Assessing the Resolution of Haplotype Distributions to Delineate Fall Armyworm (Lepidoptera: Noctuidae) Migratory Behaviors

RODNEY N. NAGOSHI,[1,2] ROBERT L. MEAGHER,[1] AND MIRIAN HAY-ROE[1,3]

J. Econ. Entomol. 107(4): 1462–1470 (2014); DOI: http://dx.doi.org/10.1603/EC14124

ABSTRACT Regions of southern Florida and southern Texas (extending into Mexico) provide the overwintering source populations for virtually all fall armyworm infestations affecting the continental United States. Understanding how these migratory populations annually disperse is important to predict and control infestations by this specific pest and to more generally investigate the environmental factors that influence the long-distance movements of flying insects. The two overwintering locations are associated with differences in the distribution of certain mitochondrial haplotypes that overlap in the region near the border separating the states of Alabama and Georgia. This provided an opportunity to test the resolution of the haplotype method by comparisons between smaller geographical areas and shorter time frames than previously examined. Correspondences were found between trap-capture numbers, fall armyworm strain proportions, and haplotype ratios calculated for individual counties and within season time periods that were generally consistent with expectations, providing confidence that those population movements could be accurately inferred. The comparison of haplotype distributions identified a migratory boundary separating the Texas and Florida populations coincident with the eastern edge of the Apalachicola–Chattahoochee–Flint River basin. Calculations of strain numbers based on genetic markers revealed similarities and differences in strain population dynamics that can be applied to study the migratory behavior of fall armyworm subpopulations. The use of this methodology for the detailed mapping of migratory pathways and the identification of factors that influence the direction and extent of pest migration are discussed.

KEY WORDS *Spodoptera frugiperda*, host strains, migration

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) represents an advantageous system to study the migration of lepidopteran pests in the United States. Fall armyworm infests many field, forage, and vegetable crops in the western hemisphere with significant economic consequences, particularly in warm winter areas such as Central and South America, where it is the primary pest of corn. This moth species is intolerant to prolonged freezing, yet its infestation range extends as far north as Canada, an indication of its migratory capacity. The primary overwintering regions are in the southern portions of Florida and Texas, extending into Mexico, and these are thought to be the source of virtually all infestations in the United States and Canada. Such behavior makes fall armyworm an excellent model for studying the long-distance aerial migration of insects. Each year's migration can be seen as a separate trial with approximately the same initial starting conditions, thereby allowing detection of recurring migratory patterns and the investigation of air transport systems presumed to direct these long-range movements (Muller 1985, Wolf et al. 1990, Johnson 1995).

Fall armyworm consists of two behaviorally distinct, but morphologically identical strains that were initially identified by differences in plant host distribution, hence their designation as rice strain and corn strain (Pashley et al. 1985, 1987; Pashley 1986, 1988). Polymorphisms in the mitochondrial *cytochrome oxidase I* (*COI*) gene provide a convenient and accurate marker for strain identity (Pashley et al. 1985, Pashley 1986). The corn strain population can be further subdivided into four haplotype subgroups (CS-h1–4; Nagoshi et al. 2007a). Surveys of populations from Brazil and the United States showed that all four subgroups were present in each area, but there were reproducible differences in their relative proportions. This was most evident when observing the ratio of the CS-h4 to CS-h2 haplotype proportions. Analysis of corn strain populations in Florida over a 5-yr period and at several locations revealed a CS-h4/CS-h2 ratio that was consistently >1.5 (Nagoshi et al. 2007a). In contrast, populations from Brazil and Texas, again sampled over multiple years and locations, consistently showed a ratio <0.5 (Nagoshi et al. 2007a, 2008).

The ability to differentiate fall armyworm populations from Texas and Florida provided a method for extrapolating migration pathways from these areas,

[1] Center for Medical, Agricultural and Veterinary Entomology, USDA–ARS, Gainesville, FL 32608.
[2] Corresponding author, e-mail: rodney.nagoshi@ars.usda.gov.
[3] Everglades Research and Education Center, University of Florida, Belle Glade, FL 33430.

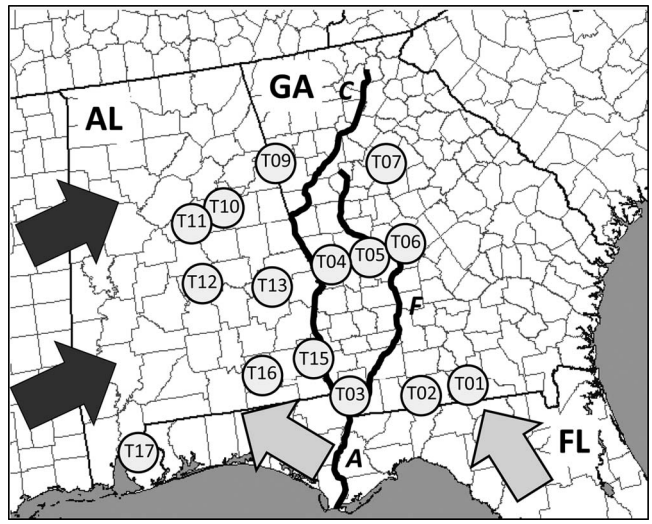

Fig. 1. Map of region surveyed in this study including Alabama (AL) and Georgia (GA). Counties are outlined. Circles indicate the 15 collection sites as described in Table 1. Arrows depict the anticipated direction of fall armyworm migrations from Texas (dark shading) and Florida (light shading) overwintering sources. The Appalachicola (*A*), Chattahoochee (*C*), and Flint (*F*) Rivers are indicated and together comprise the ACF River basin. T03 lies between the Chattahoochee and Flint Rivers.

based on the premise that the haplotype composition of a migrant population will reflect that found at its overwintering source. As a proof of concept, we showed that fall armyworm from the adjacent state of Georgia closely resembled Florida populations, while those in the more western states of Alabama, Mississippi, and Louisiana were similar to the Texas profile (Nagoshi et al. 2008). Subsequent work made practical by the efficiencies of pheromone trapping for collecting specimens led to a description of fall armyworm migration pathways in the United States (Nagoshi et al. 2009, 2012). These studies demonstrated that the CS-h4/CS-h2 ratio distribution was informative when applied over relatively large geographical regions and time frames, with comparisons mostly made between annual state averages. It has yet to be shown whether haplotype comparisons of trap collections made over smaller spatial (e.g., between counties) and shorter temporal (within season) scales are also informative.

Haplotype ratio surveys in the states of Alabama and Georgia were unusual in that these often produced haplotypes intermediate of those indicative of a Florida or Texas origin (Nagoshi et al. 2008 2010). This was interpreted as representing an area of overlap between the Texas and Florida migratory pathways such that there was a mixture of the two populations. This region is therefore of genetic relevance as it is an area where genetic exchange between the two overwintering populations can occur. Furthermore, the haplotype ratios within this region will be variable if, as we anticipate, the migratory pathways transiently shift in response to local environmental conditions and meteorological events. Such variability provides an opportunity to assess the resolving power of the haplotype ratio method, specifically whether haplotype differences between relatively small geographical areas (counties) and within-season time frames can provide insights into population movements. The capacity to monitor fall armyworm dispersal at this level of detail could allow risk assessments for local infestations and facilitate the use of preemptive mitigation strategies.

We tested the feasibility of this approach by extensive surveys of the Alabama–Georgia region over multiple collection periods within an August–September time frame, when fall armyworm populations, as indicated by pheromone trap captures, were consistently high. The haplotype ratio distribution was determined and compared with expectations from previous studies on fall armyworm movements in the region. In addition, the collection sites spanned the Apalachicola–Chattahoochee–Flint (ACF) River basin, the major geological feature in the region, allowing an assessment of its effects on insect migration. The utility and limitations of the haplotype ratio method for mapping fall armyworm migratory behavior is critically discussed.

Materials and Methods

Specimen Collections and Sites. Seventeen trapping sites were initially chosen for the agricultural regions of Alabama and Georgia in 2007. Their locations spanned the area identified as being within the region of overlap of the Florida and Texas migration pathways, as modified by practical considerations such as the location of appropriate plant hosts, cooperative growers and extension agents, and accessibility (Fig. 1). Sites T08 and T09 were located within 50 km of each other and trap captures for both were sporadic. Because of their proximity and low productivity, the data from these two sites were combined and desig-

Table 1. Locations of sites used for pheromone trap collections in 2007, 2008, or both

Site	State	County	Nearby town	Latitude	Longitude
T01[a]	GA	Lowndes	Valdosta	30° 48.566′ N	83° 21.568′ W
T02	GA	Thomas	Thomasville	30° 48.937′ N	83° 52.010′ W
T03	GA	Decatur	Brinson	30° 57.649′ N	84° 43.091′ W
T04	GA	Muscogee	Columbus	32° 23.273′ N	84° 58.459′ W
T05	GA	Taylor	Butler	32° 31.550′ N	84° 14.938′ W
T06[a]	GA	Peach	Ft. Valley	32° 33.524′ N	83° 48.376′ W
T07	GA	Newton	Covington	33° 40.483′ N	83° 52.219′ W
T09[b]	GA	Haralson	Buchanan	33° 47.930′ N	85° 16.698′ W
	AL	Cleburne	Heflin	33° 36.367′ N	85° 35.657′ W
T10	AL	Talladega	Eastaboga	33° 33.356′ N	86° 01.340′ W
T11	AL	Shelby	Vincent	33° 22.795′ N	86° 25.023′ W
T12	AL	Autauga	Prattville	32° 26.622′ N	86° 24.937′ W
T13[a]	AL	Macon	Shorter	32° 25.429′ N	85° 56.285′ W
T15	AL	Henry	Headland	31° 22.634′ N	85° 18.648′ W
T16[a]	AL	Coffee	Elba	31° 24.455′ N	86° 10.004′ W
T17	AL	Baldwin	Elberta	30° 27.706′ N	87° 36.422′ W

[a] Data from these two locations were pooled.
[b] Haplotype analyses of a subset of the 2007 collections from these locations were summarized in Nagoshi et al (2012).

nated as T09 (Table 1). Site T14 (in Russell Co., AL) was consistently unproductive and so was not included in this study. This left a total of 15 sites that provided sufficient specimens for analysis (T01–T13, T15–T17; Table 1). Within the sampling region is the ACF River basin made up of the Apalachicola (*A*), Chattahoochee (*C*), and Flint (*F*) rivers (Fig. 1). It extends from the Gulf of Mexico to the southern edge of the Appalachian Mountains. This is the major geological feature in the region and collection sites were arranged to the east (T01–02, T06–07), west (T09–13, T15–17), and within (T03–05) the basin.

Based on our previous work, we found that 20–30 specimens are required to obtain reliable strain and haplotype data (Nagoshi et al. 2007a,b). Capture numbers large enough for this analysis were not obtained in a majority of locations until August in 2007 and 2008. Collections in 2007 are described for four collection periods using day of year (DOY) dating, DOY 218–233 (6–21 August), 234–254 (22 August–11 September 11), 255–268 (12–25 September), and 269–288 (26 September–15 October), hereafter referred to as 07[1], 07[2], 07[3], and 07[4], respectively (Table 2). Sites T04, T05, and T13 were not available in 2008. Collections in 2008 were made during three periods, DOY 211–231 (30 July–19 August), 232–251 (20 August–8 September), and 252–259 (9–16 September). However, heavy storm activity damaged traps during the 232–251 period compromised the collections. Therefore, data were only available for periods 211–231 (08[1]) and 252–259 (08[2]).

Trap collections were performed using standard (green top, yellow funnel, white bucket) or all-green Universal moth traps (Unitraps; Great Lakes IPM,

Table 2. Number of moths trapped at each collection site (captures per trap per night or Cpt) during the four collection periods in 2007 (07[1–4]) and two collection periods in 2008 (08[1–2])

Site	N–S group	E–W group	Collection periods (DOY)					
			07[1] (218–233)	07[2] (234–254)	07[3] (255–268)	07[4] (269–288)	08[1] (211–231)	08[2] (252–259)
T01[a,b]	S	E	2.0	38.7	40.9	22.6	5.5	1.6
T02[a]	S	E	2.1	6.5	2.7	2.1	0.6	0.0*
T03[a,b]	S	W	33.9	31.2	30.3	25.5	60[c]	150[c]
T04	N	W	0.5*	0.3*	7.6	7.8	–	–
T05	N	W	0.0*	1.1	4.0	14.6	–	–
T06[a,b]	N	E	1.1	9.0	19.8	31.8	0.8	3.0
T07[a]	N	E	3.6	0.6	0.6	4.3	1.3	0.4*
T09	N	W	0.8	0.9	0.2*	1.8	1.8	0.2*
T10[a]	N	W	3.1	0.5	2.5	6.0	0.1*	0.8
T11	N	W	0.2*	0.2*	0.4*	1.6	0.3*	0.0*
T12[a]	N	W	7.5	1.8	14.4	56.6	0.9	0.1*
T13[a]	N	W	14.4	2.9	7.6	5.7	–	–
T15[a,b]	S	W	10.5	6.8	20.8	22.5	5.1	0.9
T16[a,b]	S	W	21.2	8.9	27.8	36.8	13.7	12.8
T17[a]	S	W	10.0	12.3	17.8	15.5	–	0.7

Collection sites were grouped according to their relative north (N) to south (S) or east (E) to west (W) locations as described in the text. Asterisk (*) indicates collections where the number of useable specimens was too low to characterize % corn strain (%CS) or h4/h2 ratios. In 2008, no collections (–) were made at several sites for technical or logistical reasons.
[a] Site provided %CS and h4/h2 data throughout 2007.
[b] Site provided %CS and h4/h2 data throughout 2008.
[c] Number is an estimate because captures surpassed capacity of one or more pheromone traps at the location.

Vestaburg, MI) baited with a commercially available fall armyworm pheromone (Scentry Biologicals Inc., Billings, MT; Trécé Inc., Adair, OK; and Suterra LLC, Bend, OR). Each trap contained insecticide strips containing 10% 2,2-dichlorovinyl dimethyl phosphate (Hercon Environmental, Emigsville, PA) to kill moths. Collections were made using 3–4 traps per site. All specimens were recorded and stored at −20°C. The collected specimens were identified as fall armyworm based on morphological criteria before molecular analysis, and the capture data were processed and recorded as captures per trap per night (Cpt).

DNA Preparation and PCR amplification. Individual specimens were homogenized in 4 ml of phosphate buffered saline (20 mM sodium phosphate, 150 mM NaCl, pH 8.0) in a 15-ml test tube using a tissue homogenizer (PRO Scientific Inc., Oxford, CT). Cells and tissue were pelleted by centrifugation at 12,000 *g* for 10 min at room temperature. The pellet was resuspended in 500 μl of cell lysis buffer (0.2 M sucrose, 0.1 M Tris-HCl at pH 8.0, 0.05 M EDTA, and 0.5% sodium dodecyl sulfate), transferred to a 1.5- or 2.0-ml microcentrifuge tube and incubated at 55°C for 10 min. Proteins were precipitated by the addition of 100 μl of 8 M potassium acetate. The supernatant was transferred to a Zymo-Spin III column (Zymo Research, Orange, CA) and processed according to manufacturer's instructions. The DNA preparation was increased to a final volume of 40 μl with distilled water. Genomic DNA preparations of fall armyworm samples were stored at −20°C and analyzed as needed.

PCR amplification of the mitochondrial *COI* gene was performed in a 30 μl reaction mix containing 3 μl of 10× manufacturer's reaction buffer, 0.5 μl of 10 mM dNTP, 0.5 μl of 20 μM primer mix, 1–2 μl of DNA template (between 0.05–0.5 μg), and 0.5 U *Taq*DNA polymerase (New England Biolabs, Beverly, MA). The thermocycling program was 94°C (1 min), followed by 33 cycles of 92°C (30 s), 56°C (45 s), 72°C (45 s), and a final segment of 72°C for 3 min. Typically, 96 PCR amplifications were performed at the same time using either 0.2-ml tube strips or 96-well microtiter plates. Amplification of the *COI* region used the primer pair *COI-893F* (5′-CACGAGCATATTTTA CATCWGCA-3′) and *COI-1303R* (5′-CAGGAT AGTCAGAATATCGACG-3′) to produce a 410-bp fragment. Primers were synthesized commercially (Integrated DNA Technologies, Coralville, IA).

Strain Identification and DNA Sequence Analysis. To each PCR reaction mix (30 μl), 5 U of the restriction enzyme EcoRV (New England Biolabs, Beverly, MA) and 4 μl of the manufacturer recommended 10× restriction enzyme buffer (final volume taken to 40 μl with water) were added. Restriction digests were incubated at 37°C for 1–3 h. For each reaction, 6 μl of 6× gel loading buffer was added and the entire sample run on a 1.8% agarose horizontal gel containing GelRed (at one-third the concentration recommended by manufacturer's instructions; Biotium, Hayward, CA) in 0.5× Tris-borate buffer (TBE, 45 mM Tris base, 45 mM boric acid, 1 mM EDTA, pH 8.0). Fragments were visualized on a long-wave UV light box. Only the rice strain-associated *COI* allele has an EcoRV site in the amplified region (Nagoshi et al. 2008). Uncut fragments were preliminarily identified as representing the corn strain and these were excised from the gel. Fragment isolation was performed using Zymo-Spin I columns (Zymo Research, Orange, CA) according to manufacturer's instructions. The isolated fragments were analyzed by DNA sequencing (University of Florida ICBR, Gainesville, FL). Species identity was confirmed by DNA sequence analysis (Nagoshi et al. 2011), and strain identification was confirmed by identifying strain-diagnostic polymorphisms in the amplified region (Nagoshi et al. 2006, 2007a).

Analyzing Capture Rate, Strains, and Haplotypes. The Cpt was calculated as the number of specimens collected at a location divided by the number of traps used and divided again by the number of nights in the collection period. The proportion of corn strain present in each collection (%CS) was determined by dividing the number of corn strain identified in a collection using molecular markers by the total number of specimens examined. A minimum of 20 specimens were required for %CS determination and in a majority of collections (66 out of 70) the sample number was >30. The number of corn strain (#CS) was calculated by the formula #CS = Cpt × %CS; and the number of rice strain (#RS) by the formula #RS = Cpt − #CS. The CS-h1–4 haplotypes were identified by the nucleotides present at sites 1164 and 1287 in the *COI* region (Nagoshi et al. 2007a, 2008). In the corn strain population, each site was empirically found to be associated with two alternative bases, producing four possible haplotypes: CS-h1 ($A_{1164}A_{1287}$), CS-h2 ($A_{1164}G_{1287}$), CS-h3 ($G_{1164}A_{1287}$), and CS-h4 ($G_{1164}G_{1287}$). The frequencies of the four haplotypes were calculated for each collection, as was the value of the CS-h4 frequency divided by the CS-h2 frequency (designated the h4/h2 ratio). Empirically, we have found that 15 corn strain specimens generated an h4/h2 frequency profile that does not substantially change with additional sampling. This threshold was met by 64 collections, of which 60 had sample numbers >20. There were two exceptions where collections with sample numbers <15 were included in Fig. 2B. In both cases (T02 in 07[4] and T15 in 08[2]) the haplotype ratio was so high that the inclusion of the additional specimens to reach the threshold minimum would not have changed the ratio category. Neither data point was included in the statistical analyses.

DNA comparisons, alignments, and restriction site mapping were performed using the Geneious Pro 5.4.6. program (Drummond et al. 2010). Statistical analyses were performed using Prism 6 for Mac OS X version 6.0D (GraphPad Software, San Diego, CA, www.graphpad.com). Pairwise comparisons were performed using two-tailed *t*-tests. Parametric analysis of variance (ANOVA) analysis with the Tukey–Kramer multiple comparisons posttest was applied when data sets met normality test criteria. Correlation tests were performed using two-tailed Pearson's correlation.

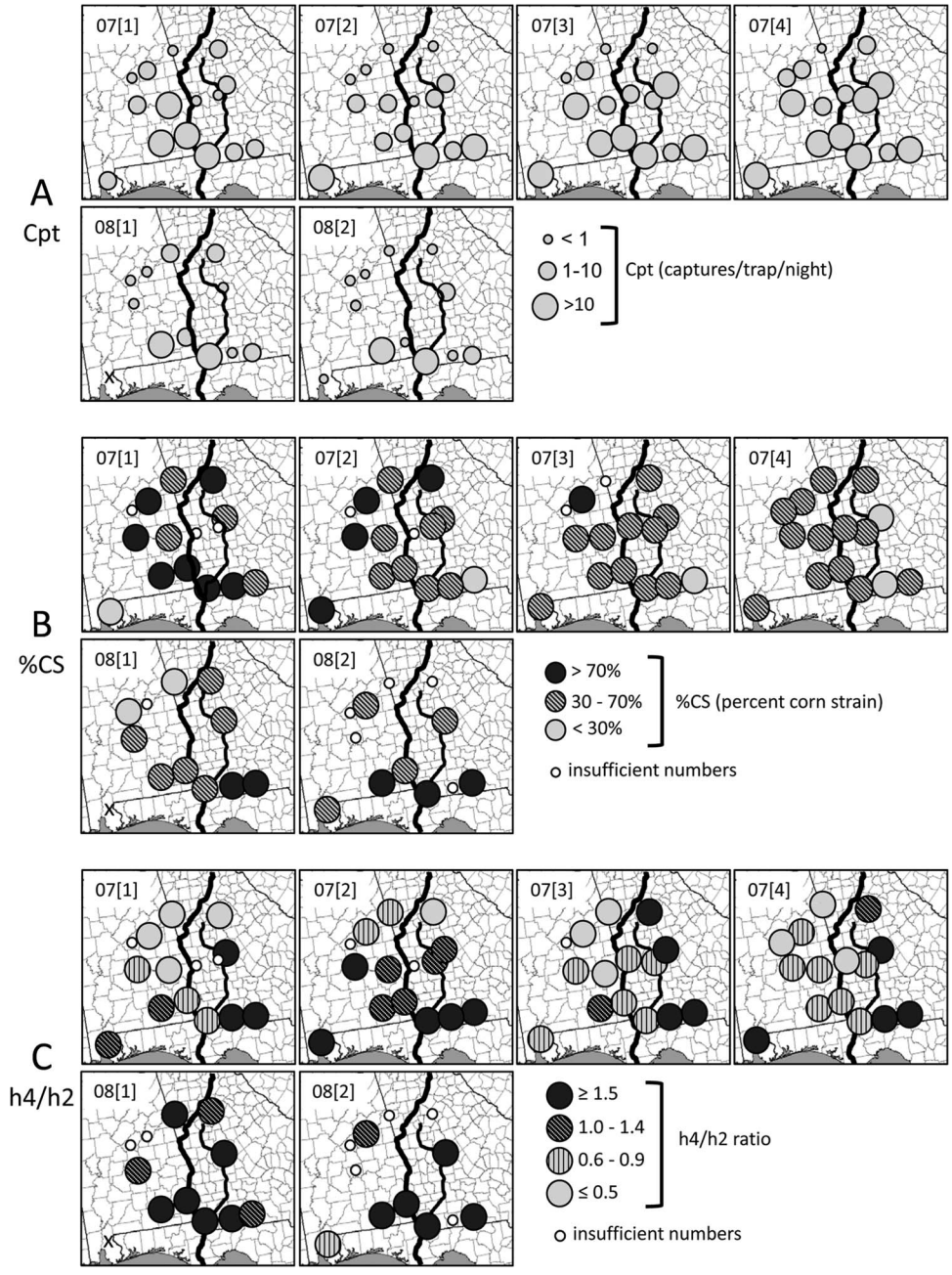

Fig. 2. Map showing the distribution of fall armyworm metrics in the AL-GA survey area at different time periods. A) Cpt (captures per trap per night). B) %CS (percent corn strain). C) h4/h2 ratios. In the period 08[1], the pheromone traps at site T17 were not functional (denoted by an X). At sites indicated as "insufficient numbers," the numbers of corn strain moths captured were too small for analysis.

Results

Analysis of Trap Capture Numbers. Capture data, recorded as Cpt, varied both regionally and over time (Fig. 2A). The most southern sites (S-group, Table 2) were distributed near the Florida border and showed a different population dynamic than the more northern (N-group) sites (Fig. 3). In 2007, mean S-group captures rose from 07[1] and peaked at 07[3], while the N-group increased from a low in 07[2] to S-group levels by 07[4]. A subset of sites used in 2007 was functional during both 2008 collection periods, five within the S-group and six from the N-group (Table 2). Cpt was consistently higher in the S-group than the N-group for all time periods (Fig. 3), with the overall

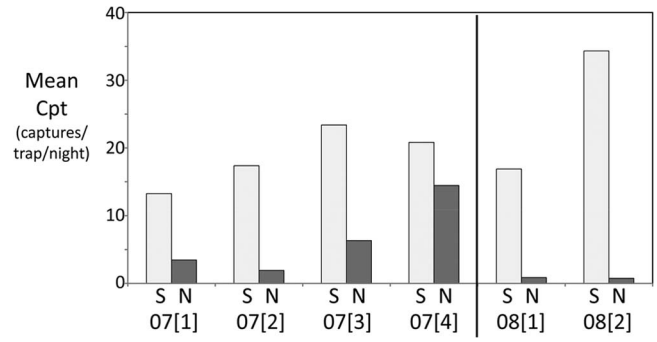

Fig. 3. Comparisons of mean Cpt (captures per night per trap) numbers in the northern (N-group) and southern (S-group) portions of the test region from 2007 and 2008. Results were limited to locations that were informative for all four sampling periods in 2007 and both periods in 2008 (Table 2).

mean S-group Cpt (mean ± SD) of 21 ± 28 significantly different from the N-group mean value of 5 ± 10 (Table 3[a]). Mean Cpt for periods 08[1–2] (12 ± 35) and the equivalent 07[1–3] time frame (11 ± 13) were not significantly different (Table 3[b]).

Changes in Strain Populations. The proportion of a given collection made up of the corn strain subpopulation was measured as %CS. Of the 83 collections from 2007 to 2008, 70 provided a sufficient number of moths to estimate %CS, with 47 (67%) having at least 50% corn strain. Mapping the %CS data showed substantial regional variation with no obvious regional biases (Fig. 2B). Temporal variation was assessed by comparing the mean %CS of each time period based on a common set of sites. Collections at 11 sites provided sufficient numbers for analysis for each of the 2007 periods (Table 2). The mean %CS declined from 07[1] to 07[4] (circles, Fig. 4), with a statistically significant difference observed by ANOVA ($F = 3.9$; $P = 0.02$; 95% CI = 0.06–0.48). In 2008, low capture rates associated with several collections resulted in more limited strain data, with five sites providing information for both 08[1] and 08[2]. No substantial change in %CS was observed (Fig. 4)

The decline in %CS from 07[1] to 07[4] coincided with a rise in overall capture rate during the same time frame (squares, Fig. 4), suggesting that the change in corn-strain frequency was primarily due to an increase in rice strain numbers. To examine this possibility, the #CS and #RS were calculated for each collection and totaled for each time period (bar graph, Fig. 4). The #RS pattern was most similar to that of the total capture rate, though both strains were sufficiently similar to exhibit a positive correlation (Table 4[a]). The significant negative correlation of %CS with #RS, but not with #CS, demonstrates that %CS in this study was primarily determined by changes in rice strain numbers (Table 4[b and c]).

Spatial Distribution of h4/h2 Ratios. Mixing of the migratory populations from Texas and Florida in the Alabama-Georgia area was confirmed in 2007 by the examination of the h4/h2 haplotype ratios (Fig. 2C). Over half of the sites (30 out of 53) had h4/h2 ratios between the Texas (≤0.5) and Florida (≥1.5) thresholds, with a mean value of 1.3 ± 1.1. In 2008, h4/h2 ratios increased substantially to a mean of 2.8 ± 2.3, with only 5 out of 16 collections showing intermediate values. Five locations (T01, T03, T06, T15, and T16) gave h4/h2 results for each of the 2007 and 2008 time periods. The mean h4/h2 ratio for these collections in 2007 was 1.6 ± 1.4, which was lower than but not quite significantly different from the mean of 3.4 ± 2.6 in 2008 (Table 3[c]). There were no significant

Table 3. Descriptions of two-tailed, unpaired parametric *t*-test (with Welch correction) comparisons made with Cpt, #CS, #RS, percentage %CS, and h4/h2 ratio data for different time periods

	Comparison 1				Comparison 2				*P* value
	Data	Traps	Time	n_1	Data	Traps	Time	n_2	
a	Cpt	S[a]	2007–2008	34	Cpt	N[b]	2007–2008	48	0.003
b	Cpt	Group[b]	07[1–3]	33	Cpt	Group[b]	08[1–2]	22	0.9
c	h4/h2	Group[c]	07[1–4]	20	h4/h2	Group[c]	08[1–2]	10	0.06
d	h4/h2	E[a]	07[1–4]	16	h4/h2	W[a]	07[1–4]	37	0.009
e	h4/h2	E[a]	08[1–2]	6	h4/h2	W[a]	08[1–2]	10	0.3
f	h4/h2	S[a]	2007–2008	35	h4/h2	N[a]	2007–2008	38	0.003
g	h4/h2	AL-NW[d]	2007–2008	19	h4/h2	Group[e]	2007–2008	45	0.001

[a] Table 2.
[b] T01–3, T06–12, and T15–16.
[c] T01, T03, T06, and T15–16.
[d] T09–13.
[e] T01–7, T15–16.

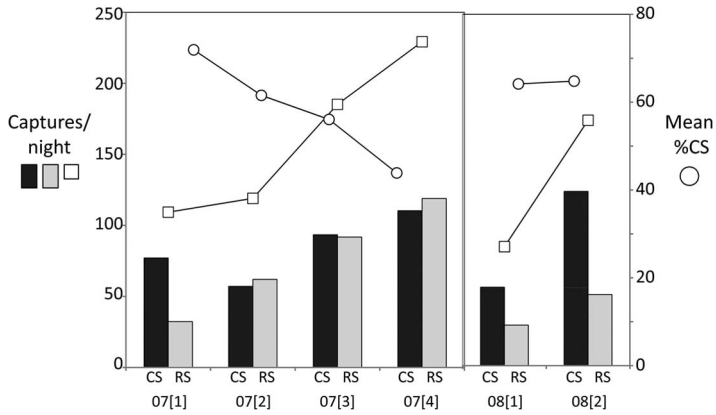

Fig. 4. Comparisons between estimated numbers of each fall armyworm strain captured and %CS. The #CS#RS captured per night was calculated for each collection and pooled for each time period (bar graph, left axis) together with total captures (square, left axis). Circles mark the mean %CS of collections from each time period (right axis). To facilitate within-year comparisons, analysis was limited to the 11 sites that provided data in each of 07[1]–07[4] and the five sites that were productive in both 08[1] and 08[2].

correlations between h4/h2 values in 2007–2008 with either Cpt or %CS (Table 4[d and e]).

Mapping of the h4/h2 ratios geographically indicated that sites to the east of the ACF river system tended to have higher h4/h2 values than more western sites (Fig. 2C). In 2007–2008, 20 of the 22 collections from the four most eastern locations (designate the E-group) had h4/h2 values >1.0, of which 16 were ≥1.5. In 2007, the mean h4/h2 for the E-group (2.1 ± 1.5) was significantly higher than that of the W-group (1.0 ± 0.6, Table 3[d]). In 2008, the mean h4/h2 values for both groups were higher than the Florida threshold of 1.5 (E-group = 2.1 ± 0.9, W-group = 3.2 ± 2.8), with the difference not statistically significant (Table 3[e]).

Discussion

Pheromone-baited traps provide an efficient method to collect fall armyworm adult males from a relatively large area with minimal supervision. However, a complication is that the adult population in a given area will be a composite of individuals recently arriving from other locations and those that developed locally. The inability to distinguish these two groups limits what can be extrapolated about the timing of migratory events from a given collection with the uncertainties likely to become increasingly significant at lower spatial and temporal scales. At question then is the resolution of the haplotype method with respect to how small an area and short a time frame can provide useable information about population movements.

Table 4. Comparative descriptions of two-tailed Pearson correlations performed for 2007 (07[1–4]) and 2008 (08[1–2]) data from 70 collections for Cpt, %CS, #CS, #RS, and 69 collections for h4/h2

	Comparison 1	Comparison 2	*n*	Pearson's *r*	*P* value
a	#CS	#RS	70	0.752	<0.0001
b	%CS	#RS	70	−0.286	0.02
c	%CS	#CS	70	0.149	0.2
d	h4/h2	Cpt	69	0.036	0.8
e	h4/h2	%CS	69	−0.101	0.4

The pattern of pheromone trap captures and haplotype ratios observed at the county level over a 2-m period in the Alabama–Georgia region were generally consistent with expectations of fall armyworm migratory behaviors. Migrant populations originating from southern Florida are expected to enter Alabama and Georgia from the south and southeast, while migrations from Texas will move into Alabama from the west and southwest (Fig. 1). This overall northward migration should result in a south-to-north gradient of trap captures with the most southern collection sites predominated by Florida populations. This is in accord with the S-group displaying consistently higher capture numbers than the N-group (Fig. 3) and significantly higher mean h4/h2 values, 2.2 ± 1.9 compared with 1.1 ± 0.7 (Table 3[f]). Furthermore, the migration pattern predicts that the proportion of Texas immigrants will be highest in the northwestern portion of the test area with consequent lower h4/h2 values in central and northern Alabama (Fig. 1). This was in fact the case as traps T09–T13 were associated with a mean h4/h2 ratio of 0.9 ± 0.7 that was significantly less than the 2.0 ± 1.8 mean of the remaining more southern and eastern sites (Table 3[g]).

Haplotype Ratios Can Delineate Migratory Boundaries. The Flint River lies at the eastern edge of the ACF river basin (Fig. 1) and generally coincides with the maximum eastern extent of the Texas migration in the survey area for the six collection periods in 2007 and 2008 (Fig. 2C). Sites immediately to the east of the Flint River (T01–2 and T06) always had h4/h2 ratios (range, 1.4–7.0) at or approaching the threshold (≥1.5) used to define the Florida population (Fig. 2C). This was the case even when nearby sites to the west of the Flint River had much lower h4/h2 values. For example, T05 and T06 are <40 km distant on

opposite sides of the Flint River (Fig. 1). During periods 07[3] and 07[4], the h4/h2 values at T05 were 0.9 and 0.7 compared with 1.6 and 1.8 at T06, respectively (Fig. 2C). Similarly, T03 and T02 are separated by the Flint River and ≈100 km. The h4/h2 ratios between the two sites differed substantially in 07[1] (T03 = 0.7 vs. T02 = 1.9), in 07[3] (1.0 vs. 1.8), and in 07[4] (0.7 vs. 3.3). Similar large discrepancies between nearby sites were not observed elsewhere.

Site T07 is an interesting case that lies just past the northern end of the Flint River, at the eastern edge of where the ACF basin narrows and merges with the Atlanta metropolitan area (Fig. 1). It shows a much more variable h4/h2 ratio with a range from 0.4 to 1.5 (Fig. 2C). Even with this variation, the four sites of the E-group (T01–2 and T06–7) lying east of the Flint River had a significantly higher mean h4/h2 in 2007 than those within or west of the ACF basin (W-group, Table 3[d]). In 2008, this east-west difference disappeared, as the majority of all sites displayed high h4/h2 values.

These observations suggest that the Flint River approximates the eastward limit of the Texas migration into the southeastern United States. The ACF River system has previously been implicated in creating discontinuities in the distribution of genetically distinct subspecies, though examples have been largely limited to land animals (Soltis et al. 2006, Newman and Rissler 2011). Why this geographical feature would be associated with a fall armyworm migratory boundary is not clear because even a large river would not be expected to significantly impede long-distance moth flight, which can extend for hundreds of kilometers when influenced by favorable air transport systems (Rose et al. 1975, Westbrook and Sparks 1986, Wolf et al. 1990, Mitchell et al. 1991, Johnson 1995). It is possible that in 2007, the interface of the air transport pathways directing the long distance components of the respective Texas and Florida migrations approximated the ACF basin to create a region of overlap and potential mixing. We speculate that the Flint River could then have acted as a water barrier to impede more local dispersion behaviors, thereby maintaining partial segregation of the two populations. In comparison, the 2008 h4/h2 data suggest a westward shift in the migration boundary such that most of the test area, including the ACF basin, now appeared to be predominantly occupied by fall armyworm from Florida (Fig. 2C).

The Two Strains Show Similar Patterns of Population Changes. The preferences of the two fall armyworm host strains for different plant hosts suggest that strain-specific migration in response to host availability is possible, if not likely. The relative movements of the two strains can be estimated by observing changes in their numbers over time and calculating whether these correlate with patterns of Cpt and %CS. The positive correlation between the two strains indicates similar population dynamics (Table 4[a]), providing evidence against large differences in migration behavior.

An unexpected finding was that the %CS metric was significantly correlated with changes in rice strain numbers, but not with those of the corn strain (Table 4[b and c]). This indicates that even in areas predominated by corn strain-preferred host plants the rice strain population can be sufficiently high and variable that it becomes the primary determinant of %CS. Therefore, the measure of fall armyworm corn strain proportion in a given area is an unreliable indicator of infestation pressure on nearby corn acreage if not combined with information on population density from which the relative size of the corn strain population can be calculated.

Trap Captures in 2007 and 2008 Peaked Relatively Late in the Migration Season. Fall armyworm infestations in the Alabama–Georgia test region can be detected as early as late March (Pair et al. 1989), yet we were not able to get capture numbers sufficient for genetic analysis from a majority of the collection sites until August (data not shown). The reason for this discrepancy is not known, but suggests that the pheromone trapping method is relatively insensitive to either early season fall armyworm or low population density. It may be that a large local population must first be established before consistent pheromone trapping occurs. In any case, this inconsistency with efficient trap captures earlier in the season limits the usefulness of pheromone trapping for this type of analysis and illustrates the need for the development of improved collection methods. These could include alternative pheromone blends or the use of alternative attractants such as floral volatiles.

In conclusion, despite limitations associated with the use of pheromone trapping, most notably the inability to distinguish between migrant and local specimens and apparent inefficiencies in collecting moths at certain times of the season, our results affirmed the utility of using systematic field collections combined with the characterization of genetic haplotypes to monitor fall armyworm population dynamics at the county level. The patterns of trap capture numbers and h4/h2 ratios were generally consistent with expectations of fall armyworm migration behavior based on earlier studies, providing confidence that intraseasonal population movements could be accurately inferred in the relatively small survey area. The h4/h2 distribution map identified a migratory boundary separating the Texas and Florida populations that implicated the eastern edge of the ACF River basin as a physical barrier. Calculations of strain numbers based on genetic markers revealed similarities and differences in strain population dynamics that can be applied to study the migratory behavior of fall armyworm subpopulations. Studies are ongoing to map haplotype ratio distributions from additional years (2011 to the present) and locations in the southeastern United States for comparisons with synoptic and seasonal wind patterns to generate a more detailed description of migratory pathways and the meteorological factors that influence their direction and extent.

Acknowledgments

We thank Kathy Flanders (Auburn University), and the regional and county extension agents and growers in Alabama and Georgia for assistance in locating sample sites. We thank Jane Sharp and Jean Thomas at U.S. Department of Agriculture–Agricultural Research Service (USDA-ARS) for excellent technical support and assistance. We are grateful to Sandra Allan (USDA-ARS) and Steven Valles (USDA-ARS) for helpful comments on the manuscript. The use of trade, firm, or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the U.S. Department of Agriculture or the Agricultural Research Service of any product or service to the exclusion of others that may be suitable. This material is based upon [work] supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, under Agreement 2011-67003-30209.

References Cited

Drummond, A. J., B. Ashton, S. Buxton, M. Cheung, A. Cooper, C. Duran, M. Field, J. Heled, M. Kearse, S. Markowitz, et al. 2010. Geneious v5.5. (http://www.geneious.com).

Johnson, S. J. 1995. Insect migration in North America: synoptic-scale transport in a highly seasonal environment, pp. 31–66. *In* V. A. Drake and A. G. Gatehouse (eds.), Insect migration: Tracking resources through space and time. Cambridge University Press, Cambridge, United Kingdom.

Mitchell, E. R., J. N. McNeil, J. K. Westbrook, J. F. Silvain, B. Lalannecassou, R. B. Chalfant, S. D. Pair, V. H. Waddill, A. Sotomayorrios, and F. I. Proshold. 1991. Seasonal periodicity of fall armyworm, (Lepidoptera, Noctuidae) in the Caribbean basin and northward to Canada. J. Entomol. Sci. 26: 39–50.

Muller, R. A. 1985. The potential for the atmospheric transport of moths from the perspetive of synoptic climatology. *In* D. R. MacKenzie, C. S. Barfield, G. G. Kennedy, and R. D. Berger (eds.), The movement and dispersal of agriculturally important biotic agents. Claitor's Publisher, Baton Rouge, LA.

Nagoshi, R. N., R. L. Meagher, J. J. Adamczyk, S. K. Braman, R. L. Brandenburg, and G. Nuessly. 2006. New restriction fragment length polymorphisms in the *cytochrome oxidase I* gene facilitate host strain identification of fall armyworm (Lepidoptera : Noctuidae) populations in the southeastern United States. J. Econ. Entomol. 99: 671–677.

Nagoshi, R. N., P. Silvie, and R. L. Meagher Jr. 2007a. Comparison of haplotype frequencies differentiate fall armyworm (Lepidoptera: Noctuidae) corn-strain populations from Florida and Brazil. J. Econ. Entomol. 100: 954–961.

Nagoshi, R. N., P. Silvie, R. L. Meagher, J. Lopez, and V. Machados. 2007b. Identification and comparison of fall armyworm (Lepidoptera: Noctuidae) host strains in Brazil, Texas, and Florida. Ann. Entomol. Soc. Am. 100: 394–402.

Nagoshi, R. N., R. L. Meagher, K. Flanders, J. Gore, R. Jackson, J. Lopez, J. S. Armstrong, G. D. Buntin, C. Sansone, and B. R. Leonard. 2008. Using haplotypes to monitor the migration of fall armyworm (Lepidoptera:Noctuidae) corn-strain populations from Texas and Florida. J. Econ. Entomol. 101: 742–749.

Nagoshi, R. N., S. J. Fleischer, and R. L. Meagher. 2009. Texas is the overwintering source of fall armyworm in central Pennsylvania: Implications for migration into the northeastern United States. Environ. Entomol. 38: 1546–1554.

Nagoshi, R. N., R. L. Meagher, and D. A. Jenkins. 2010. Puerto Rico fall armyworm has only limited interactions with those from Brazil or Texas but could have substantial exchanges with Florida populations. J. Econ. Entomol. 103: 360–367.

Nagoshi, R. N., J. Brambila, and R. L. Meagher. 2011. Use of DNA barcodes to identify invasive armyworm *Spodoptera* species in Florida. J. Insect Sci. 11: 154 (431 insectscience.org/11.154).

Nagoshi, R. N., R. L. Meagher, and M. Hay-Roe. 2012. Inferring the annual migration patterns of fall armyworm (Lepidoptera: Noctuidae) in the United States from mitochondrial haplotypes. Ecol. Evol. 2: 1458–1467.

Newman, C. E., and L. J. Rissler. 2011. Phylogeographic analyses of the southern leopard frog: the impact of geography and climate on the distribution of genetic lineages vs. subspecies. Mol. Ecol. 20: 5295–5312.

Pair, S. D., J. R. Raulston, A. N. Sparks, S. R. Sims, R. K. Sprenkel, G. K. Douce, and J. E. Carpenter. 1989. Pheromone traps for monitoring fall armyworm, Spodoptera frugiperda (Lepidoptera:Noctuidae), populations. J. Entomol. Sci. 24: 34–39.

Pashley, D. P. 1986. Host-associated genetic differentiation in fall armyworm (Lepidoptera, Noctuidae) - a sibling species complex. Ann. Entomol. Soc. Am. 79: 898–904.

Pashley, D. P. 1988. Quantitative genetics, development, and physiological adaptation in host strains of fall armyworm. Evolution 42: 93–102.

Pashley, D. P., S. J. Johnson, and A. N. Sparks. 1985. Genetic population structure of migratory moths: the fall armyworm (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 78: 756–762.

Pashley, D. P., T. C. Sparks, S. S. Quisenberry, T. Jamjanya, and P. F. Dowd. 1987. Two fall armyworm strains feed on corn, rice and bermudagrass. LA Agric. Mag. 30: 8–9.

Rose, A. H., R. H. Silversides, and O. H. Lindquist. 1975. Migration flight by an aphid, Rhopalosiphum maidis (Hemiptera: Aphididae) and a noctuid, *Spodoptera frugiperda* (Lep.: Noctuidae). Can. Entomol. 107: 567–576.

Soltis, D. E., A. B. Morris, J. S. McLachlan, P. S. Manos, and P. S. Soltis. 2006. Comparative phylogeography of unglaciated eastern North America. Mol. Ecol. 15: 4261–4293.

Westbrook, J. K., and A. N. Sparks. 1986. The role of atmospheric transport in the economic fall armyworm (Lepidoptera:Noctuidae) infestations in the southeastern United States in 1977. Fla. Entomol. 69: 494–502.

Wolf, W. W., J. K. Westbrook, J. Raulston, S. D. Pair, and S. E. Hobbs. 1990. Recent airborne radar observations of migrant pests in the United States. Philos. Trans. R. Soc Lond. S. B Biol. Sci. 328: 619–630.

Received 25 March 2014; accepted 29 May 2014.