

Is *Heliothis virescens* (Lepidoptera: Noctuidae) a long-distance migrant?

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Abstract

Heliothis virescens (Hüfnagel) (Lepidoptera: Noctuidae) is an important polyphagous pest of beans, cotton, maize, and alfalfa worldwide. *H. virescens* is a long-distance migrant, and if so, what pattern of seasonal migration this species exhibits in northern China remains unknown. In this study, in order to determine the seasonal migration of *H. virescens* in northern China, the combination of searchlight trapping and ovarian dissection was carried out on an isolated small island in the center of the Bohai Strait during 2003–2014. The results confirmed that *H. virescens* undertakes long-distance migration on the prevailing winds of the East Asian monsoon airflows. This species exhibited a regular pattern of seasonal migration across the sea from May to October, but there was considerable yearly and monthly variation in the trapped numbers, with the majority being trapped in summer ($67.99 \pm 6.54\%$). The mean period when migration was detectable at the island was 116.5 ± 5.6 days from 2003 to 2014, with the shortest time span of 74 days in 2013 and the longest of 144 days in 2005. Trapped females in May and June showed a relatively higher mating rates and some degree of ovarian development when compared with July, August and September, suggesting the migration of this species is not completely bound by the ‘oogenesis-flight syndrome’. These findings will be helpful to improve the forecasting system and managing strategies of *H. virescens*.

Keywords: *Heliothis virescens*, seasonal pattern, searchlight trapping, sexual maturation, trans-regional migration

(Accepted 22 May 2016; First published online 14 June 2016)

Introduction

Insects, with the most species and abundant biodiversity, have evolved two general strategies to cope with habitat changes: diapause and migration, which have been referred to as the ‘here later’ and ‘there now’ strategies (Dingle, 2014). Long-distance migration, a seasonal to and from movement of insects between regions where conditions are favorable or unfavorable, plays a key role in the life-cycle of many insect species (Dingle & Drake, 2007). Billions of insects migrate (over land, or through air, or across water) within and between continents every year, often responsible for sudden

outbreaks of crop pests and insect-vectored plant diseases (Chapman et al., 2011, 2015). A good understanding of insect migration facilitates the development of forecasting systems and management strategies, however the general patterns of how populations migrate across different regions and seasons are largely unknown in most insect species (Stefanescu et al., 2013).

Heliothis virescens (Hüfnagel) (Lepidoptera: Noctuidae), one of the major crop pests in the world, is widely distributed in European, Africa and Asia (Kravchenko et al., 2005), occupying climates ranging from tropical to temperate (Weigand, 1996; Kahrarian et al., 2012). In China, *H. virescens* is widely distributed from northeastern through central to the Tibetan Plateau, and occasionally the occurrence range can extend to southeastern provinces. There are 2–4 generations of this species each year, increasing as the effective accumulative temperature increases from north to south (He et al., 1997).

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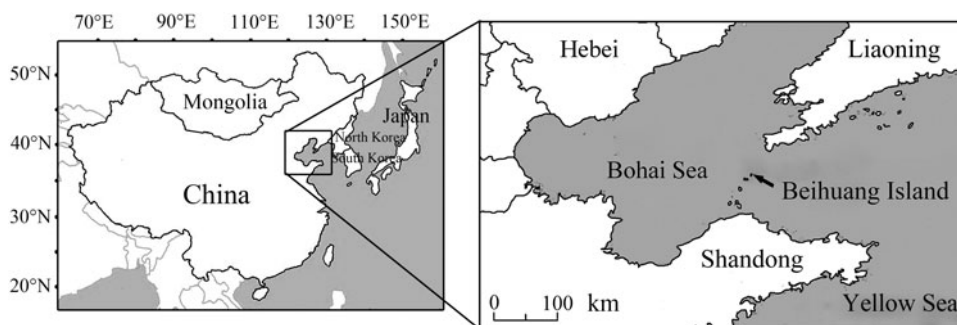


Fig. 1. Maps showing the position of Beihuang (BH) Island, the searchlight trapping site, relative to the Bohai and Yellow Sea.

H. virescens larvae are polyphagous with 78 host plants including >20 cultivated crops. They mainly damage peas in spring; cotton, maize and soybean in summer; alfalfa, sunflower and sugar beet in autumn; and overwinter as pupae in the soil (Cui et al., 1997; He et al., 1997; Liu et al., 2010). It is hard to prevent the damage and to control this pest species because it can silk and roll plant leaves, and it has also developed resistance to chemical pesticides (Mu et al., 2004). In the past decade, a series of major outbreaks of *H. virescens* has been reported in Asian soybean, maize, and chickpea fields, and severe infestations commonly reduce yields by 40–90% (Mu et al., 2004; Liu et al., 2010; Kahrarian et al., 2012).

Although it is commonly accepted that the wide distribution of *H. virescens* is due to its strong flight ability (He et al., 1997), so far there is no direct evidence that this species is a long-distance migrant. And if so, it is still unclear (1) whether the migration of this species is a regular occurrence, and (2) what the pattern and physiological states of seasonal migration is in this species. In the current study, the combination of searchlight trapping and ovarian dissection was used to monitor wind-borne migration of *H. virescens* for 12 consecutive years on a small isolated island located in the center of the Bohai Strait, China. Findings from this study will be helpful in developing sound management strategies against *H. virescens*.

Materials and methods

Searchlight trapping and field observation

The searchlight trapping studies were carried out each year from April to October 2003–2014 on Beihuang island (BH, 38° 24'N, 120°55'E), the northern most island of Changdao county in Shandong province. BH ($\approx 2.5 \text{ km}^2$) is located in the center of the Bohai Strait at a distance of $\approx 60 \text{ km}$ from the mainland to the south and $\approx 40 \text{ km}$ to the north (fig. 1). A vertical-pointing searchlight trap was placed on a platform $\approx 8 \text{ m}$ above sea level, and was used to attract and capture high-altitude migrants (up to $\approx 500 \text{ m}$ above the ground level; Feng et al., 2009). The trap was equipped with a 1000 W metal halide lamp (model JLZ1000BT; Shanghai Yaming Lighting Co. Ltd., Shanghai, China), which produces a vertical beam of light with a luminous flux of 105,000 lm, a color temperature of 4000 K, and a color rendering index of 65.

The searchlight trap was turned on at sunset and turned off at sunrise on all nights from April to October (Zhai, 2004). Incomplete data sets caused by power cuts or heavy rains were excluded from the analysis, while those nights in

which the light trapping was carried out normally but no *H. virescens* was captured were given a 'zero' count in the analysis. Trapped insects were collected with a nylon net bag (60 mesh) beneath the trap, which was changed manually every 2 h each night. The trapped insects were kept in a freezer at -20°C for 4 h before being identified and *H. virescens* females were dissected.

There are some pine trees and graminaceous weeds on BH, but no arable lands or host crops of *H. virescens*. To confirm whether there is a local population of *H. virescens* (e.g., on the weeds), visual observations were carried out daily to detect larvae of this species on any potential wild host plants from spring throughout autumn 2003–2014.

Ovarian dissection

To test the hypothesis of an 'oogenesis-flight syndrome', a subsample of 20 *H. virescens* females (or all individuals if the total capture of females was <20) was randomly taken each night, and was dissected under a stereomicroscope (model JNOEC-Jsz4; Motic China Group Co.Ltd., Xiamen, China) to determine the development level of the ovaries from 2009 to 2014. The level of ovarian development 1–5 was estimated according to the criteria described in table 1. These data were used to generate an average monthly level of ovarian development (i.e., the sum of individual levels of ovarian development divided by the number of females dissected). Females with ovarian development level 1–2 were regarded as 'sexually immature individuals', and others with level 3–5 were regarded as 'sexually mature individuals' (Zhang et al., 1979). Moreover, mating frequency and mating occurrences of *H. virescens* were determined by the number of spermatophores in the female spermatheca (Zhang et al., 1979).

Meteorological data

Daily wind directions on Bohai Strait from May to October 2003–2014 were obtained from China Meteorological Data Sharing Service System (<http://cdc.cma.gov.cn/>).

Data analysis

Dates of trap catches reported in this paper indicate the period from sunset of that day to sunrise of the next day. Differences in the number of *H. virescens* captured in the searchlight trap, the monthly mean proportion of females, mated females, and sexually mature females (the proportion data were arcsine square root transformed), were analyzed

Table 1. Criteria of ovarian development level of *H. viriplaca* moths.

Development level	Characteristics of ovary
1	Undeveloped thin and short oviducts, with transparent and light milky white ovary
2	Developed longer oviducts and ovarioles, with developing oocytes but no mature eggs
3	Well-developed yellowish green ovary with 1–3 chorionated eggs stored in the egg calyx
4	Well-developed the biggest oviducts, some eggs have been laid and interspaces appeared among mature eggs
5	The ovary has atrophied and contains no eggs

by using generalized linear mixed models, with month as the fixed effect and year as the random effect (Chaves, 2010; Tang, 2010; Tang & Zhang, 2013). If the analysis of variance (ANOVA) indicated a significant difference between months, Tukey's HSD (honestly significant difference) test was employed to distinguish significantly different monthly means. Sex ratio (females: males = 1:1) in each month from 2009 to 2014 was compared using chi-square tests. Differences of the mean proportion between mated and unmated females, sexually mature and immature females, were compared by using *t*-test (the proportion data were arcsine square root transformed). In order to distinguish mass or weak invasion years, the annual total catches of *H. viriplaca* were analyzed by hierarchical cluster analysis (clustering distance: Euclidean distance; clustering method: nearest-neighbor method) (Zhang *et al.*, 2015). All statistical analysis was performed by SPSS software, except for the sex ratio, which was analyzed by SAS software (SAS Institute, 1990).

Results

Annual and seasonal pattern of migration

During the study period of 2003–2014, no *H. viriplaca* larvae were found on BH by field investigations although some graminaceous weeds were available as potential wild host plants. However, *H. viriplaca* moths were regularly captured in the searchlight trap, which strongly suggests that these moths immigrate from the mainland rather than emerging locally, and that they migrated at least 40–60 km (and probably much greater distances) to reach the trapping site across the Bohai Strait. The strength of this over-sea migration did not differ significantly across years ($F_{11, 1836} = 1.78$, $P = 0.052$; table 2), but month \times year interaction ($F_{55, 1836} = 1.75$, $P < 0.001$; table 2) was significant. The results of the hierarchical cluster analysis indicated that the annual total trap catches could be divided into three groups – 2005, 2008, and the other 10 years clustered together (table 3). Specifically, mass migrations occurred in 2005 and 2008 (with the annual total catches reaching 1363 and 1144 individuals, respectively), while weak migrations occurred in the other years (with the annual total catches of *H. viriplaca* ranging between 40 and 250 individuals; fig. 2).

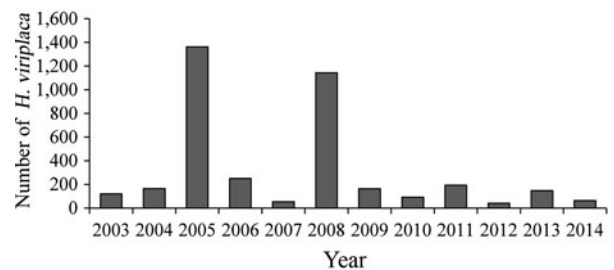
The number of *H. viriplaca* captured in the searchlight trap was not significantly different across months ($F_{5, 1836} = 1.21$, $P = 0.317$; table 2 and fig. 3), but month \times year interaction

Table 2. Two-way ANOVA analysis on the number of *H. viriplaca* captured in the searchlight trap on BH from May to October 2003–2014.

Source	Type III sum of squares	df	Mean squares	F-values	P
Month	5560.80	5	1112.1605	1.21	0.317
Year	10315.71	11	937.79	1.78	0.052
Month \times year	50573.04	55	919.51	1.75	<0.001
Error	964810.97	1836	525.50		
Total	1036388.81	1907			

Table 3. Hierarchical clustering analysis on the annual total numbers of *H. viriplaca* captured in the searchlight trap on BH from May to October 2003–2014.

Group	Year	Observed value	Distance to the center	Group mean	SD
First group	2003	120	9	129.0	38.8
	2004	165	36		
	2006	250	121		
	2007	54	75		
	2009	163	34		
	2010	92	37		
	2011	193	64		
	2012	41	88		
	2013	148	19		
	2014	64	65		
Second group	2008	1144	0	1144.0	–
Third group	2005	1363	0	1363.0	–
R^2		0.98			
Pseudo_F		236.28			
P		<0.001			

Fig. 2. Annual number of *H. viriplaca* captured in the searchlight trap on BH from 2003 to 2014.

($F_{55, 1836} = 1.75$, $P < 0.001$; table 2) was significant. Throughout the early summer (May–July), southerly winds (ESE to WSW, $65.4 \pm 1.6\%$) were the prevailing airstream over the Bohai Strait (fig. 4), and the mean proportion of *H. viriplaca* trapped in this period was $39.83 \pm 7.48\%$. In contrast, throughout the late summer and the autumn (August–October), northerly winds (WNW to ENE, $51.0 \pm 2.2\%$) became the prevailing airstream (fig. 4), and the mean proportion of *H. viriplaca* trapped in this period reached to $60.2 \pm 7.5\%$.

The mean period when over-sea migration was detectable on the island was 116.5 ± 5.6 days (ranged from 74 to 144 days) during 2003–2014, with the earliest and latest trapping occurred on 1 May and 4 October 2005, respectively (table 4).

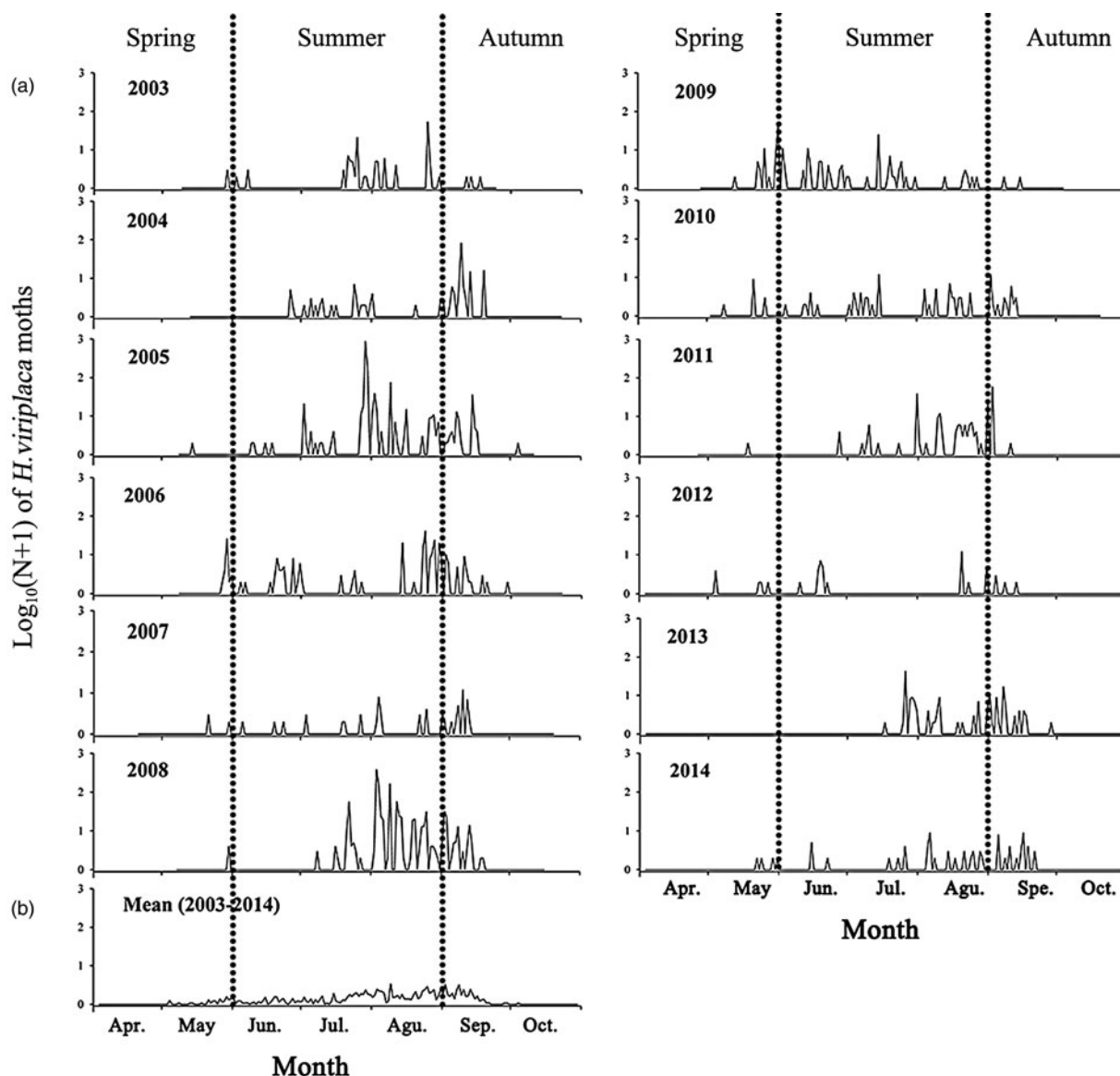


Fig. 3. Nightly catch (a) and annually mean number (b) of *H. virescens* in the searchlight trap on BH from April to October during 2003–2014.

Sex ratio, mating frequency, and ovarian development

From May to September 2009–2014, the vast majority of trapped *H. virescens* were females (fig. 5a), and the proportion of trapped females was significantly greater than that of males in each month (chi-square tests: May: $60.1 \pm 6.8\%$, $\chi^2 = 11.76$, $df = 1$, $P < 0.01$; June: $57.9 \pm 3.8\%$, $\chi^2 = 13.13$, $df = 1$, $P < 0.01$; July: $66.8 \pm 1.1\%$, $\chi^2 = 32.76$, $df = 1$, $P < 0.01$; August: $66.7 \pm 2.37\%$, $\chi^2 = 34.57$, $df = 1$, $P < 0.01$; September: $62.2 \pm 1.75\%$, $\chi^2 = 21.62$, $df = 1$, $P < 0.01$) (fig. 5b). The monthly mean proportion of *H. virescens* females captured in the searchlight trap did not differ significantly across months ($F_{4, 141} = 0.44$, $P = 0.780$; table 5) or years ($F_{5, 141} = 0.80$, $P = 0.550$; table 5), and there was no significant month \times year interaction ($F_{17, 141} = 1.55$, $P = 0.085$; table 5) during 2009–2014 (fig. 5b).

In May and June, most of the trapped *H. virescens* females were mated individuals (May: $90.3 \pm 4.0\%$, $t = 5.19$, $df = 6$,

$P < 0.01$; June: $76.2 \pm 6.4\%$, $t = 2.59$, $df = 8$, $P < 0.01$). There was no significant difference between the proportion of mated ($45.3 \pm 4.1\%$) and unmated ($54.71 \pm 4.1\%$) females in July ($t = 0.71$; $df = 8$; $P = 0.50$). However, in August and September, the majority of the trapped *H. virescens* females was unmated individuals (August: $90.4 \pm 1.5\%$, $t = 9.49$, $df = 10$, $P < 0.01$; September: $93.1 \pm 1.0\%$, $t = 10.61$, $df = 10$, $P < 0.01$). The monthly mean proportion of mated females was not significantly different across months ($F_{4, 141} = 0.01$, $P = 0.999$; table 6) or years ($F_{5, 141} = 1.32$, $P = 0.261$; table 6), but month \times year interaction ($F_{17, 141} = 2.18$, $P = 0.007$; table 6) was significant. Overall, the seasonal variation in the mean proportion of mated females showed a significant downward trend from May to September (logistic regression model: $y = 96.18 / (1 + 6.01 \times 10^{-5} e^{1.41})$, $R^2 = 0.94$, $n = 5$, $F = 33.02$, $P = 0.03$) (fig. 5d). The majority ($65.1 \pm 6.7\%$) of the mated females had mated once, about a third ($33.5 \pm 6.6\%$)

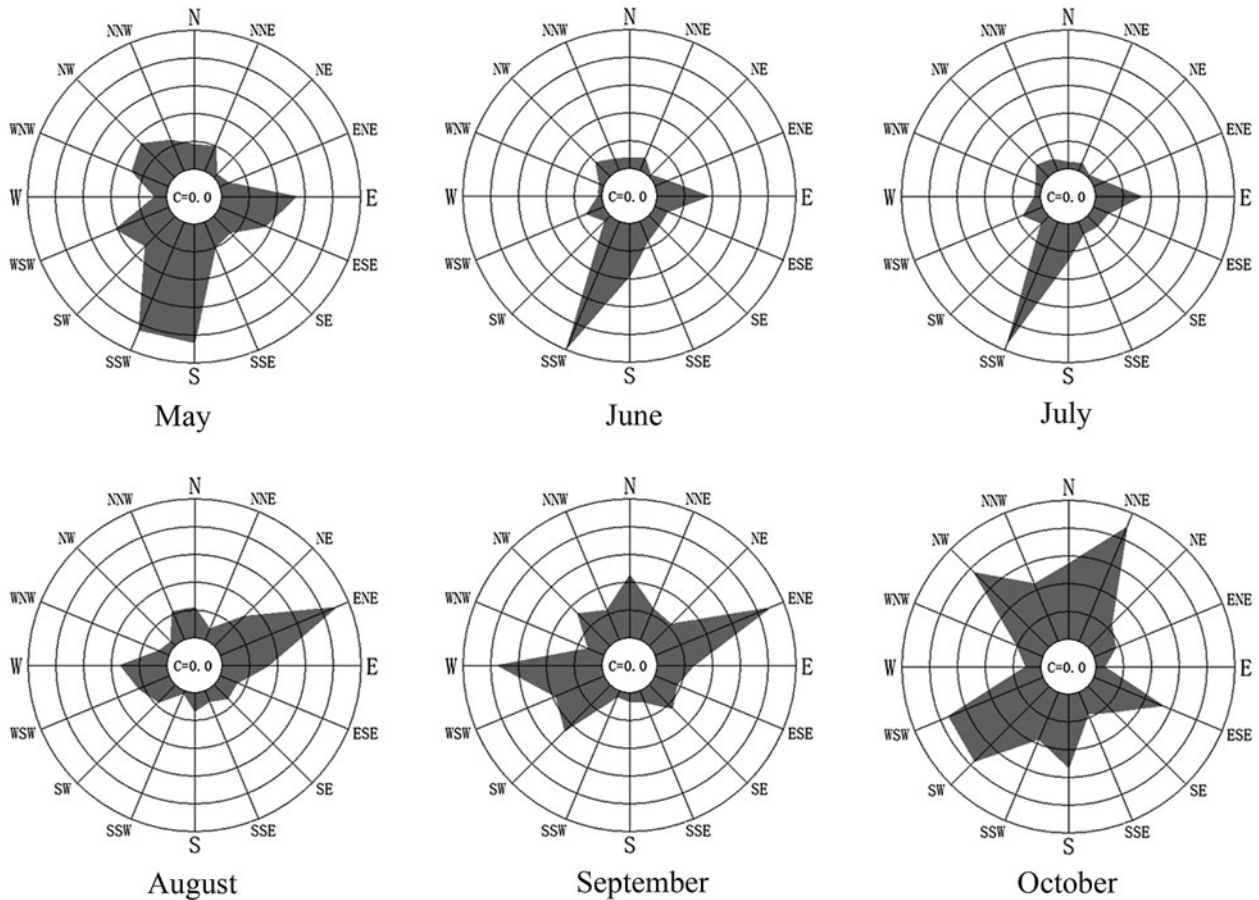


Fig. 4. Frequency distribution of wind direction on BH during 2003–2014. Concentric circles indicate the frequency of wind direction, and each circle means difference value of 4% in May, 7% in June, 8% in July, 6% in August, 4% in September, and 3% in October.

Table 4. Duration and peak catches of *H. virescens* moths caught in the searchlight trap on BH from May to October 2003–2014.

Year	Date of first capture (no.)	Date of final capture (no.)	Duration (d)	Date of peak catches (no.)
2003	29 May (2)	17 September (1)	112	25 August (52)
2004	26 June (4)	19 September (15)	86	09 September (81)
2005	01 May (1)	04 October (1)	144	29 July (880)
2006	27 May (1)	29 September (1)	126	24 August (40)
2007	21 May (2)	13 September (1)	116	10 September (11)
2008	30 May (3)	19 September (1)	113	03 August (380)
2009	13 May (1)	15 September (1)	126	01 June (40)
2010	08 May (1)	13 September (2)	129	15 July and 02 September (11)
2011	18 May (1)	09 September (1)	116	02 September (58)
2012	04 May (3)	13 September (1)	133	20 August (11)
2013	17 July (1)	29 September (1)	74	26 July (42)
2014	22 May (1)	21 September (2)	123	06 Aug. and 16 September (8)

had mated twice, only a small proportion ($1.4 \pm 0.8\%$) had mated three times, and no individuals mated more than this (fig. 6).

In May and June, the vast majority of the trapped *H. virescens* females were sexually mature individuals (May: $77.4 \pm 7.5\%$, $t = 2.52$, $df = 6$, $P = 0.04$; June: $85.8 \pm 3.2\%$, $t = 4.90$, $df = 8$, $P < 0.01$). There was no significant difference between the proportion of sexually mature ($53.2 \pm 3.8\%$) and immature ($56.8 \pm 3.8\%$)

females in July ($t = 0.55$; $df = 8$; $P = 0.60$). However, in August and September, most of the trapped *H. virescens* females were sexually immature individuals (August: $77.3 \pm 2.5\%$, $t = 6.14$, $df = 10$, $P < 0.01$; September: $86.6 \pm 1.8\%$, $t = 6.45$, $df = 10$, $P < 0.01$). The monthly mean proportion of sexually mature females was not significantly different across months ($F_{4, 141} = 0.04$, $P = 0.996$; table 7) or years ($F_{5, 141} = 1.60$, $P = 0.165$; table 7), but month \times year interaction ($F_{17, 141} = 2.61$, $P = 0.001$; table 7)

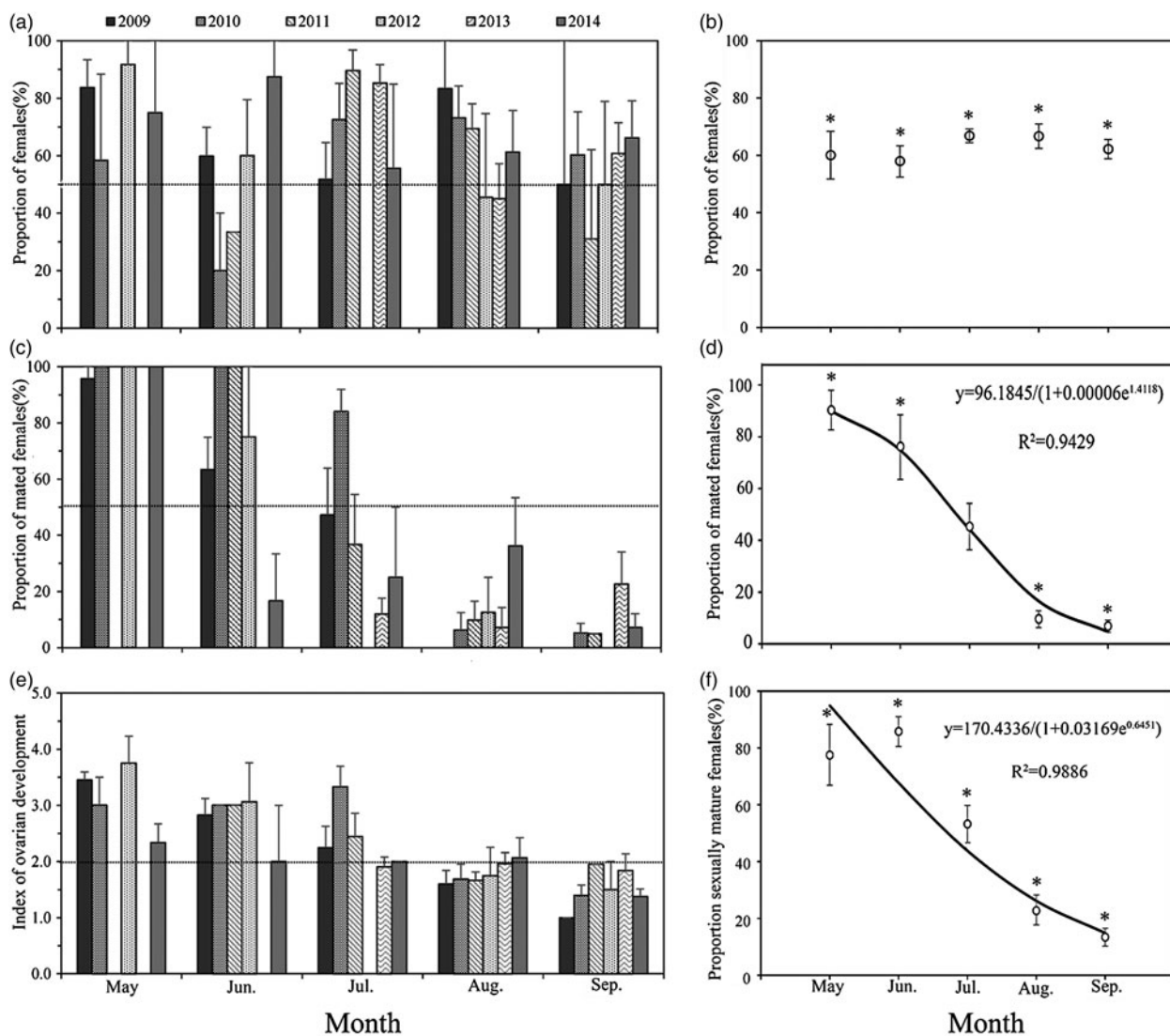


Fig. 5. Proportion of females (a, b), mated females (c, d) and sexually mature females (e, f) of *H. virescens* captured in the searchlight trap from May to September 2009–2014. The histograms in (a) and (c) indicate the mean proportion in each month. The histograms in (e) indicate the mean ovarian development level in each month. Vertical bars in (a), (c), and (e), represent Standard errors between days in that month. Dots in (b), (d), and (f) indicate the monthly mean proportion from 2009 to 2014. Vertical bars in (b), (d), and F represent Standard errors between years in that month. Single asterisk (*) above a bar in (b) indicates the sex ratio (female: male) was >1:1 in that month at the 5% level of significance as determined by chi-squared test. Single asterisk (*) above a bar in (d) and (f), indicates there was significant difference between the monthly mean proportion of mated and unmated females, and that of sexually mature and immature females at the 5% level of significance as determined by *t*-test in (d) and (f).

Table 5. Two-way ANOVA analysis on the monthly mean proportion of *H. virescens* females captured in the searchlight trap on BH from May to September 2009–2014 (the proportions were arcsine square root transformed).

Source	Type III sum of squares	Df	Mean squares	F values	P
Month	12.70	4	3.18	0.44	0.7797
Year	18.74	5	3.75	0.80	0.5502
Month × year	123.38	17	7.26	1.55	0.0853
Error	659.32	141	4.68		
Total	802.11	167			

Table 6. Two-way ANOVA analysis on the monthly mean proportion of mated *H. virescens* females captured in the searchlight trap on BH from May to October 2009–2014 (the proportions were arcsine square root transformed).

Source	Type III sum of squares	Df	Mean squares	F values	P
Month	0.26	4	0.07	0.01	0.9998
Year	19.53	5	3.91	1.32	0.2605
Month × year	109.72	17	6.45	2.18	0.0072
Error	418.29	141	2.97		
Total	761.20	167			

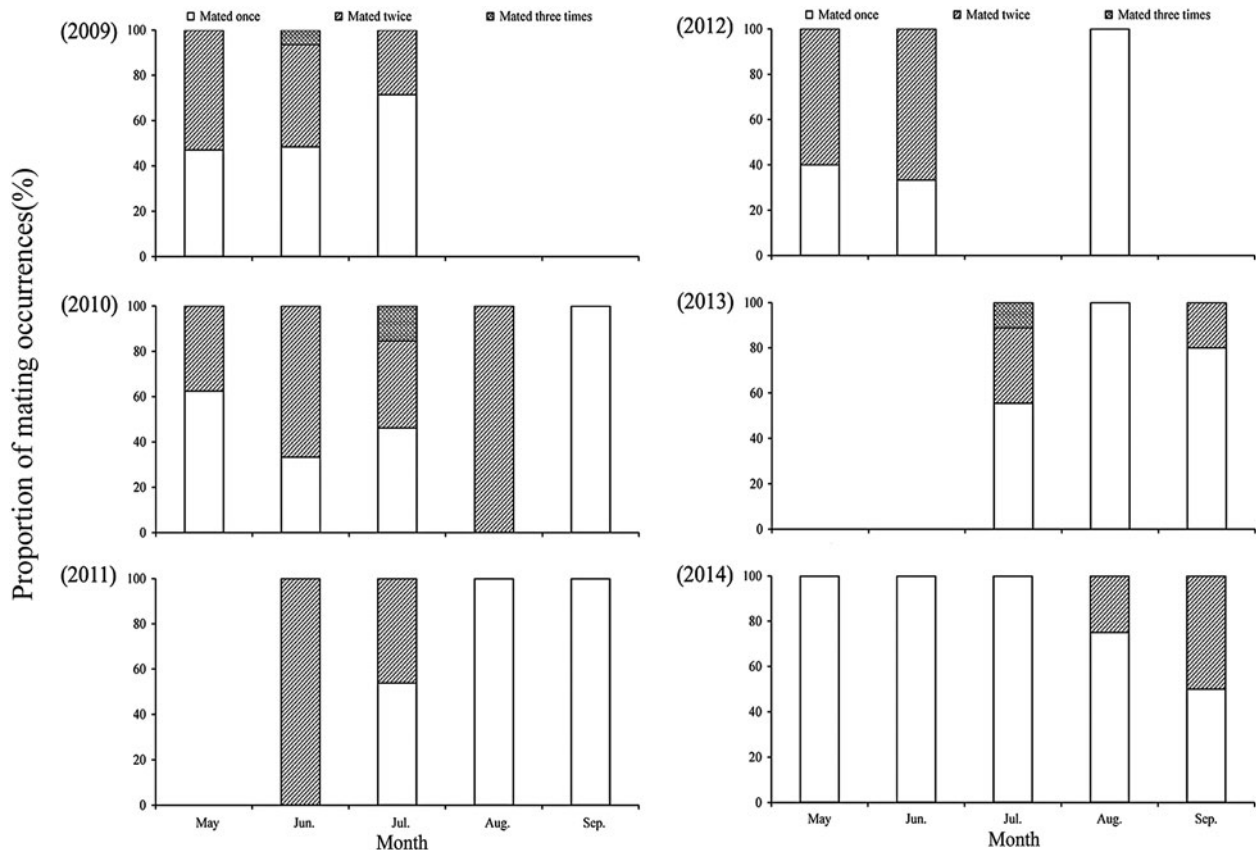


Fig. 6. Proportion of mating occurrences of *H. virescens* females captured in the searchlight trap on BH from May to September 2009–2014.

Table 7. Two-way ANOVA analysis on the monthly mean proportion of sexually mature *H. virescens* females captured in the searchlight trap on BH from May to October 2009–2014 (the proportions were arcsine square root transformed).

Source	Type III sum of squares	Df	Mean squares	F values	P
Month	1.27	4	0.32	0.04	0.9963
Year	23.09	5	4.62	1.60	0.1651
Month × year	128.39	17	7.55	2.61	0.0011
Error	408.07	141	2.89		
Total	731.87	167			

was significant. Overall, the seasonal variation on the mean proportion of sexually mature females showed a significant downward trend (logistic regression model: $y = 170.43 / (1 + 0.032 * e^{0.65x})$, $n = 5$, $F = 173.56$, $P < 0.01$, $R^2 = 0.99$) from May to September 2009–2014 (fig. 5f).

Discussion

Migratory insects have been studied for many years because of their economic and ecological importance, and a better understanding of the migration behavior of crop pests is essential for the development of forecasting systems and sustainable Integrated Pest Management strategies (Irwin 1999;

Wu & Guo, 2005; Wu et al., 2006). *H. virescens* is one of the most destructive crop pests in East Asia, and there has been a long debate about whether this species is a migrant or not (Wu et al., 1998). In the present long-term study, the results from the combination of searchlight trapping, field investigations and ovarian dissection on BH Island provide direct evidence that *H. virescens* is a long-distance migrant. The seasonal population dynamics of this species observed in this study was similar to previous observations for other migratory insects in the orders of Lepidoptera, Odonata, and Coleoptera made over the sea (Feng et al., 2003, 2004a, b, 2005, 2006, 2008, 2009; Wu et al., 1998, 2006).

The East Asian monsoon airflows in temperate regions provide an advantageous carrier for long-distance insect migration (Drake & Farrow, 1988). In the semi-arid temperate climate zone (Liaoning province, which is located to the north of BH), the average of daily minimum temperature in April is generally $< 6^\circ\text{C}$. Given the low temperature, *H. virescens* moths cannot overwinter or produce a new generation in spring (Kahrarian et al., 2012). In the current study, *H. virescens* moths could be captured on BH island as early as May, and considering the prevailing southerly winds during this season, these individuals should be coming from the mainland in south of BH by windborne migration, in order to exploit temporary habitats in northeastern agricultural regions of China, while the spring maize and soybean widely planted there plays an important role in the survival of *H. virescens* larvae. Similar wind-related migration has been observed in

Helicoverpa armigera (Hübner), *Spodoptera exigua* Hübner, *Mythimna separata* (Walker), and *Loxostege sticticalis* L. at the same site (Feng et al., 2004a, b, 2005, 2008, 2009).

In autumn (August–October), prevailing northerly winds, caused by the prevailing temperature gradient, blowing from east Siberia to northern China, and the updraft airflows that generally occur in the northeastern agricultural region of China, promote large number of offspring produced by summer breeders emigrating to the south. Characteristics of the backward migration observed in the present study were rather similar to those windborne migrations of *Empoasca fabae* (Harris) (Taylor & Reeling, 1986), *Nilaparvata lugens* (Stål) (Riley et al., 1994), *Cnaphalocrocis medinalis* Guenée (Riley et al., 1995), *Agrotis ipsilon* (Rottemberg) (Showers, 1997), *Vanessa atalanta* (L.) (Mikkola, 2003), *M. separata* (Feng et al., 2008), *H. armigera* (Feng et al., 2009), and *Autographa gamma* (L.) (Chapman et al., 2012), and it has been postulated that this behavior facilitated return movements to the southern overwintering areas of these species. Understanding the seasonal migration pattern of *H. virescens* is also of economic importance, as this species becomes a major pest in many Asian crop fields during outbreak years. Just how representative *H. virescens* is of other migrant insects is a matter for further study, but given the similarities in the migration strategies of *H. virescens* to those of other insects in Asia, it is very likely that the results of this study will be applicable to a wide range of migrants.

The monthly mean proportion of mated females and that of sexually mature females showed a significant downward trend from May to September 2009–2014. Trapped females in May and June showed a relatively higher mating rates and some degree of ovarian development when compared with July, August, and September, which might be due to these moths emigrating from sites far away from the trapping site and therefore having several successive nights of migration. This trend was similar to previous observations in *M. separata* (Zhao et al., 2009), *Athetis lepigone* (Fu et al., 2014), *S. litura* (Fu et al., 2015), *A. ipsilon* (Liu et al., 2015), *Agrotis segetum* (Guo et al., 2015), *Mamestra brassicae* (Wu et al., 2015) and *Ctenoplia agnata* (Li et al., 2014) at the same site. However, the majority of the trapped *H. virescens* females from August to September have little or no ovarian development, supporting the idea that the onset of migration is initiated by sexually immature individuals (Riley et al., 1995). The relationship between long-duration flight and the state of oogenesis appears to be similar to that of *A. ipsilon* in North America (Showers, 1997). Here the northward-moving spring migrants developed reproductively, and it was suggested that there was no need to shut down reproductive development because the movement takes place rapidly, aided by the low-level jet stream. The southward movement in autumn is generally much slower (8–15 nights) due to the slow-speed winds, and in this case the moths may enter reproductive diapause (Showers, 1997).

The current study provides direct evidence that *H. virescens* can migrate across the Bohai Strait, thus the hypothesis that *H. virescens* is a long-distance migrant is confirmed. These findings will contribute to a better understanding of the occurrence of this species in northern China, and also to make managing this pest more efficient. However, further study is needed to characterize the population dynamics of *H. virescens* on the Chinese mainland. In addition, migration behavior in high-altitude and trajectory analysis would be beneficial to determine the seasonal pathway of this pest.

Acknowledgements

The authors thank Jianglong Guo, Xiao Wu, Hong Chang, and Limei He from Institute of Plant Protection, Chinese Academy of Agricultural Sciences; Ning Liu, HaoHao Li from College of Plant Protection, Henan Agricultural University; and Congzheng Yuan, Shengyuan Zhao from College of Agronomy and Plant Protection, Qingdao Agricultural University, for their contributions in field investigation. This research was supported by Special Fund for Agro-scientific Research in the Public Interest of China (grant number 201403031).

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