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Genetic shifts in *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) over a year in the Dawson/Callide Valleys

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Abstract. Microsatellites were used to analyse 68 collections of *Helicoverpa armigera* in the Dawson/Callide Valleys in central Queensland. The study aimed to evaluate the genetic structure in this region over a 12-month period (September 2000–August 2001). The results detected genetic shifts in *H. armigera* collections, with genetic changes occurring month by month. Collections in any month were genetically distant from the preceding month's collections. There was no observed difference between collections of *H. armigera* from the Biloela region and those found in the Theodore region of central Queensland. The data support the current area-wide management strategies for *H. armigera* by reinforcing the importance and contribution of local management practices. The study also indicates a need for the continuation of regional or Australia-wide approaches to management of the low levels of immigration that are occurring, and for future high pest pressure years.

Additional keywords: molecular markers, migration, molecular biology, resistance management.

Introduction

The cotton bollworm *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) is a widely distributed pest (in regions including Africa, the Middle East, India, Australia, and Asia), which often develops resistance to agricultural insecticides (Zhou *et al.* 2000). Its ability to develop resistance, and its polyphagous nature, make *H. armigera* a significant pest on cotton, grains, and other crops. In Australia, *H. armigera* has 4–5 generations between September and April. The second generation arising between December and February often causes the most problems for cotton growing (Wardhaugh *et al.* 1980; Maelzer and Zalucki 1999).

An understanding of the genetic structure of *H. armigera* on an Australia-wide scale, or more locally, can be of significant benefit to the agricultural industry, as it enables a reduction of insecticide application and resistance build-up (Stokes *et al.* 1997). Examination of gene flow in *H. armigera* is an efficient means to establish these population movements and structure.

Gene flow can be quantified directly by measuring dispersal distance and the breeding contribution, or inferred by measuring gene frequencies (Slatkin 1987; Zhou *et al.* 2000). Previous genetic studies on Australian *H. armigera* have used isozymes (Daly and Gregg 1985), mitochondrial DNA variation (McKechnie *et al.* 1993), and sodium channel gene alleles (Stokes *et al.* 1997) to examine population structure. Additional molecular studies on *H. armigera* have also been done in the Mediterranean using random amplified polymorphic DNA (Zhou *et al.* 2000), and in Africa and Europe using isozymes (Nibouche *et al.* 1998). The pattern and quantity of genetic variation in *H. armigera* has allowed for inference on the amount and direction of migration. Using isozymes, Daly and Gregg (1985) found small genetic distances between widely separated populations in Australia, which led to the suggestion that extensive long distance migration was occurring in *H. armigera*. However, Stokes *et al.* (1997) found a difference in the allele frequencies of a sodium channel gene in *H. armigera* between the Namoi Valley (New South Wales) and the St George (Queensland) irrigation area. The Namoi and St George regions are 300 km apart with no physical barriers to prevent migration. Only the absence of suitable host crop species appears to limit movement. The Stokes *et al.* (1997) study showed a clear

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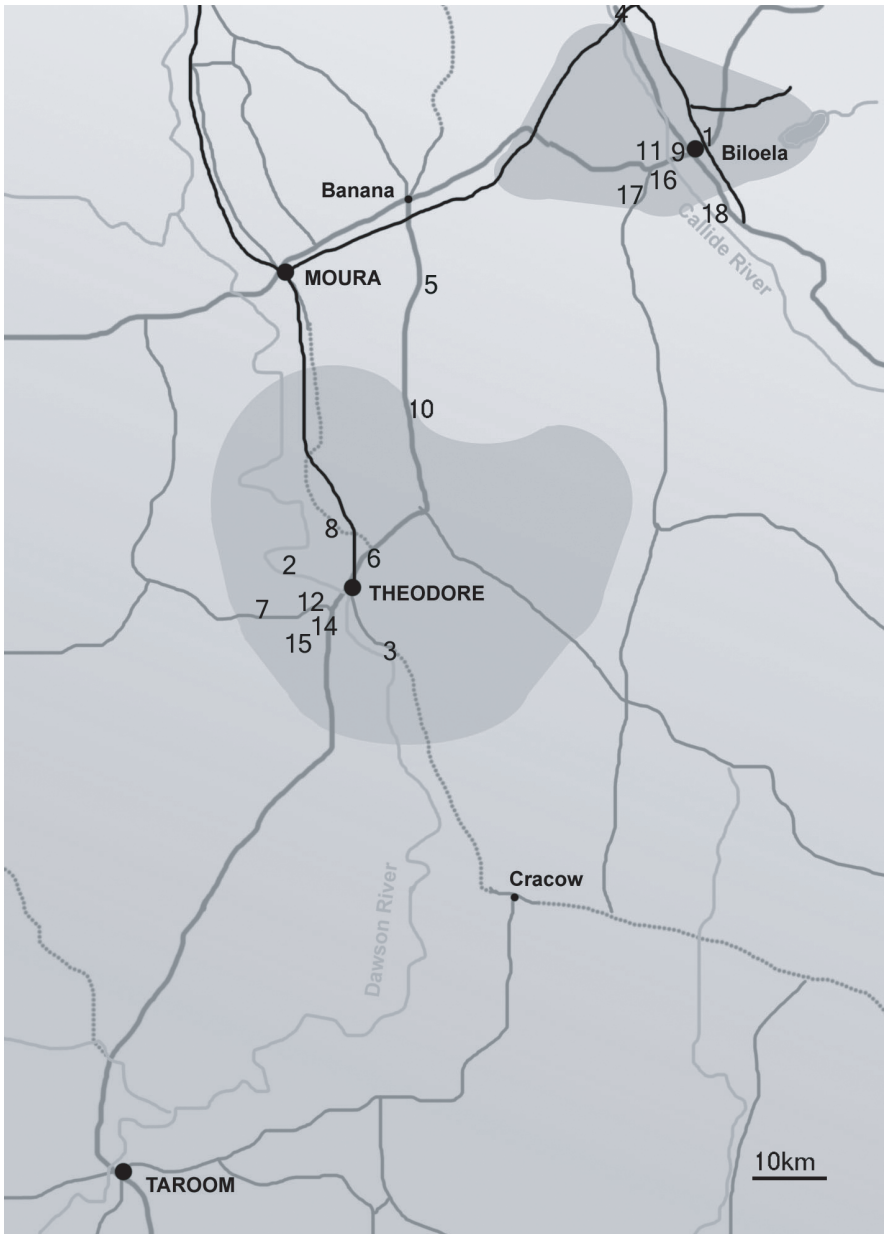

Fig. 1. Dawson/Callide Valleys map showing the sample locations. Site numbers 1–18 relate to site details as shown in Table 1. Shaded areas mark the cotton-growing regions.

restriction to free and fast gene flow between the regions, which differed from the Daly and Greg (1985) work. Our Dawson/Callide cotton-growing area over a 12-month period, to evaluate how management practices altered the genetic profile of a region and to establish population structure in this area.

Materials and methods

Sample source and DNA extraction

Heliothis armigera larvae and moths were collected from the Dawson/Callide Valleys in central Queensland (Fig. 1, Table 1). Sampling was over a 12-month period (September 2000–August 2001). Due to variation in pest pressure, the number of samples obtained each month varied over the year. No samples could be collected in April or May due to a low pest incidence. Samples were taken from both chickpea and cotton crops.

DNA for microsatellite analysis was extracted from individual moth heads or larval posterior prolegs using a 96-well modification of the Miller *et al.* (1988) protocol. The remaining insect was stored individually in an ethanol vial, and cross-referenced to the DNA extraction. A diagnostic PCR (developed by the Centre for Identification and Diagnostics) was then utilised to determine if each individual was either *H. armigera* or *H. punctigera*. The species diagnostic ensured that microsatellite analysis was performed on study was aimed at assessing the genetic structure in the

Table 1. Sample locations, map references, GPS co-ordinates, collection details, and sample numbers from the Dawson/Callide Valleys

Location	Map ref.	GPS	Date collected	Crop	No. of individuals
Biloela – Research Station	1	S24 22 11.3 E150 31 06.1	1 Sept. 00	Chickpea	15
Theodore – J. Anderson	2	S24 57 02.6 E150 05 29.7	4 Sept. 00	Cotton	13
Theodore – G. Holmes	3	S24 55 24.7 E149 58 35.7	4 Sept. 00	Chickpea	8
Jambin – H. McInnes	4	S24 13 28.3 E150 24 27.9	5 Sept. 00	Chickpea	15
Biloela – Research Station	1	S24 22 11.3 E150 31 06.1	5 Sept. 00	Chickpea	15
Banana – M. Ballentine	5	S24 36 23.8 E150 07 22.7	7 Sept. 00	Chickpea	15
Theodore – P. French	6	S24 55 18.2 E150 04 16.9	11 Sept. 00	Cotton	12
Banana – M. Ballentine	5	S24 36 23.8 E150 07 22.7	11 Sept. 00	Chickpea	15
Theodore – G. Holmes	3	S24 55 24.7 E149 58 35.7	11 Sept. 00	Chickpea	15
Theodore – T. Brownley	7	S24 55 48.8 E149 48 53.3	13 Sept. 00	Cotton	15
Jambin – H. McInnes	4	S24 13 28.3 E150 24 27.9	19 Sept. 00	Chickpea	15
Biloela – Research Station	1	S24 22 11.3 E150 31 06.1	19 Sept. 00	Chickpea	8
Banana – M. Ballentine	5	S24 36 23.8 E150 07 22.7	20 Sept. 00	Chickpea	14
Theodore – H. Anderson	8	S24 57 27.3 E150 05 04.1	20 Sept. 00	Cotton	15
Theodore – T. Brownley	7	S24 55 48.8 E149 48 53.3	27 Sept. 00	Cotton	3
Biloela – Research Station	1	S24 22 11.3 E150 31 06.1	29 Sept. 00	Chickpea	15
Theodore – J. Anderson	2	S24 57 02.6 E150 05 29.7	2 Oct. 00		15
Banana – M. Ballentine	5	S24 36 23.8 E150 07 22.7	2 Oct. 00	Cotton	15
Biloela – Research Station	1	S24 22 11.3 E150 31 06.1	3 Oct. 00	Chickpea	15
Biloela – Research Station	1	S24 22 11.3 E150 31 06.1	3 Oct. 00	Chickpea	11
Theodore – J. Anderson	2	S24 57 02.6 E150 05 29.7	16 Oct. 00	Cotton	9
Theodore – T. Brownly	7	S24 55 48.8 E149 48 53.3	23 Oct. 00	Cotton	15
Biloela – Research Station	1	S24 22 11.3 E150 31 06.1	29 Oct. 00	Trap crop	15
Biloela – T. Manwearing	9	S24 22 34.0 E150 29 32.0	16 Nov. 00	Chickpea	15
Theodore – A. Edwards	12	S24 55 24.7 E149 58 35.7	23 Nov. 00	Cotton	2
Banana – M. Ballentine	5	S24 36 23.8 E150 07 22.7	27 Nov. 00	After chickpea	15
Theodore – H. Anderson	8	S24 46 14.8 E149 58 45.5	27 Nov. 00		15
Theodore – H. Anderson	8	S24 46 14.8 E149 58 45.5	27 Nov. 00		7
Theodore – P. Durkin	10	S24 57 02.6 E150 05 29.8	4 Dec. 00	Chickpea	15
Biloela – T. Sullivan	11	S24 22 34.0 E150 28 42.6	5 Dec. 00	Cotton	15
Theodore – H. Anderson	8	S24 46 14.8 E149 58 45.5	12 Dec. 00	Cotton	7
Biloela – T. Manwearing	9	S24 22 34.0 E150 29 32.0	19 Dec. 00	Chickpea	6
Theodore – J. Anderson	2	S24 57 02.6 E150 05 29.7	20 Dec. 00	Cotton	13
Theodore – A. Edwards	12	S24 55 24.7 E149 58 35.7	5 Jan. 01	Cotton	15
Biloela – T. Sullivan	11	S24 22 34.0 E150 28 42.6	5 Jan. 01	Cotton	15
Theodore – T. Brownly	7	S24 57 52.0 E150 04 40.4	12 Jan. 01	Cotton	11
Theodore – T. Brownly	7	S24 57 52.0 E150 04 40.4	29 Jan. 01	Cotton	15
Theodore – J. Anderson	2	S24 57 02.6 E150 05 29.7	6 Feb. 01	Cotton	15
Theodore – A. Edwards	12	S24 55 24.7 E149 58 35.7	16 Feb. 01	Cotton	3
Alton Downs – P. Foxwell[A]	13		22 Feb. 01	Cotton	15
Theodore – H. Anderson	8	S24 46 14.8 E149 58 45.5	6 Mar. 01	Cotton	15
Theodore – P. French	6	S24 55 18.2 E150 04 16.9	6 Mar. 01	Cotton	15
Theodore – P. Gee	14	S24 55 38.1 E150 04 25.1	6 Mar. 01	Cotton	15
Theodore – G. Austin	15	S24 56 17.6 E149 59 56.6	6 Mar. 01	Cotton	15
Theodore – T. Brownly	7	S24 55 48.8 E149 48 53.3	6 Mar. 01	Cotton	15
Theodore – A. Edwards	12	S24 55 24.7 E149 58 35.7	6 Mar. 01	Cotton	15
Theodore – H. Anderson	8	S24 57 27.3 E150 05 04.1	6 Mar. 01	Cotton	8
Theodore – G. Austin	15	S24 56 17.6 E149 59 56.6	13 Mar. 01	Cotton	15
Theodore – P. French	6	S24 55 18.2 E150 04 16.9	13 Mar. 01	Cotton	15
Theodore – T. Brownly	7	S24 55 48.8 E149 48 53.3	13 Mar. 01	Cotton	15
Theodore – A. Edwards	12	S24 55 24.7 E149 58 35.7	13 Mar. 01	Cotton	4
Theodore – P. French	6	S24 55 18.2 E150 04 16.9	13 Mar. 01	Cotton	2
Theodore – T. Brownly	7	S24 55 48.8 E149 48 53.3	21 Mar. 01	Cotton	8
Theodore – A. Edwards	12	S24 55 24.7 E149 58 35.7	21 Mar. 01	Cotton	2
Theodore – T. Brownly	7	S24 57 52.0 E150 04 40.4	27 June 01	Chickpea	15
Biloela – P. Jenson	16	S24 22 43.2 E150 29 31.8	27 June 01	Chickpea	5
Biloela – Research Station	1	S24 22 11.3 E150 31 06.1	27 June 01	Chickpea	2
Jambin – H. McInnes	4	S24 13 28.3 E150 24 27.9	31 July 01	Chickpea	15
Biloela – P. Jenson	16	S24 22 43.2 E150 29 31.8	31 July 01	Chickpea	12
Jambin – H. McInnes	4	S24 13 28.3 E150 24 27.9	31 July 01	Chickpea	15
Biloela – I. Kennedy	17	S24 26 29.8 E150 31 31.6	31 July 01	Chickpea	6
Theodore – H. Anderson	8	S24 46 14.8 E149 58 45.5	6 Aug. 01	Chickpea	15
Theodore – P. Gee	14	S24 55 38.1 E150 04 25.1	6 Aug. 01	Chickpea	5
Theodore – H. Anderson	8	S24 46 14.8 E149 58 45.5	6 Aug. 01	Chickpea	15
Biloela – P. VanItalie	18	S24 25 42.3 E150 32 14.7	27 Aug. 01	Chickpea	14
Biloela – I. Kennedy	17	S24 26 29.8 E150 31 31.6	27 Aug. 01	Chickpea	8
Biloela – P. Jenson	16	S24 22 43.2 E150 29 31.8	27 Aug. 01	Chickpea	15
Biloela – P. VanItalie	18	S24 25 E150 32	27 Aug. 01	Chickpea	8

[A]Note that this location is outside the Dawson/Callide Valleys

H. armigera individuals only, as morphological determination of species after storage in ethanol can be problematic. *H. armigera* individuals totalling 811 were analysed from 68 collections consisting of 18 geographic locations and multiple collection dates (Table 1). Some variation occurred in the number of individuals analysed per collection due to differing proportions of *H. armigera* and *H. punctigera* present in the samples taken at each location.

Microsatellite analysis

Microsatellites were selected as the marker system in this study as they covered multiple loci, were economic for large scale studies, were transferable to other *Helicoverpa*, and were co-dominant and highly polymorphic, suiting them well to the task of measuring genetic structure in *H. armigera* in Australia. Five microsatellite loci (170 alleles) were used to analyse the 811 *H. armigera* individuals. The loci were HaB60, HaD25, HaD47, HaC87, and HaC14 (Centre for Identification and Diagnostics, unpubl. data; for primer sequences please contact the authors). The microsatellite amplification conditions were as follows: 25 ng DNA, 1.5 mM $MgCl_2$, 0.2 μM of each primer (forward primer labelled with Hex), 20 mM Tris-HCl, 100 mM KCl, 1 unit of *Taq* polymerase (Qiagen; Clifton Hill, Vic.), and 0.2 mM of dNTPs (Amrad/Biotech; Boronia, Vic.) in a 20-μL reaction volume. Cycling conditions in a Corbett Research PC960 Thermal Cycler (Corbett Research, Mortlake, NSW) were 94°C for 1 min, 50°C for 1 min, and 73°C for 1 min, for 35 cycles. Microsatellite scoring was on a 7% native acrylamide gel (as per Corbett Research GS2000 manual) in a Corbett Research GS2000 Genetic Analyser.

Statistical analysis

Microsatellite alleles were scored using ONE-Dscan (Ver. 1.33, Scanalytics Inc.; Billerica, MA). Allele sizes were entered into Excel (Microsoft Corp.; North Ryde, NSW) and analysed using GenAlEx (Peakall and Smouse 2001). Nei distance between collections was calculated as in Weir (1990), and pairwise genetic distances were calculated as in Peakall *et al.* (1995). Allele frequencies and heterozygosity calculations followed the formulae of Hartl and Clark (1997). N*m* was estimated using the private allele method of Slatkin (1985) and Slatkin and Barton (1989). Analysis of molecular variance (AMOVA) analysis was as for Excoffier *et al.* (1992), Peakall *et al.* (1995), and Michalakis and Excoffier (1996). Principal co-ordinate analysis used the algorithm published by Orloci (1978).

Table 2. A month by month estimate of migrants (N*m*) into the Dawson/Callide Valleys using the private alleles method (Slatkin 1985; Slatkin and Barton 1989)

Month	N*m*	Month	N*m*
Sept.	1.06	Jan./Feb.	0.97
Oct.	1.58	Mar.	2.09
Nov.	1.50	June/July	0.96
Dec.	0.53	Aug.	1.44

Results

There was significant differentiation between collections with an AMOVA of 14% between collections. Month by month estimates of N*m* using the private alleles approach of Slatkin (1985) and Slatkin and Barton (1989) gave N*m* values from 0.53 to 2.09 (with N = local population size, m = average rate of migration) (Table 2).

Nei's genetic distance (Table 3) showed that month by month the collections generally became more genetically distant from what they were in the preceding months. The smallest Nei distances between months occurred in September, October, November, and December, when population sizes were largest, and also between March, July, and August, when populations entered and exited winter.

Principal co-ordinate analysis (Fig. 2) of Nei's genetic distance between all 68 collections illustrates that the genotypes of *H. armigera* shifted every few months in the Dawson/Callide region.

In each geographic location sampled, the genetic structure of *H. armigera* changed month by month, with the collection in a subsequent month being genetically distant from the preceding month's collection. There was no observed difference between collections of *H. armigera* from the Biloela region and those from the Theodore region; AMOVA between regions was 0% (i.e. Theodore *v.* Biloela), within a region (between collections) was 14%, and within collections 86% ($P = 0.01$).

Discussion

Analysis of *H. armigera* using 5 microsatellite loci showed significant differentiation between sampled collections in the Dawson/Callide Valleys (AMOVA 14% variation between collections) and low migration levels (N*m* = 0.53–2.09; Table 2). This is consistent with the findings of Daly and Gregg (1985), where *H. armigera* was more restricted to cropping areas in comparison with the more mobile *H. punctigera*. However, there was no significant

Table 3. Nei genetic distance between all samples of *H. armigera* in each month in the Dawson/Callide Valleys

	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	June	July
Oct.	0.073								
Nov.	0.126	0.106							
Dec.	0.114	0.152	0.218						
Jan.	0.252	0.230	0.291	0.357					
Feb.	0.480	0.467	0.378	0.728	0.410				
Mar.	0.319	0.324	0.575	0.425	0.320	0.931			
June	0.546	0.477	0.780	0.527	0.642	1.256	0.320		
July	0.646	0.545	0.976	0.727	0.514	1.312	0.124	0.376	
Aug.	0.572	0.516	0.813	0.519	0.496	1.172	0.099	0.261	0.089

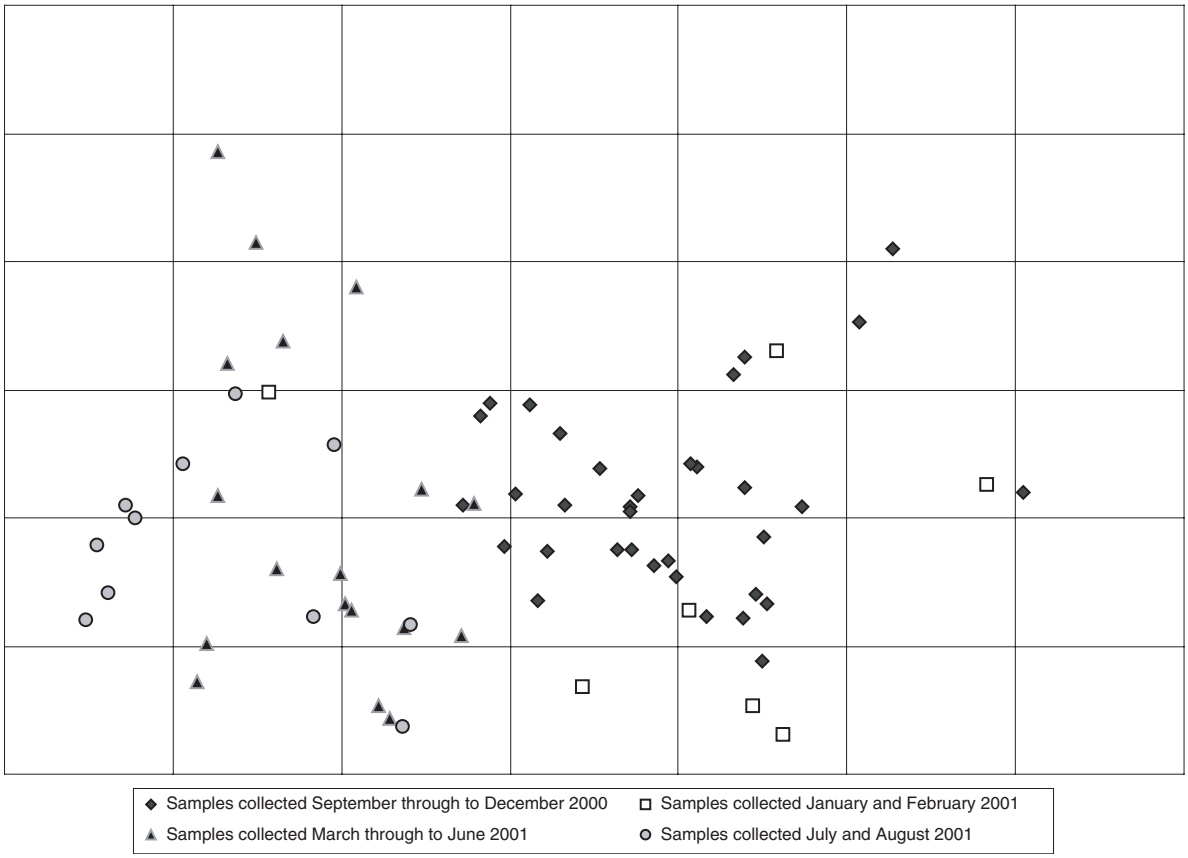

Fig. 2. Principal co-ordinate analysis of Nei's genetic distance between collections. Collections were made from September 2000 to August 2001 in the Dawson/Callide Valleys.

differentiation between the collections from the Theodore area and those collections from the Biloela region in the 2000–01 season (AMOVA 0% variation between regions).

The high level of collection differentiation seen here is indicative of low levels of gene flow (i.e. small migration rates) coupled with high selection pressure in the period of study. Low levels of migration can either increase or reduce the accumulation of resistance to insecticide (Korman *et al.* 1993). For example, low levels of migration will reduce the speed and likelihood of movement of resistant moths to new areas; however, low migration will also mean that if resistance is accumulating, there will be no dilution of this accumulation by susceptible moths immigrating from other areas (Korman *et al.* 1993).

In our 2000–01 study of *H. armigera* in the Dawson/Callide Valleys, genetic structure was shown to vary seasonally, as genotypes were shifting as the months progressed (Fig. 2). A similar phenomenon was seen in Monarch butterflies (Eanes and Koehn 1978). Eanes and Koehn (1978) explained their change by either regional drift after 'founder effect' or differentiation resulting from regionally differing selection regimes. This is also likely to be the case in this study, as *H. armigera* is not distributed uniformly over space or time, and the population size is not constant. Large fluctuations occur over the season making a 'founder-like effect' possible. Furthermore, *H. armigera* is subject to high levels of local selection due to insecticide usage, agricultural practices, and seasonal change.

The small genetic distances occurring among *H. armigera* in the months of September, October, November, and December (Table 3) were when the pest pressure and population sizes were the highest. At times when the pressure is high, the physical distance between normally geographically separated collections is likely to be reduced, so more moths will have contributed to a mixing of the gene pool (i.e. smaller genetic distance). In March, July, and August the genetic distances were also small. The smaller genetic distances at this time may be due to larvae that are going into diapause in March becoming mostly the same moths that would emerge and contribute to the first *H. armigera* generation in July and August. However, since diapause is not observed to occur to any great extent in the Dawson/Callide Valleys, an alternative is that the development of *H. armigera* is slowed by reduced temperatures and day length, to the extent that a similar effect to diapause is observed in the genetic structure. Other

possible reasons for the smaller genetic distances at this time may be reduced selection pressure with reduced insecticide usage over winter, or smaller population sizes (i.e. fewer in the effective population) reducing the genetic diversity through the contribution from relatively fewer females and males.

In conclusion, the results from this study indicate that the management of *H. armigera* should be focused at the local/regional level in the Dawson/Callide Valleys, as most *H. armigera* were of local origin. However, small amounts of migration into the region are still occurring so broad-scale management should also be maintained. Maintenance of broad-scale management is also vital, as the data presented here describe the genetic structure in the 12-month period from September 2000 to August of 2001, and the levels of migration into the area may change from year to year.

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