragment contains regions which are suitable for low-level phylogenetic investigations (Lunt et al. 1996). DNA was extracted using a genomic DNA purification kit (Dingguo Co., Ltd, Beijing, China).

The PCRs was performed in 50 µL of PCR mix with 25 µL of 2×Taq PCR (Dingguo Co., Ltd, Beijing, China), 1 µL of each primer, 1 µL of DNA template and 22 µL of ddH2O. The cycling parameters were 94 °C for 5 min, 35 cycles of 94 °C/30 s; 56 °C/30 s; 72 °C/40 s, followed by 10 min at 72 °C. The reactions were conducted with PCR-Cycler ABI 9700 and sequenced on an ABI 3730 automatic sequencer (Applied Biosystems, Foster City, CA, USA). Raw sequences were aligned and assembled by Chromas and DNAMAN software. The sequences obtained were deposited at GenBank as accession numbers MH900379, MH900381, MH900383, MH900385-386, and MH900388-411. Mega 4.0 was used to determine the variable sites, nucleotide compositions and haplotype distances (Tamura et al. 2007). Neighbor-joining (NJ) analysis was performed using the Kimura 2-parameter method. Haplotype networks
were constructed by Network version 4.1.1.1 (Bandelt et al. 1999). DNASP5 (Librado and Rozas 2009) was used to calculate several key population parameters: haplotype diversity \((Hd)\), nucleotide diversity \((Pi)\), and the average number of nucleotide differences \((K)\). The amount of genetic variation within and among populations was examined using the Analysis of Molecular Variance (AMOVA) framework in Arlequin v3.5.1.2 (Excoffier et al. 2005).

**Results**

**Systemic monitoring**

During the 5-year monitoring, catches of armyworm moths in Gongzhuling suggested that third-generation adults were present from late August to mid-October and peaked in early and middle September. Although the catch number was relatively low, the emergence period was also obvious in Meihekou and Taonan. Moths of Meihekou were regularly captured from mid-August to late September. Moths of Taonan were regularly captured from late August to late September. Altogether, there was an intact adult period from late August to September in Jilin Province (Fig. 1). Although this was a longer duration, most females had low ovarian development (Fig. 2). Moths with level 1 ovarian development accounted for 98.4%, and the other levels showed a relatively lower proportion. Most females caught were unmated (mating rate 0.64–1.64%) between 2015 and 2019.

**Analysis of meteorology data**

*Mythimna separata* have an optimum temperature for flight of 17 °C and the temperature range is 11–32 °C (Zhang and Li 1985). During the monitoring years, temperatures at the 925 hpa level ranged between 15.64 and 21.25 °C. These temperatures are well above the lower threshold for flight. The average wind speeds at this level were 5.22–9.33 m/s. During emigration periods, the winds come from a variety of directions. The northeasterly winds (9.6%) provided an approximate direction for the moths to return southward (Online Resource, see Supplementary). The number of days with rainfall accounted for 29.85% of the sampling dates.

**Radar observations**

During the observed period, the echo’s height with daily trap catches greater than 10 is depicted in Table 3. Radar observations indicated that the migratory populations were over a wide height range and were generally located below 600 m. The moths formed high-density layer concentrations and these often extended throughout a deep 500–1400 m boundary layer and showed a peak height at 2000 m. The moths migrated frequently and were distributed over a range of directions. The PPI displays a notable “dumbbell pattern” as shown in Fig. 3. The moths exhibited downwind displacements and headed in various directions including the northeast-southwest direction. Return movements were detected by radar observations during 3 d of northerly winds (Fig. 3). (i) Moth layers were present at 18.35 h at 750–1300 m on 8 September 2017. (ii) A strong layer formed by 22.41 h at approximately 700–900 m on 7 September 2018. (iii) Migrants typically concentrated into layers at height of 1300 m at 20.52 h on 10 September 2019.

**Analysis of insect trajectory**

Based on the data from 2015 to 2019, the forward trajectory simulations, therefore, suggest that the moths dispersed over a wide area. There were endpoint differences among the

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**Table 2**

| Generation of Insects Collected | Population Code | Collection Locality | Geo-coordinates | Sampling Size | Sampling Date |
|--------------------------------|-----------------|---------------------|-----------------|---------------|---------------|
| 2016-III                       | NA-2016         | Nongan              | 44°10′19″ N, 125°09′25″ E | 20            | 2016–8        |
| 2016-III                       | SL-2016         | Shuangliao          | 43°41′50″ N, 123°57′75″ E | 20            | 2016–8        |
| 2016-III                       | LS-2016         | Lishu               | 43°33′00″ N, 124°24′92″ E | 20            | 2016–8        |
| 2016-III                       | CL-2016         | Changling           | 44°22′91″ N, 124°17′40″ E | 20            | 2016–8        |
| 2017-II                        | HL-2017         | Helong              | 44°02′53″ N, 125°12′97″ E | 30            | 2017–6        |
| 2017-II                        | TN-2017         | Taonan              | 45°19′54″ N, 122°50′71″ E | 14            | 2017–7        |
| 2017-II                        | SY-2017         | Songyuan            | 44°54′31″ N, 125°02′05″ E | 26            | 2017–6        |
| 2017-II                        | LS-2017         | Lishu               | 43°20′51″ N,124°20′50″ E | 27            | 2017–6        |
| 2017-II                        | MHK-2017        | Meihekou            | 42°38′23″ N, 125°50′40″ E | 30            | 2017–6        |
| 2017-II                        | GZL-2017        | Gongzhuling         | 43°31′69″ N, 124°49′28″ E | 30            | 2017–6        |

To determine the population source of the third-generation *M. separata* in Jilin Province, the 2017 samples combined with other molecular data were used in our previous study (Sun et al. 2022). This study brought has reported new findings in the estimation of migration destinations of the third-generation moths, by comparison.
Fig. 1 Daily catches of *Mythimna separata* in the ground light traps in Jilin Province from 2015 to 2019. a Gongzhuling; b Meihekou; c Taonan

Fig. 2 Ovarian development status of female of *Mythimna separata* moths in the food-lure traps during the migration period. The sampling location was Gongzhuling. 1: 2015-08-29 ~ 2015-09-11, 75; 2: 2015-09-12 ~ 2015-09-25, 80; 3: 2017-08-25 ~ 2017-09-11, 52; 4: 2017-09-12 ~ 2017-09-29, 22; 5: 2018-09-04 ~ 2018-09-26, 102; 6: 2018-09-27 ~ 2018-10-19, 95; 7: 2019-08-27 ~ 2019-09-21, 233; 8: 2019-09-22 ~ 2019-10-17, 91. The sampling period and the number of tests were according the serial number.
three monitoring regions, and among the sampling years. Possible endpoints were scattered across the Russian Far East, Korea Peninsula, and Northern China (i.e. in Inner Mongolia, Heilongjiang, Jilin and Liaoning Provinces) (Table 4). Trajectory pass over the Bohai sea was detected, but the endpoints did not reach the region (33–34°N) where possible for overwintering. Thus, most of the sink areas were located in the far east region of Russia (34.74%), Heilongjiang (20.19%), Jilin (18.78%), and Liaoning Provinces (10.33%). To visually display the moths’ flight pathways, five typical trajectories are calculated in Fig. 4.

MtDNA analysis

Sixty hundred and five bp fragments were successfully amplified and sequenced from 237 individuals. No additions or deletions were observed. The fragments contained 580 conserved sites and 25 variable sites. There were 9 parsimonious informative sites and 16 single variable sites. The average composition was $T = 42.7\%$, $A = 29.1\%$, $C = 14.5\%$ and $G = 13.7\%$. The average A + T contents were rich (71.8%). Transitions occurred at 23 sites, while transversions occurred at two sites. Most of the transitions were C-T. The overall transition/transversion ratio $R = 20.2$.

In this study, 29 haplotypes were detected (e.g., H1, H3, H5, H7-8, and H10-H33). The range of haplotype distances was 0.002–0.01 (average 0.005). As shown in the haplotype network (Fig. 5), haplotype 1 was widespread and predominant with a frequency of 0.553, while haplotypes H3 and H8 had frequencies of 0.181 and 0.08, respectively. No geographical pattern can be discerned from the NJ tree and network (Fig. 6). Six haplotypes (20.70%) were shared between 2016 and 2017. Seven haplotypes were detected in samples from 2016, while the other 16 haplotypes were sampled in 2017. A total of 13 haplotypes were detected in the 80 individuals from 2016. A total of 22 haplotypes were detected in the 157 individuals from 2017.

Based on the data from 2016 (Table 5), the number of haplotypes ranged from 2 to 8 and had a mean of 5. SL-2016 populations exhibited a higher value than the other populations. The mean $Hd$ was 0.6618 (range 0.4790–0.7474). SL-2016 populations exhibited a higher degree of variation than the other populations. The $Pi$ ranged from 0.0008 to 0.0030, with an average value of 0.0018. Among the populations, the $K$ was between 0.4790 and 1.8211 (average of 1.0961). The AMOVA results showed that nearly all of these genetic variations resided within the populations (94.56%, $P = 0.00317$), whereas only a small proportion came from the populations (5.44%, $P = 0.01347$). Based on the data from 2017, the number of haplotypes ranged from 4 to 10 and had a mean of 6.5. Furthermore, the GZL-2017 population exhibited a higher value than the other populations. The mean $Hd$ was 0.6074 (range 0.2764–0.8901). TN-2017 populations exhibited a higher degree of variation than the

| Survey Year | Date (mm-dd) | Echo’s Height (m) | Survey Year | Date (mm-dd) | Echo’s Height (m) |
|-------------|--------------|-------------------|-------------|--------------|-------------------|
| 2015        | 9–4          | 850               | 2018        | 9–15         | 1200              |
|             | 9–5          | 400–600           |             | 9–17         | 400–600           |
|             | 9–6          | 1000–2000         |             | 9–19         | 400–600           |
|             | 9–7          | 400–600           |             | 9–24         | 500               |
|             | 9–8          | 500–700           | 2019        | 9–27         | 500               |
|             | 9–9          | 600               |             | 9–3          | 400–600           |
|             | 9–10         | 400–600           |             | 9–4          | 400–600           |
|             | 9–11         | 500–600           |             | 9–10         | 1300              |
|             | 9–12         | 400–600           |             | 9–11         | 400–500           |
|             | 9–13         | 400–600           |             | 9–12         | 400–500           |
|             | 9–14         | 400–500           |             | 9–14         | 400–600           |
|             | 9–15         | 400–600           |             | 9–16         | 600               |
|             | 9–16         | 400               |             | 9–18         | 500–600           |
|             | 9–17         | 400–600           | 2017        | 9–19         | 400–900           |
|             | 9–5          | 1400              |             | 9–20         | 400–500           |
|             | 9–6          | 1400              |             | 9–21         | 500–800           |
|             | 9–8          | 750–1300          |             | 9–27         | 500–800           |
|             | 9–16         | 400–500           |             | 9–29         | 400–500           |
|             | 9–18         | 500               |             |              |                   |
| 2018        | 9–14         | 500               |             |              |                   |

Only clear and no-rain nights with daily trap catches (light trap or food-lure trap) greater than 10 were shown.

Table 3 The echo height observed by radar from 2015 to 2019
Fig. 3 Image of the radar digital data-acquisition system showing the moths’ return trajectory. Each range ring was 400 m and the distance to the edge was 2 km. The elevation angle was 45°. (a) The layer extends between heights of 750–1300 m at 18:35 h on 8 September, 2017; (b) The layer extends between heights of 700 and 900 m at 22:41 h on 7 September, 2018; (c) The layer at height of 1300 m at 20:52 h on 10 September, 2019

Table 4 Distribution of trajectory endpoints starting from the three monitoring regions from 2015 to 2019

| Location | Provinces of China | Russia | Korea |
|----------|--------------------|--------|-------|
|          | HLJ | LN | JL | IM | SD |     |     |
| GZL      | 30  | 9  | 24 | 3  | 0  | 43  | 16  |
| MHK      | 4   | 8  | 8  | 1  | 3  | 7   | 3   |
| TN       | 9   | 5  | 8  | 8  | 0  | 24  | 0   |

The analytical period for trajectory analysis was confirmed using the light trap occurrence dates as follows. Dates of zero moths caught were not evaluated during these periods:
- Gongzhuling: 2015-9-1 ~ 2015-9-24, 2017-8-24 ~ 2017-9-24, 2018-9-3 ~ 2018-10-13 and 2019-8-26 ~ 2019-10-3
- Meihekou: 2015-8-17 ~ 2015-9-10, 2016-8-16 ~ 2016-8-25 and 2017-8-16 ~ 2017-9-29
- Taonan: 2015-9-7 ~ 2015-9-22, 2017-8-19 ~ 2017-9-26, 2018-8-17 ~ 2018-9-25 and 2019-8-31 ~ 2019-9-29

The acronym is the same as Fig. 4. The number represents the days of trajectory endpoints.
Fig. 4  Typical flight pathways of forward trajectories showing the migration of *Mythimna separata*. ● destination; a Gongzhuling, 2015–09-18; b Meihekou, 2015–08-22; c Taonan, 2015–09-19; d Meihekou, 2017–09-13; e Taonan, 2017–09-18; GZL Gongzhuling, MHK Meihekou, TN Taonan, HLJ Heilongjiang, LN Liaoning, JL Jilin, IM Inner Mongolia, SD Shandong, HB Hebei. The same as Table 4

Fig. 5  Median-joining network for the COI haplotypes in *Mythimna separata* populations. Circles represent different haplotypes and the sizes are proportional to the sample sizes. Colors represent geographical groups. The haplotypes H1, H3, H5, H8, H10, H12, and H18–H33, combined with other haplotype data, were used in our previous study to determine the population source of the third-generation *M. separata* in Jilin Province (Sun et al. 2022). By comparison, this study provided novel findings (The same as Fig. 6)
other populations. The $P_i$ ranged from 0.0005 to 0.0024, with an average value of 0.0015. Among the populations, the $K$ was between 0.2906 and 1.4725 (average of 0.9025). Analysis of molecular variance (AMOVA) results also showed that most of the genetic variation resided within the populations (98.23%, $P < 0.0001$), with only a small proportion among the populations (1.77%, $P = 0.05287$). Considering samples from 2016 to 2017 as a separate group, 98.02% of genetic variation was within the populations ($P < 0.0001$), and 1.98% of genetic variation was among the populations ($P = 0.01271$).

### Discussion

The research clarified the period of third-generation adults based on 5-year data. The period coincided with the emergence period of third-generation moths across China (Jiang et al. 2011). Jilin’s spring moth (first-generation moth) is considered an immigrant pest (Chen et al. 1989). In our previous study, we utilized the scanning entomological radar and molecular marker to prove that the summer moth (second-generation moth) is considered a mixed (emerged, migrants, and retained) population (Sun et al. 2022). In this study, analysis of ovarian development revealed that the degree of ovarian development and mating rates were low. These results suggest that the third-generation moths can be attributed to local emergence, ready for emigration. Thus, the moths were identified as an “emigration population.”

Based on meteorological data, the wind speeds were greater than the moths’ flight speed (4 m/s), so the moths could not displace of fly in a certain direction (Chen et al. 1995). Thus, the third-generation moths possibly moved downwind. Generally, the temperatures and wind speeds were optimum for the moths to take off and presented a downwind component for the long-distance migration tracks (Jiang et al. 2011). Our study confirmed that emigrant migration does occur by radar observation. However, for armyworm moths, their overwintering areas are located at lower latitudes (< 33°N), Chinese studies have indicated that $M. separata$ do not have the ability to survive the prevailing winter in all life stages in Jilin (Pan et al. 2014). Autumn is the harvest season in Jilin. Temperatures there are getting cold. Rain can stop the armyworm moths from taking off at dusk (Cheng et al. 2005). For the third-generation moths, whether the return movement was successful is crucial.

### Table 5

| Population code | Number of haplotypes ($H$) | Haplotype diversity ($Hd$) | Nucleotide diversity ($P_i$) | Average number of nucleotide differences ($K$) |
|-----------------|---------------------------|---------------------------|------------------------------|----------------------------------|
| NA-2016         | 5                         | 0.7000                    | 0.0019                       | 1.1632                           |
| SL-2016         | 8                         | 0.7474                    | 0.0030                       | 1.8211                           |
| LS-2016         | 5                         | 0.7211                    | 0.0015                       | 0.9211                           |
| CL-2016         | 2                         | 0.4790                    | 0.0008                       | 0.4790                           |
| HL-2017         | 5                         | 0.4460                    | 0.0009                       | 0.5540                           |
| TN-2017         | 8                         | 0.8901                    | 0.0024                       | 1.4725                           |
| SY-2017         | 6                         | 0.6800                    | 0.0015                       | 0.8954                           |
| LS-2017         | 4                         | 0.2764                    | 0.0005                       | 0.2906                           |
| MHK-2017        | 6                         | 0.5793                    | 0.0014                       | 0.8644                           |
| GZL-2017        | 10                        | 0.7724                    | 0.0022                       | 1.3379                           |
Scanning entomology radar has proven to be a powerful tool for monitoring insect migration (Hao et al. 2020; Jeffries et al. 2013; Riley et al. 2007). Observed flight parameters combined with trajectory simulation could improve the accuracy of migration analysis (Chapman et al. 2004; Drake and Reynolds 2012; Wood et al. 2006). Compared to previous studies (Sun et al. 2018), this study provides a wide distribution range and the destinations of third-generation moths, including (i) the southward return, (ii) other areas of Jilin Province, and neighboring provinces, and (iii) other countries. Additionally, there is verifiable evidence for the trajectory model as follows.

(i) In previous studies, southward migration of the species has been studied on a small island in the Bohai Sea. The authors of these studies observed unequivocal examples of *M. separata* migration from Northeast China on northeastern winds (Feng et al. 2008). Southward migratory movements were also observed in our study on some nights with strong northeasterly winds. A large numbers of moths emigrating from northern regions overflew the radar site in layers. There were trajectories passing over the Bohai Sea during the measurement period, but the destination was still far from the region where overwintering is possible.

(ii) The trajectories showed the moths’ movements to neighboring areas (other areas of Jilin Province and neighboring provinces). This result indicated that movements occurred during our monitoring and the neighboring areas. Thus, moths from the neighboring areas could also migrate to the monitoring areas. The analysis of ovarian development fully supports the above observation. Female moths with a lower proportion of irregular ovarian levels were assumed to be emigrants from the neighboring areas. It seems that the newly emerged moths from the neighboring area terminated their migration and landed in the monitoring areas after days of flight. The movements occur from the neighboring areas to the monitoring areas, proving that the emigrants from the monitoring areas could have gotten to the neighboring areas.

(iii) The forward trajectories also suggest that the moths could have arrived in the foreign countries; for example, some moths could reach as far north as Russia. However, such movements were poorly documented, but some indirect evidence was proposed by Jiang et al. (2018). They set many vertical-pointing search light traps to investigate the population dynamics of *M. separata* across China. In autumn, the lack of catch number correlation between the north and south strongly indicated that the third-generation moths migrate to places other than southern China.

Taken together, trajectory analyses showed that the destination regions were far from their overwintering areas. Regions such as the Russian Far East and Korea (with cold winters) provide adverse conditions for the species’ survival. However, unsuccessful migrations seem to be “wrong-way” flight, which is known as the “pied piper” effect (Pedgley et al. 1995). Third-generation moths were doomed to be extinguished by the harsh winter and would not be population sources in the following year. These results are consistent with a previous study for the second generation of the oriental armyworm in northeastern China (Pan et al. 2014). In growing seasons, the northward movement of this pest is known as multi-generation migration with a specific synoptic weather pattern (Lee and Uhm 1995). By comparison, the autumn movement lacks a clear return pathway. It is difficult for the moths to complete return migrations without help from persistent northerly airflows. Rainfall and prevailing winds hindered southward migration. If the second-generation moths did not make a return flight to northern China (i.e., in Shandong and Hebei Provinces), there are few opportunities for this species to return to overwintering areas in just one migration generation due to great geographical distance. Although we do not have sufficient information for return migrations in the sampling years, there is some evidence that they did perform return migrations in some years (Feng et al. 2008). Otherwise, moths originating from Liaoning Province, which is in the southern part of northeast China, might have more opportunities to return southward. Moreover, if a small fraction of moths return successfully, their numbers would be very large because of the pests’ high reproductive rate (Sun et al. 2018).

The genetic diversity of *M. separata* was estimated by the partial COI gene. Only 6 haplotypes were shared by the two sampling years. The significantly different haplotypes suggest different origin sources. These results were confirmed by our previous research which showed that the spring emigration populations in Jilin are proved to possess different origin sources in different years (Sun et al. 2019a). The haplotype analysis showed similar content in the two sampling years. We found similar high genetic diversity levels within the two sampling generations in 2016 and 2017. This high index was similar to that reported in previous studies (Li et al. 2019). Genetic diversity is assumed to be an important indicator of environmental adaption and migratory ability (Souza et al. 2017; Sun et al. 2015; Wongsa et al. 2017). Genetic diversity, therefore, was not reduced by the unsuccessful southward return. This result suggests the genetic polymorphism at mtDNA level of the oriental armyworm population in China is maintained without return of northeastern autumn populations. The result could be for three reasons:

(i) Many Chinese entomologists believe that the summer generation (second-generation moths) in northeastern China makes a return flight to northern China (i.e., in Shandong and Hebei Provinces). After they complete one generation there, the autumn generation (third-generation moths) begins to appear and fly to the lower latitudes (e.g. Zhejiang, Anhui) for overwintering (Lin and Zhang 1964). Therefore, if the summer return migration happens, the
“wrong-way” autumn flight might be unimportant to the species in the overall migratory strategy. Thus, the haplotype diversity could be maintained. Furthermore, according to a systematic study by Chen et al. (2019), in the Henan Province (central-northern area in China) due to climate change, more first-generation moths, which should have migrated northward to Northeast China, have become residents in recent decades. This indicates that these moths would retain and damage summer corn there. Therefore, such populations would make a return migration in autumn and could mainly contribute to the genetic flow.

(ii) Migratory behavior of this pest produced large numbers of effective migrants in lower latitude ranges. Perhaps, this was sufficient to overcome the migratory genes lost and maintained the migration genotype.

(iii) Another possible explanation is the widely distributed range and gene flow of this species. Research has shown that some populations exist in the Indochinese Peninsula, Malaysia and Philippines produce insect sources for China (Jiang et al. 2018) and summer populations originating in northeastern China could extended to Japan (Hirai 1995). Gene flow among these areas could increase genetic diversity.

In conclusion, this study’s findings support the notion that third-generation moths are perhaps unimportant as population sources in the following year. Therefore, pest management actions should emphasize on the occurrence of moths from spring to summer in the Jilin Province.

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Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

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