Population Structure in *Heliothis virescens* (Lepidoptera: Noctuidae): An Estimate of Gene Flow

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ABSTRACT Starch gel electrophoresis was used to evaluate the genetic population structure of the tobacco budworm, *Heliothis virescens* (F.). A total of 1,836 adult male moths were collected from 60 trap sites throughout Texas, Louisiana, Mississippi, Arkansas, and Georgia during a 4-wk period in 1989. An average of 30.6 (±0.4) moths was electrophoretically analyzed per trap. Genotypic proportions showed no significant departures from Hardy-Weinberg expectations, suggesting random mating within populations. Population differentiation, measured by the standardized gene frequency variance, F_{st}, was low ($F_{st} = 0.002 \pm 10^{-4}$) but highly significant. This indicates an average local population size, Nm, of 135 (±10 using a jackknife estimate), the highest value we can find reported. This figure suggests a combination of high mobility (m) and high population size (N). Hierarchical F statistics were estimated using three levels: (1) traps within localities, (2) localities (traps within 8 km of each other) within regions, and (3) regions (localities within 80 km of each other). There was significant heterogeneity at all distance scales; however, gene frequency variance between regions and variance between localities within regions was low compared with variance within localities. These F statistics indicate that the extent of a local panmictic population has an average diameter on the order of 8 km or less.

KEY WORDS *Heliothis virescens*, gene flow, F statistics

THE TOBACCO BUDWORM, *Heliothis virescens* (F.), is an important pest of cotton in the southeastern United States. It is a multivoltine and highly polyphagous pest and because it has evolved resistance to most commonly used insecticides, it is especially difficult to control. Many factors are involved in the evolution of resistance to insecticides, such as rates of insecticide applications against other pests (e.g., in cotton: the boll weevil, *Anthonomus grandis grandis* Boheman; cotton aphids, *Aphis gossypii* Glover), the number of generations spent on treated host plants, availability of alternate hosts, and migration to and from areas with greater or lesser levels of insecticide treatment (Campanhola & Plapp 1989, Plapp et al. 1990).

In attempting to manage resistance to pyrethroids, a voluntary program is in place in much of the southeastern United States to limit use of pyrethroids in the early season (Graves et al. 1989). In some regions of the cotton belt, this program has achieved low compliance, in part because growers perceive the program to entail some risk at the local level to benefit cotton farming as a whole. In contrast, many results indicate that population pressure and insecticide resistance in *H. virescens* is a local problem that may have a distance scale on the order of a few fields or farms rather than the entire cotton belt. Essentially this is an argument about the population structure of *H. virescens*. Insecticide resistance management is an "area-wide" insect management program, and it is important to understand the spatial structure of populations to carry out area-wide management plans (Schneider 1989).

Heliothine moths are known to be highly mobile (Haile et al. 1975, Raulston et al. 1982, Sparks et al. 1986, Farrow & Daly 1987, Daly 1989). In spite of this knowledge, it has been difficult to gain accurate information on move-

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ment of particular species. Some data indicate that per generation movement in *H. virescens* is on the order of 10 km (Schneider et al. 1989), but other workers believe that movement is often much more extensive (>100 km) (Raulston et al. 1982, Sparks et al. 1986). Schneider et al. (1989) based their estimates of movement on a massive mark-recapture study over a limited area, and it is not clear whether this work can be generalized to *H. virescens* across the cotton belt.

Examining evidence for gene flow, the genetic consequences of immigration and successful reproduction of immigrant individuals is an alternative means of understanding movement. Recent theoretical advances make such an approach possible based on geographic analyses of polymorphic loci (Slatkin 1987, Slatkin & Barton 1989). Resistance genes can be used as markers; for example, resistance problems in *H. virescens* in Texas cotton, together with data on wind patterns, suggested that resistance originated in other parts of the state where insecticide pressure was high (Westbrook et al. 1990). Although many electrophoretic analyses of population structure have been published, not many attempts have been made to estimate spatial scales of gene flow. Here we apply these techniques to estimation of population structure in *H. virescens*. Because so much is already known about its biology, *H. virescens* is an extremely suitable organism for testing novel methods for estimating gene flow. In addition, better knowledge of movement and gene flow in this species could lead to better means of managing populations and insecticide resistance.

Materials and Methods

A total of 1,836 adult male moths were collected from 60 pheromone traps located throughout Texas, Louisiana, Mississippi, Arkansas, and Georgia (Table 1; Fig. 1). Texas moths were collected from 12 June through 29 June 1989. All other moths were collected from 23 June through 12 July 1989. These nearly simultaneous collections are necessary to obtain estimates of gene frequency variance within a unit of time of a single generation. Moths were shipped frozen or transported live to Mississippi State University and stored at −70°C. Voucher specimens will be deposited in the Mississippi Entomological Museum, Department of Entomology, Mississippi State University, Mississippi State, MS.

Starch gel electrophoresis (Harris & Hopkinson 1976, Mallet et al. 1993) was used to identify individual genotypes at 13 enzyme loci (acid phosphatase [*Acph*], aconitase [*Acon*], adenylate kinase [*Ak*], alcohol dehydrogenase [*Adh*], glucose phosphate isomerase [*Gpi*], glutamate-oxaloactetate transaminase [*Got-1*], hydroxyacid dehydrogenase [*Had*], isocitrate dehydrogenase [*Idh-1, Idh-2*], mannose phosphate isomerase [*Mpi*], phosphoglucomutase [*Pgm*], 6-phosphogluconate dehydrogenase [*6Pgd*], 3-phosphoglycerate dehydrogenase [*3Pgd*]). An average of 30.6 (±0.4) moths was electrophoretically analyzed per trap.

Table 1. Sampling areas for collections of *H. virescens* (June–July 1989)

Region	Locality, County, State	No. of trap sites
1	Tift, GA-1	1
	Tift, GA-2	2
	Tift, GA-3	1
2	Monroe, MS	6
	Oktibbeha, MS	3
3	Union, MS	2
4	Leflore, MS-1	5
	Leflore, MS-2	2
	Leflore, MS-3	1
	Leflore, MS-4	2
	Leflore, MS-5	1
5	Drew & Desha, AR	2
	Desha, AR	1
	Chicot, AR	1
	Washington, MS-1	1
	Washington, MS-2	2
	Washington, MS-3	5
	Washington, MS-4	1
	Washington, MS-5	1
	Washington, MS-6	1
6	Richland Parish, LA	1
	Tensas Parish, LA-1	1
	Tensas Parish, LA-2	1
	Franklin Parish, LA-1	1
	Franklin Parish, LA-2	1
	Ouachita Parish, LA	1
	Madison Parish, LA	1
7	Bossier Parish, LA	4
8	Natchitoches Parish, LA-1	1
	Natchitoches Parish, LA-2	1
9	E. Baton Rouge Parish, LA	1
10	Burleson, TX-1	1
	Burleson, TX-2	1
11	Hildalgo, TX-1	1
	Hildalgo, TX-2	1
	Hildalgo, TX-3	1

NOTE: Localities contain trap sites within 8 km of each other. Regions are groups of localities within 80 km of each other.

Mobilities of all variants were scored relative to the most common allele, and homogenates of individuals from earlier runs were used as standards in subsequent runs. Possible variants with very subtle mobility differences were pooled together (see Mallet et al. 1993). The data were stored as individual genotypes and were analyzed using tests for Hardy-Weinberg deviations, contingency tests, and F statistics using BIOSYS-1 (Swofford & Selander 1981).

Allele frequencies were calculated for each trap site. Genotypic proportions were tested for conformity to Hardy-Weinberg expectations within each site by χ^2 tests. Population differentiation was determined using Wright's F statistics (Wright 1978) and tested for significance using contingency χ^2 tests. F_{st} (Wright 1978), the standardized variance of allele frequencies across populations, measures the extent of population differentiation ($F_{st} = \sigma_p^2/p(1 - p)$; σ_p^2 =

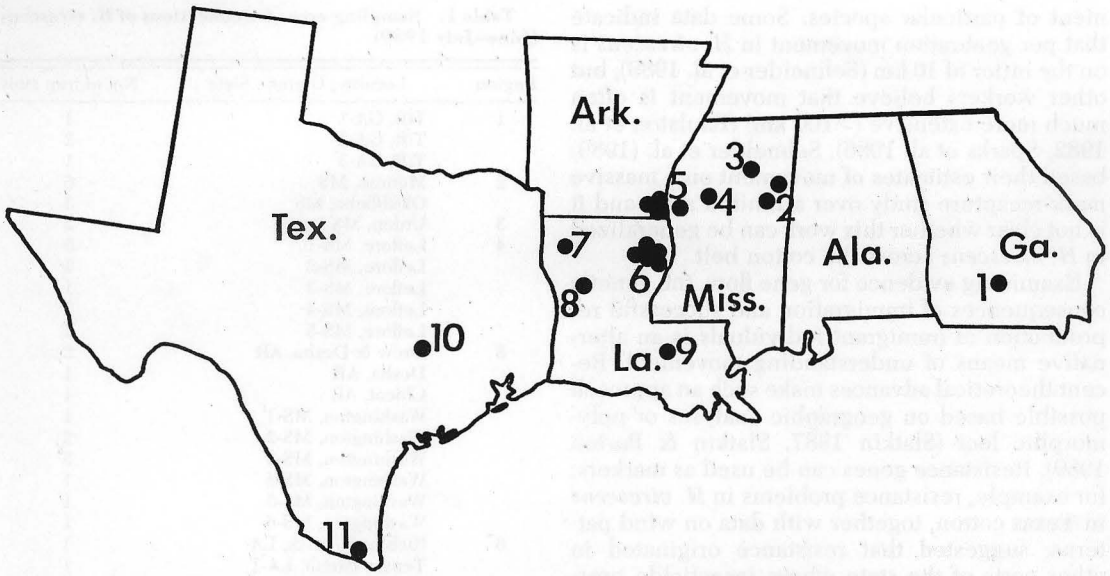

Fig. 1. Distribution of sampling sites for *H. virescens* collected from pheromone traps in 1989. Sampling regions are numbered 1–11; counties are represented by filled circles. The trapping hierarchy is listed in Table 1.

variance in allele frequency among populations; p = average allele frequency). Because the gene frequency variance in samples is a composite of the variance due to small sample size and the actual variance of gene frequency, we used the WRIGHT78 step in the BIOSYS-1 program, which includes a correction for sampling error. Standard error of F_{st} was obtained from jackknife estimates across loci.

The data were analyzed based on a hierarchy of trap locations. Traps within 8 km of each other were pooled into 36 localities. This distance was chosen because there is some evidence that suggests that most movement in *H. virescens* is limited to several kilometers. Schneider et al. (1989) found that marked moths tend to concentrate within 7.5 km of the release site. Localities within 80 km of each other were pooled into 11 nonoverlapping regions (Table 1; Fig. 1). *F* statistic analyses were performed based on this hierarchical arrangement of traps, localities, and regions. Homogeneity of gene frequencies across loci was determined by contingency χ^2 tests.

We used Wright's (1943) island model to estimate the effect of gene flow, represented as the local panmictic population size, Nm; N = local population size, m = average rate of immigration. For this method, it is assumed that alleles are neutral and that migrants disperse randomly to any other population. However, it has been shown that estimates of Nm are robust to small deviations from complete neutrality and from the island model (Slatkin & Barton 1989). Population subdivision, measured by F_{st}, can be used to estimate Nm as follows:

$$Nm \approx [(1/F_{st}) - 1]/4$$

(Wright 1943, Slatkin & Barton 1989).

Results

Overall allele frequencies are given in Mallet et al. (1993). Because gene frequency variance between populations was extremely low compared with sampling error, we do not present gene frequencies from all 60 populations. In χ^2 tests for deviations from Hardy-Weinberg expectations, few significant departures from expected genotypic proportions were observed [56 of 665 (8.4%) locus × population χ^2 tests]. However, when χ^2 values were summed over populations, there was no overall significant deviation from expected values (the summed χ^2 = 2,703.8; df = 3,022; $P > 0.05$) (Table 2). One locus, *Had*, showed a highly significant deviation from expected genotypic proportions across populations. These deviations were the result of apparent homozygotes for very rare alleles possibly because of undetected null alleles. χ^2 tests at this locus were significant in 8 of 57 (14%) populations in which this locus was variable.

Population structure was evaluated by means of Wright's *F* statistics (Wright 1978). Treating each trap site as a separate population gave an overall $F_{st} = 0.002 \pm 10^{-4}$ (Table 3), an order of magnitude lower than previously reported for this species and the lowest value reported in insects to date (Pashley et al. 1985, McCauley & Eanes 1987, Daly 1989). F_{st} is the observed variance expressed as a proportion of the maximum possible, and F_{st} can therefore vary between 0

Table 2. Tests for Hardy-Weinberg equilibrium in *H. virescens* populations; χ^2 values were summed across populations to give overall tests at each locus

Locus	χ^2	df
Acon	326.6	346
Acph	275.6	262
Adh	545.9	603
Ak	13.4	43
Got-1	0.1	14
Gpi	214.5	264
Had	334.7	213*
Idh-1	0.8	48
Idh-2	66.5	94
Mpi	446.2	562
3pgd	121.0	149
6pgd	135.6	145
Pgm	222.9	279
Total	2,703.8	3,022

* $P << 0.0005$.

(complete panmixia) and 1 (fixation for different alleles among populations). Thus, our measurement indicates that the gene frequency variance was only 0.2% of the possible maximum. In spite of the small size of overall gene frequency variance, contingency table analyses indicate that this differentiation is highly significant overall (χ^2 = 4,518; df = 3,953; $P << 0.0001$; Table 3). Gene frequency variance was significantly different from zero at 6 of 13 loci: *Acon, Acph, Adh, Gpi, Had,* and *Idh-2* (Table 3).

We found that sample size for loci within populations can easily distort the results of the F_{st} calculations when using BIOSYS-1. Earlier analysis of these data resulted in an overall F_{st} = 0.006. The larger F_{st} was chiefly due to the effect of the *6Pgd* locus, which had a considerably higher F_{st} (0.024) than any other locus (Table 3). Selection at the *6Pgd* locus could have been responsible for the inflated F_{st}, but the higher F_{st} was actually found to be the result of including two populations for which there was only one record at the *6Pgd* locus. A single homozygote in each population therefore implied fixation for those two populations, thus driving estimates of F_{st} away from 0. Deleting those two populations from the input data had the same effect on overall F_{st} as deleting the *6Pgd* locus from the data; F_{st} dropped from 0.006 to 0.002 in both cases. In the interest of preserving information at all other loci for these populations, the single homozygotes were replaced with heterozygotes for the two most common alleles. The mean frequencies (across populations) of the two most common alleles at *6Pgd* are 0.451 and 0.535 (Mallet et al. 1993). Replacement of a single homozygous individual with one heterozygote to be representative of the entire population at that locus gives frequencies of 0.5 and 0.5 for the most common alleles. These frequencies for the two populations are similar to averages across all populations. Jackknife estimates across loci after this adjustment showed that no single locus was outstanding in estimating overall variance. In 12 of 13 jackknife estimates, F_{st} = 0.002. Elimination of the *Acph* locus reduced F_{st} to 0.001, but contingency tests indicated that F_{st} remained significant (contingency χ^2 total = 4,218; df = 3,717; $P \leq 0.0001$). This observation agrees with the expectation that, in the absence of selection, each locus should provide a similar independent measurement of variance among populations.

We tested the sensitivity of our estimates to misscoring of similar alleles by performing an analysis similar to that of Eanes & Koehn (1978). Alleles were lumped into three classes: faster than the common allele, the common allele, slower than the common allele. The contingency test for these data was not significant, although close to significance (χ^2 = 1,547; df = 1,475; P = 0.09), reflecting the reduced power of the test. Overall F_{st} = 0.001.

Local population size, Nm, is 125 given F_{st} = 0.002. From jackknife estimates of F_{st} performed to remove bias, mean Nm = 135 (±10). Nm is simply a re-expression of the F_{st} result, implying that whatever the local population size, about

Table 3. Locus by locus analysis of F_{st}

Locus	No. of alleles	F_{st}	Contingency χ^2	df	P $F_{st} = 0$
Acon	7	0.002	449	354	0.0005
Acph	5	0.005	299	236	0.0035
Adh	8	0.004	680	413	<<0.0001
Ak	4	0.000	163	177	0.7621
Got-1	5	0.001	230	236	0.5932
Gpi	7	0.001	405	354	0.0319
Had	8	0.002	473	413	0.0226
Idh-1	5	0.003	264	236	0.1045
Idh-2	5	0.002	281	236	0.0239
Mpi	7	0.000	330	354	0.8180
3Pgd	7	0.000	333	354	0.7807
6Pgd	7	0.000	397	354	0.0560
Pgm	5	0.000	214	236	0.8491
Overall		0.002 (±10-4)[a]	4,518	3,953	<<0.0001

[a] Standard error obtained from jackknife estimates across loci.

Table 4. Hierarchical analysis of *F* statistics among populations of *H. virescens*

Hierarchical level X within Y	F_{XY}	Contingency χ^2	df	P $F_{XY} = 0$
Traps within localities	0.002	1,064	959	<0.01
Localities within regions	0.001	1,559	1,413	<0.01
Between regions	−0.001[a]	1,895	1,581	<0.0001
Overall between traps	0.002	4,518	3,953	<0.0001

[a] Variances must be positive, and therefore variance components should actually be positive. In this case there is strong evidence for significantly positive variance between regions in a contingency test, and yet estimation of variance components gives a negative estimate. This is a common problem with the estimation of variance components where subtraction is used, as in BIOSYS-1, to estimate some components (Swofford & Selander 1981).

135 individuals will be exchanged with other populations. This is considered to be a rather large number (Slatkin 1987). If $Nm >> 1.0$, as here, gene flow will homogenize gene frequencies across populations to a substantial degree.

The variance in gene frequency (F_{st}) was split into hierarchical components as a function of different distance scales, and contingency table analysis was performed to determine the significance of each level in the hierarchy. All components (within localities, between localities within regions, between regions) had F_{XY} significantly different from 0 (Table 4). The increased significance of tests for $F_{XY} = 0$ at successively higher levels of the hierarchy are probably due to greater sample sizes, which increased the power of the tests. The magnitudes of F_{XY} at various spatial scales are more interesting for understanding population structure. A problem arises in estimating variance components in that some of the estimated components may be less than zero when the magnitude of a variance component is less than the value expected from sampling error. Subtraction of sampling variance will then give a negative component. This problem surfaces in our results for the between-regions level (estimated $F_{st} = -0.001$), in spite of the fact that contingency table analysis shows the variance to be significantly positive (Table 4). Ignoring this problem of estimation, we find that the lowest level of the hierarchy—traps within localities—contributes most to overall F_{st}. That is, fluctuations in gene frequency are mostly picked up at this spatial scale. This seems to indicate that placement of traps within areas <8 km in diameter is extremely critical for evaluating spatial scales of variance. Regions and localities ($F_{XY} = -0.001$ and 0.001, respectively) have low levels of differentiation because average gene frequencies about which local populations fluctuate are similar everywhere. These results imply that Wright's (1951) "neighborhood area" (the area of a local panmictic population in a continuum) for *H. virescens* is less than about 8 km in diameter.

Discussion

Although our results concur with the general conclusion of Sluss & Graham (1979) that there is little genetic differentiation among populations, these results do indicate that overall geographic differentiation may be much less (although highly significant) than earlier studies suggest. F_{st} and Nm calculated from published frequencies (Sluss & Graham 1979) were determined to be 0.048 and 5, respectively (Pashley et al. 1985, Daly 1989). Sluss & Graham (1979) calculated genetic distances and performed a cluster analysis but found limited geographic structure among 13 populations. We also found that UPGMA (unweighted pair-group method with arithmetic averaging cluster analysis) phenograms produced from genetic distance calculations of these 60 populations were geographically unstructured (J. M. & A.K.K., unpublished data). Discrepancies between our F_{st} values and those estimated from Sluss & Graham (1979) may be due to Sluss & Graham misscoring of alleles with very slight (1%) differences in mobility (see Mallet et al. 1993). In *Heliothis/Helicoverpa*, whole-body homogenates of adult moths result in inadequate resolution at some enzyme loci; therefore, abdomina were routinely ground separately from the rest of the body (Mallet et al. 1993). Sluss & Graham (1979) used larval homogenates for electrophoresis and there is the possibility that larval homogenates may produce different electrophoretic results than ground tissue obtained from adults.

Population structure may vary seasonally (e.g., the monarch butterfly, *Danaus plexippus* [L.] [Eanes & Koehn 1978]; the house fly, *Musca domestica* L. [Black & Krafsur 1986]) and spatially (e.g., the forest day mosquito, *Aedes albopictus* [Skuse] [Black et al. 1988a,b]; the red milkweed beetle, *Tetraopes tetrophthalmus* [Forster] [McCauley & Eanes 1987]). The data presented here were obtained from samples collected over 12 yr since the earlier study and also were representative of different locations. Therefore, we must also consider the possibility that differences between this study and that of Sluss & Graham (1979) may be due to changing population structure.

Gene flow usually constrains the forces (e.g., genetic drift, selection) that would otherwise make populations more dissimilar. An Nm value of much more than one is sufficient to prevent much heterogeneity among populations because of genetic drift alone. On the other hand, selection for a novel trait like insecticide resistance can either be enhanced or retarded by gene flow. New adaptive genes may be introduced into populations through gene flow; however, the influx

of genes may hinder the adaptation of populations to local selection pressures (Slatkin 1987). Evolution of insecticide resistance in *Helicoverpa armigera* (Hübner) and sustained insecticide susceptibility in *Helicoverpa punctigera* (Wallengren) are possible illustrations of this principle. High gene flow is common in both species (Daly 1989), but *H. armigera,* which has evolved resistance to insecticides, seems to be restricted to cropping systems while the more mobile *H. punctigera* maintains large populations on unsprayed alternative host plants (Daly & Gregg 1985). Of course, *H. punctigera* may continue to be susceptible to insecticides because it has no genetic variation for resistance rather than because of the swamping effect of gene flow. A parallel situation may exist in the two heliothine pests in the southern United States. *Helicoverpa zea* (Boddie) and *H. virescens* are both found in cotton fields, but *H. zea* probably utilizes more alternative host plants, which are not subjected to insecticide applications; *H. virescens* is apparently more restricted to treated cropping areas. The rapid evolution of insecticide resistance in *H. virescens* has promoted its primary pest status, but *H. zea* can still be controlled by conventional insecticide applications.

Estimates of gene flow can be obtained in several ways from allele frequency data. Slatkin & Barton (1989) compared methods of estimating gene flow (F_{st}, rare alleles, and maximum likelihood) and determined that F_{st} is the most useful under many circumstances. Here we estimated gene flow by calculating Nm, the number of individuals that immigrate (population size × migration rate), after first determining the standardized variance of allele frequencies, F_{st}. This calculation assumes an island model of population structure, where discrete populations of size N receive migrants at rate m from a mainland with infinite population size. However, details of population structure are relatively unimportant in estimating Nm; for populations in a continuum, the equivalent of Nm is neighborhood size, N_b (Wright 1943, 1951; Slatkin & Barton 1989).

Although gene flow tends to be higher in the more mobile species, the relationship between mobility and gene flow is not necessarily a direct one (see Daly 1989). Dispersion at any spatial scale will not contribute to gene flow without subsequent reproduction. As noted above, population structure may not be constant within species. Genetic differentiation among populations reflects the organisms' life history and ecology. For example, even though the monarch butterfly is a migratory species, significant F_{st} has been detected in sedentary summer populations; however, migration in the fall reduces differentiation to a low level (Eanes & Koehn 1978). Habitat (Liebherr 1988) and host plant distribution (McCauley & Eanes 1987) also contribute to differentiation among populations.

Insect movement is often difficult to evaluate directly. Some evidence suggests that *H. virescens* is capable of movement over distances >10 km (Haile et al. 1975, Raulston et al. 1982, Sparks et al. 1986, Schneider et al. 1989). With regard to evaluation of management tactics for insect pests, Schneider et al. (1989) suggested that measuring the "typical distance traveled" will be more beneficial than studying the extreme limits of mobility. Measurement of dispersal of *H. virescens* in Mississippi using mark-recapture methods indicated that movements of <10 km are most common (Schneider et al. 1989). This agrees with our results showing that variance among populations at a range of ≈8 km from each other (traps within localities) contributes more to population differentiation than variance found among localities within regions (>8 km, <80 km) or variance among regions (>80 km). The low but highly significant F_{st} does demonstrate that *H. virescens* is not a single panmictic population. Further studies on gene flow of *H. virescens* at different times of the year are now in progress.

Population structure may have a great impact on control programs. Our preliminary results imply that local groups of growers could work together to manage *H. virescens* and its insecticide resistance, and can ignore insecticide use in remote locations (>100 km away). Most population and insecticide-resistance problems arise locally. Extremely effective genes for insecticide resistance may spread to remote locations fairly rapidly at low frequency by long-distance dispersal, but management of the problem caused by such genes will involve local collective action within areas that appear to be less than about 8 km in diameter.

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References Cited

Black, W. C. & E. S. Krafsur. 1986. Seasonal breeding structure in house fly, *Musca domestica* L., populations. Heredity 56: 289–298.

Black, W. C., J. A. Ferrari, K. S. Rai & D. Sprenger. 1988a. Breeding structure of a colonizing species: *Aedes albopictus* (Skuse) in the United States. Heredity 60: 173–181.

Black, W. C., W. A. Hawley, K. S. Rai & G. B. Craig, Jr. 1988b. Breeding structure of a colonizing species:

Aedes albopictus (Skuse) in peninsular Malaysia and Borneo. Heredity 61: 439–446.

Campanhola, C. & F. W. Plapp, Jr. 1989. Managing pyrethroid resistance in the tobacco budworm: bioassays, resistance mechanisms and biological constraints, pp. 352–359. *In* Proceedings, Beltwide Cotton Production and Research Conference, Nashville, TN.

Daly, J. C. 1989. The use of electrophoretic data in a study of gene flow in the pest species *Heliothis armigera* (Hübner) and *H. punctigera* Wallengren (Lepidoptera: Noctuidae), pp. 115–141. *In* H. D. Loxdale & J. den Hollander [eds.], Electrophoretic studies on agricultural pests. Syst. Assoc. Spec. Vol. 39.

Daly, J. C. & P. Gregg. 1985. Genetic variation in *Heliothis* in Australia: species identification and gene flow in two pest species *H. armigera* (Hübner) and *H. punctigera* Wallengren (Lepidoptera: Noctuidae). Bull. Entomol. Res. 75: 169–184.

Eanes, W. F. & R. K. Koehn. 1978. An analysis of genetic structure in the monarch butterfly, *Danaus plexippus* L. Evolution 32: 784–797.

Farrow, R. A. & J. C. Daly. 1987. Long-range movements as an adaptive strategy in the genus *Heliothis* (Lepidoptera: Noctuidae): a review of its occurrence and detection in four pest species. Aust. J. Zool. 35: 1–24.

Graves, J. B., B. R. Leonard, A. M. Pavloff, T. C. Sparks, S. Micinski, E. Burris & K. J. Ratchford. 1989. Monitoring pyrethroid resistance in tobacco budworms. La. Agric. 32: 4–5, 20.

Haile, D. G., J. W. Snow & J. R. Young. 1975. Movement by adult *Heliothis* released on St. Croix to other islands. Environ. Entomol. 4: 225–226.

Harris, H. & D. A. Hopkinson. 1976. Handbook of enzyme electrophoresis in human genetics. North Holland, Amsterdam.

Liebherr, J. K. 1988. Gene flow in ground beetles (Coleoptera: Carabidae) of differing habitat preference and flight-wing development. Evolution 42: 129–137.

Mallet, J., A. Korman, D. G. Heckel & P. King. 1993. Biochemical genetics of *Heliothis* and *Helicoverpa* (Lepidoptera: Noctuidae) and evidence for a founder effect in *Helicoverpa zea*. Ann. Entomol. Soc. Am. 86: 189–197.

McCauley, D. E. & W. F. Eanes. 1987. Hierarchical population structure analysis of the milkweed beetle, *Tetraopes tetraophthalmus* (Forster). Heredity 58: 193–201.

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

Plapp, F. W., Jr., J. A. Jackman, C. Campanhola, R. E. Frisbie, J. B. Graves, R. G. Luttrell, W. F. Kitten & M. Wall. 1990. Monitoring and management of pyrethroid resistance in the tobacco budworm (Lepidoptera: Noctuidae) in Texas, Mississippi, Louisiana, Arkansas, and Oklahoma. J. Econ. Entomol. 83: 335–341.

Raulston, J. R., W. W. Wolf, P. D. Lingren & A. N. Sparks. 1982. Migration as a factor in *Heliothis* management, pp. 61–73. *In* Proceedings, International Workshop on *Heliothis* Management, 15–20 November 1981. Patancheru, India.

Schneider, J. C. 1989. Role of movement in evaluation of area-wide insect pest management tactics. Environ. Entomol. 18: 868–874.

Schneider, J. C., R. T. Roush, W. F. Kitten & M. L. Laster. 1989. Movement of *Heliothis virescens* (Lepidoptera: Noctuidae) in Mississippi in the spring: implications for area-wide management. Environ. Entomol. 18: 438–446.

Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. Science (Washington, DC) 236: 787–792.

Slatkin, M. & N. H. Barton. 1989. A comparison of three indirect methods for estimating average levels of gene flow. Evolution 43: 1349–1368.

Sluss, T. P. & H. M. Graham. 1979. Allozyme variation in natural populations of *Heliothis virescens*. Ann. Entomol. Soc. Am. 72: 317–322.

Sparks, A. N., R. D. Jackson, J. E. Carpenter & R. A. Muller. 1986. Insects captured in light traps in the Gulf of Mexico. Ann. Entomol. Soc. Am. 79: 132–139.

Swofford, D. L. & R. B. Selander. 1981. BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J. Hered. 72: 281–283.

Westbrook, J. K., C. T. Allen, F. W. Plapp, Jr. & W. Multer. 1990. Atmospheric transport and pyrethroid-resistant tobacco budworm, *Heliothis virescens* (Lepidoptera: Noctuidae), in western Texas in 1985. J. Agric. Entomol. 7: 91–101.

Wright, S. 1943. Isolation by distance. Genetics 28: 114–138.

1951. The genetical structure of populations. Ann. Eugen. 15: 323–354.

1978. Evolution and the genetics of populations, vol. 4. Variability within and among natural populations. University of Chicago Press, Chicago.

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