Scott, K. D.; Wilkinson, K. S.; Lawrence, N.; Lange, C. L.; Scott, L.; Merritt, M. A.; Lowe, Andrew John; Graham, G. C..
Gene-flow between populations of cotton bollworm Helicoverpa armigera (Lepidoptera: Noctuidae) is highly variable between years, *Bulletin of Entomological Research*, 2005; 95 (4):381-392.

Bulletin of Entomological Research (2005) **95**, 381–392 DOI: 10.1079/BER2005369

Gene-flow between populations of cotton bollworm *Helicoverpa armigera* (Lepidoptera: Noctuidae) is highly variable between years

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Abstract

Both large and small scale migrations of *Helicoverpa armigera* Hübner in Australia were investigated using AMOVA analysis and genetic assignment tests. Five microsatellite loci were screened across 3142 individuals from 16 localities in eight major cotton and grain growing regions within Australia, over a 38-month period (November 1999 to January 2003). From November 1999 to March 2001 relatively low levels of migration were characterized between growing regions. Substantially higher than average gene-flow rates and limited differentiation between cropping regions characterized the period from April 2001 to March 2002. A reduced migration rate in the year from April 2002 to March 2003 resulted in significant genetic structuring between cropping regions. This differentiation was established within two or three generations. Genetic drift alone is unlikely to drive genetic differentiation over such a small number of generations, unless it is accompanied by extreme bottlenecks and/or selection. *Helicoverpa armigera* in Australia demonstrated isolation by distance, so immigration into cropping regions is more likely to come from nearby regions than from afar. This effect was most pronounced in years with limited migration. However, there is evidence of long distance dispersal events in periods of high migration (April 2001–March 2002). The implications of highly variable migration patterns for resistance management are considered.

Keywords: migration, molecular biology, pest management, resistance management, gene-flow, *Helicoverpa armigera*, Australia

Introduction

Cotton bollworm, *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae), is a widely distributed species, occurring in Africa, the Middle East, India, Australia and Asia (Fitt, 1989), and is a significant pest of cotton, grains and horticultural crops due to its polyphagous nature, its ability to rapidly develop large pest populations and a propensity to develop resistance to agricultural insecticides (Zhou *et al*., 2000). The effective management of *Helicoverpa* in crops is complicated by variability in infestation levels between regions and between seasons (Rochester *et al*., 1996). The capacity *H. armigera* has for extensive movement also complicates the understanding of the population dynamics of this pest, because local populations may consist of elements of diverse origins at any one time (Fitt *et al*., 1995). To improve the effectiveness of managing this significant pest, it is essential that a greater understanding of the genetic structure and migratory behaviour of *H. armigera* be achieved.

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Adult *H. armigera* are known to show their highest flight abilities from 3 to 6 days old (Wu & Guo, 1996), with a peak flight ability at 4 days old (Coombs, 1997). Both male and female *H. armigera* are capable of multiple mating (Hou & Sheng, 1999), although mated females show progressively reduced flight ability with each day post-mating (Saito, 2000). Highest mating frequency is from day 4 to 6 after emergence (Singh & Rembold, 1989).

Diverse approaches, both traditional and molecular, have been employed to measure dispersal, migration and gene-flow of *H. armigera* across major agricultural growing regions within Australia. The results of these studies have produced mixed interpretations, identifying either low levels of migration, high levels of long-range dispersal, or a combination thereof.

Relatively low dispersal distances were described in Australian mark–recapture experiments, where most of the released *H. armigera* colonized crops within 10 km of their emergence site (Fitt & Dillon, 1993). Analysis of variation in a sodium channel gene (Stokes *et al*., 1997), identified restrictions to free and fast gene-flow between the Lower Namoi Valley and St George (approximately 300 km) in Australia, indicating that extensive migration was not occurring between these regions. In contrast, other studies have indicated high levels of dispersal for *H. armigera*. Analysis of pheromone trap catches (Fitt & Daly, 1990) found that both local and immigrant *H. armigera* were represented. Analysis of pollen carried on *H. armigera* (Gregg, 1993) indicated that the species was capable of travelling vast distances over eastern Australia. Genetic analysis using isozymes (Daly & Gregg, 1985) identified small genetic distances, consistent with significant long distance dispersal. Similar analyses of *H. armigera* in the Mediterranean region of Europe using random amplified polymorphic DNA (Zhou *et al*., 2000), and in Africa and Europe using isozymes (Nibouche *et al*., 1998) have shown high migration rates and small genetic distances between disjunct populations of this species.

Despite the suitability of microsatellites for population studies within species, they have not been applied to *H. armigera* prior to Scott *et al*. (2003), as they are a relatively recent development in the taxon (Tan *et al*., 2001; Ji *et al*., 2003). Some research groups have reported difficulties with the isolation of microsatellites from lepidopteran species, due to low frequency of repeats, false positives during library screening and low repeat numbers (Ji *et al*., 2003). However, recent studies have shown that large numbers of microsatellites can be easily isolated from four lepidopteran species including *H. armigera, H. punctigera* Wallengren (Lepidoptera: Noctuidae) (Scott *et al*., 2004), *Chiasmia assimilis* Warren (Lepidoptera: Geometridae) (Wardill *et al*., 2004), and *Danaus plexippus* Linnaeus (Lepidoptera: Nymphalidae) (unpublished). The present study applied these markers and aimed to measure *H. armigera* gene-flow and migration between the major agricultural regions of Australia over several consecutive years. The results from this study may assist the management programmes for the species.

Materials and methods

Sample source and DNA extraction

In total, 3142 *H. armigera* larvae and moths were collected from 16 localities in eight regions over a 38-month period (fig. 1 and table 1). The eight regions included the Central Highlands, the Dawson-Callide Valleys, the Darling Downs, Macintyre Valley, Lower Namoi Valley, Murrumbidgee Valley, Victoria and Northern Territory. Although a stratified sampling strategy was proposed, fluctuating pest pressure resulted in variations in the availability and number of samples obtained at different sites and at various times. Samples were obtained from a variety of crops: peanut *Arachis hypogaea* L., pigeon pea *Cajanus cajan* L., chickpea *Cicer arietinum* L., field pea *Pisum sativum* L., soybean *Glycine max* (L.) Merr., faba bean *Vicia faba* L., mungbeans *Vigna radiata* (L.) R. Wilczek (all Fabaceae), cotton *Gossypium hirsutum* L. (Malvaceae), watermelon *Citrullus vulgaris* L. (Cucurbitaceae), sunflower *Helianthus annuus* L. (Asteraceae), barley *Hordeum vulgare* L., millet *Panicum miliaceum* L., sorghum *Sorghum bicolour* (L.) Moench., wheat *Triticum aestivum* L., corn *Zea mays* L. (all Poaceae), and tomato *Lycopersicon esculentum* L. (Solanaceae).

DNA for microsatellite analysis was extracted from individual moth heads or larval posterior prolegs using a 96-well modification of the protocol developed by Miller *et al*. (1988). The remainder of each insect was stored individually in an ethanol vial, and cross-referenced to the DNA extraction. A polymerase chain reaction (PCR) based diagnostic (developed at the School of Integrative Biology, unpublished data; for details please contact authors) was utilized to determine whether each individual was *H. armigera* or *H. punctigera*. The species diagnostic ensured microsatellite analysis was performed only on *H. armigera* individuals, as morphological determination of species after storage in ethanol was problematic.

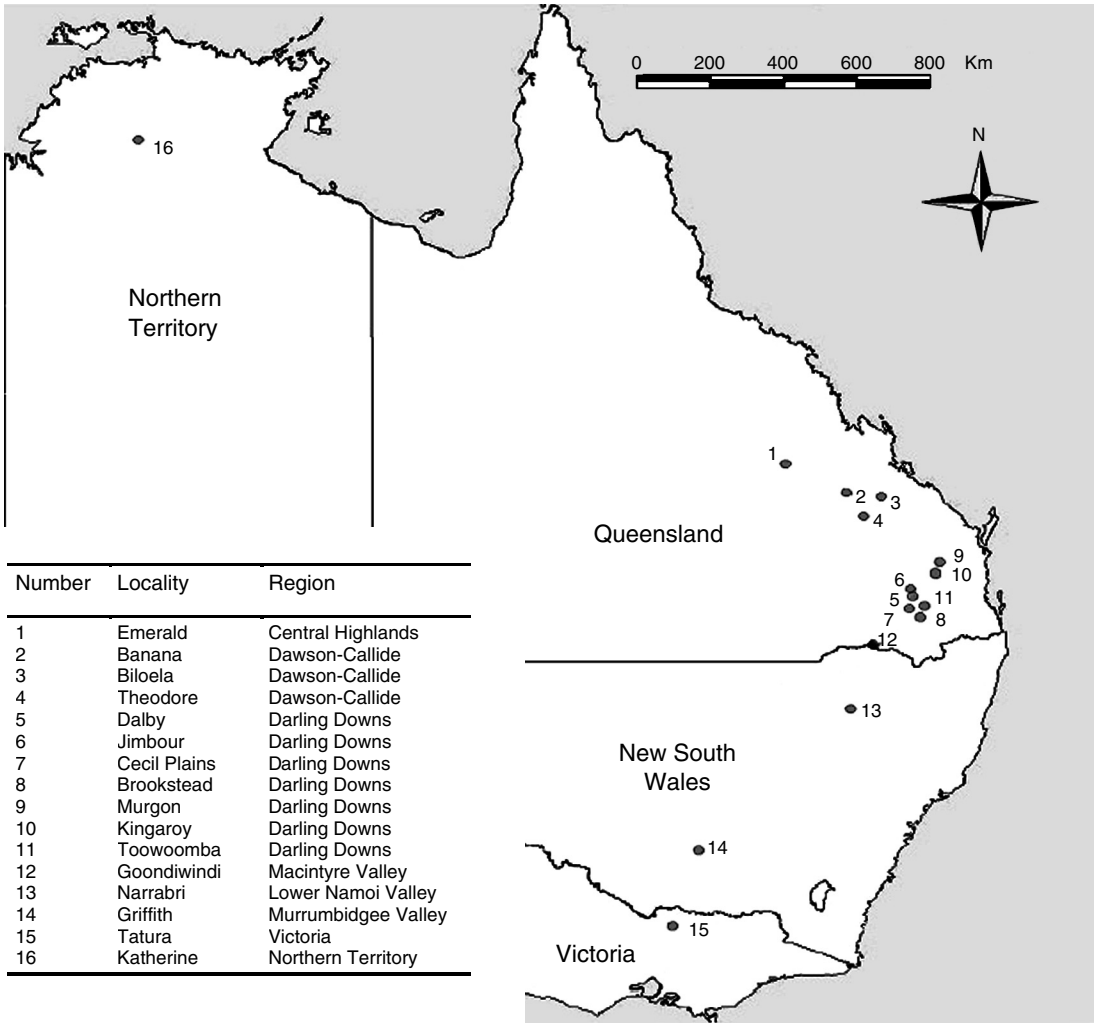
Microsatellite analysis

Five highly variable microsatellite loci were used to analyse the 3142 *H. armigera* individuals collected (Scott *et al*., 2004). The loci were HaB60 (25 alleles, expected heterozygosity (*He*) = 0.45, observed heterozygosity (*Ho*) = 0.04), HaD25 (58 alleles, *He* = 0.73, *Ho* = 0.36), HaD47 (85 alleles, *He* = 0.75, *Ho* = 0.24), HaC87 (33 alleles, *He* = 0.50, *Ho* = 0.17) and HaC14 (32 alleles, *He* = 0.65, *Ho* = 0.48). Microsatellites were *Hex* labelled with PCR amplification conditions and gel separation as published in Scott *et al*. (2003).

Statistical analysis

Microsatellite alleles were scored using ONE-Dscan (Ver 2.05, Scanalytics Inc., Billerica, Massachusetts, USA). Allele sizes were entered into Excel (Microsoft Corp.) and analysed using GenAlEx (Peakall & Smouse, 2001). Nei genetic distance between collections was calculated using Peakall *et al*. (1995). Allele frequencies and heterozygosity calculations followed the method of Hartl & Clark (1997). Analysis of molecular variance (AMOVA) was as for Excoffier *et al*. (1992), Peakall *et al*. (1995) and Michalakis & Excoffier (1996). The correlation of geographical distance to genetic distance was performed using the Mantel test of Smouse *et al*. (1986) and Smouse & Long (1992).

Assignment tests were performed in GeneClass2.3 (Piry *et al*., 2004) with 1000 Monte-Carlo resampling of gametes to preserve linkage disequilibrium from recent immigrations (Paetkau *et al*., 2004). Assignment tests were completed in three month blocks. The challenge in undertaking assignment testing in a species where migration may be common,



Number	Locality	Region
1	Emerald	Central Highlands
2	Banana	Dawson-Callide
3	Biloela	Dawson-Callide
4	Theodore	Dawson-Callide
5	Dalby	Darling Downs
6	Jimbour	Darling Downs
7	Cecil Plains	Darling Downs
8	Brookstead	Darling Downs
9	Murgon	Darling Downs
10	Kingaroy	Darling Downs
11	Toowoomba	Darling Downs
12	Goondiwindi	Macintyre Valley
13	Narrabri	Lower Namoi Valley
14	Griffith	Murrumbidgee Valley
15	Tatura	Victoria
16	Katherine	Northern Territory

Fig. 1. Map of eastern Australia showing the major localities and regional groupings of samples outlined in table 1.

is having enough genetic differentiation between source and sink populations to be able to identify immigrants. If the genetic differentiation is limited, a large proportion of individuals will not be assigned at a significant level. Assignment criteria for populations of less than 40 individuals used a 1% error rate, and for populations with greater than 40 individuals an error rate of 5% was applied. This was to account for the increase in type 1 errors when using smaller sample sizes. The assignment test used in this study was that of Paetkau *et al*. (2004) which enables the identification of immigrant individuals in the current generation. This differs from many methods such as Wilson & Rannala (2003) that estimate migration over several generations. Other assignment test methods such as Rannala & Mountain (1997) and Cornuet *et al*. (1999) also resample alleles rather than gametes, randomly distributing migrant alleles across the population (Paetkau *et al*., 2004). These later methods represent unrealistic approaches for a species such as *H. armigera* that has continuous migration, and would lead to an excess of 'locals' being wrongly identified as immigrant (Type 1 error). It is acknowledged that the assumption of Hardy-Weinberg equilibrium is breached; however, the results are consistent with all other measures of migration and appear to be informative.

Results

Data analysis was performed on an annual basis, with each 'year' beginning at the end of the summer growing season (April) and extending through to March of the following year. As with other Lepidoptera (Meglecz *et al*., 1998; Britten & Glasford, 2002), *H. armigera* collections were not in Hardy-Weinberg equilibrium, showing a deficit of heterozygotes. *Helicoverpa armigera* is subject to substantial and continuous selection and migration is relatively common. Therefore, it would be expected that populations of *H. armigera* will deviate from Hardy-Weinberg equilibrium. Microsatellite analysis of *H. armigera* showed high differentiation between collections over the three years of study, and genetic structures

Table 1. List of analysed sample regions, locations, collection date and sample numbers.

Region	Locality	Date	Number	Region	Locality	Date	Number
1999–2000							
Central Highlands	Emerald	29 Feb 00	25		Brookstead	24 Feb 00	15
Darling Downs	Brookstead	04 Nov 99	15		Brookstead	03 Mar 00	15
	Jimbour	02 Feb 00	30		Jimbour	08 Mar 00	15
	Brookstead	04 Feb 00	15		Brookstead	15 Mar 00	15
	Brookstead	14 Feb 00	45		Brookstead	16 Mar 00	15
	Brookstead	17 Feb 00	15		Jimbour	21 Mar 00	15
	Jimbour	18 Feb 00	15		Jimbour	22 Mar 00	15
2000–2001							
Dawson-Callide Valleys	Biloela	27 Jul 00	14	Dawson-Callide Valleys	Theodore	12 Dec 00	7
	Banana	28 Jul 00	15		Biloela	19 Dec 00	6
	Biloela	31 Jul 00	15		Theodore	20 Dec 00	13
	Banana	01 Aug 00	15		Biloela	05 Jan 01	15
	Theodore	04 Aug 00	8		Theodore	05 Jan 01	15
	Biloela	11 Aug 00	15		Theodore	12 Jan 01	11
	Biloela	21 Aug 00	15		Theodore	29 Jan 01	15
	Banana	21 Aug 00	15		Theodore	06 Feb 01	15
	Biloela	24 Aug 00	11		Theodore	06 Mar 01	98
	Theodore	25 Aug 00	10		Theodore	13 Mar 01	45
	Banana	25 Aug 00	15		Theodore	21 Mar 01	8
	Biloela	01 Sep 00	15	Darling Downs	Brookstead	02 Oct 00	34
	Theodore	04 Sep 00	13		Brookstead	11 Oct 00	15
	Theodore	04 Sep 00	8		Jimbour	18 Oct 00	15
	Biloela	05 Sep 00	15		Cecil Plains	02 Mar 01	15
	Banana	07 Sep 00	15		Jimbour	08 Mar 01	15
	Banana	11 Sep 00	15		Toowoomba	08 Mar 01	15
	Theodore	11 Sep 00	12	Lower Namoi Valley	Narrabri	20 Mar 01	9
	Theodore	11 Sep 00	15		Narrabri	04 Apr 00	15
	Theodore	13 Sep 00	15		Narrabri	06 Apr 00	15
	Theodore	20 Sep 00	15		Narrabri	10 Apr 00	13
	Biloela	19 Sep 00	8		Narrabri	11 Apr 00	15
	Banana	20 Sep 00	14		Narrabri	12 Apr 00	15
	Biloela	29 Sep 00	15		Narrabri	12 Apr 00	15
	Theodore	02 Oct 00	15		Narrabri	12 Apr 00	15
	Banana	02 Oct 00	15		Narrabri	12 Apr 00	15
	Biloela	03 Oct 00	26		Narrabri	13 Apr 00	15
	Theodore	16 Oct 00	9		Narrabri	14 Apr 00	7
	Theodore	23 Oct 00	15		Narrabri	19 Apr 00	15
	Biloela	29 Oct 00	15		Narrabri	19 Apr 00	15
	Biloela	16 Nov 00	15		Narrabri	20 Dec 00	10
	Theodore	27 Nov 00	22		Narrabri	23 Jan 01	12
	Banana	27 Nov 00	15		Narrabri	02 Mar 01	15
	Biloela	05 Dec 00	15				
2001–2002							
Central Highlands	Emerald	17 Sep 01	15	Dawson-Callide Valleys	Theodore	25 Jan 02	15
	Emerald	18 Sep 01	15		Theodore	04 Feb 02	23
	Emerald	19 Sep 01	14		Theodore	11 Feb 02	26
	Emerald	20 Sep 01	30		Theodore	22 Feb 02	23
	Emerald	28 Nov 01	5	Darling Downs	Jimbour	17–20 Sept 01	15
	Emerald	16 Jan 02	6		Brookstead	03 Oct 01	15
	Emerald	26 Feb 02	15		Toowoomba	14 Oct 01	13
	Emerald	01 Mar 02	14		Brookstead	09 Nov 01	9
	Emerald	08 Mar 02	10		Jimbour	12–27 Nov 01	10
	Emerald	13 Mar 02	15		Brookstead	03 Dec 01	15
Dawson-Callide Valleys	Theodore	27 Jun 01	15		Brookstead	18 Jan 02	15
	Biloela	27 Jun 01	5		Jimbour	20 Feb 02	10
	Biloela	31 Jul 01	18		Jimbour	21 Feb 02	13
	Theodore	06 Aug 01	20	Lower Namoi Valley	Narrabri	02 Apr 01	15
	Biloela	27 Aug 01	45		Narrabri	03 Apr 01	15
	Theodore	06 Sep 01	47		Narrabri	03 Apr 01	9
	Banana	13 Sep 01	10		Narrabri	05 Apr 01	24
	Theodore	13 Sep 01	25		Narrabri	05 Apr 01	13
	Theodore	13 Sep 01	116		Narrabri	05 Apr 01	28
	Theodore	22 Sep 01	12		Narrabri	10 Apr 01	5
	Biloela	11 Oct 01	15		Narrabri	07 Jun 01	6
	Biloela	15 Oct 01	14		Narrabri	01 Nov 01	8
	Theodore	22 Oct 01	6		Narrabri	09 Nov 01	11

Table 1. Continued.

Region	Locality	Date	Number	Region	Locality	Date	Number
	Theodore	15 Nov 01	29		Narrabri	26 Mar 02	15
	Theodore	26 Nov 01	13	Murrumbidgee Valley	Griffith	01 Oct 01	15
	Theodore	03 Dec 01	15		Griffith	11 Oct 01	8
	Theodore	12 Dec 01	6		Griffith	23 Jan 02	10
	Theodore	31 Dec 01	9		Griffith	20 Feb 02	15
	Theodore	10 Jan 02	24		Griffith	14 Mar 02	15
	Theodore	17 Jan 02	20				
2002–2003							
Northern Territory	Katherine	08 May 02	15	Darling Downs	Toowoomba	20–25 Nov 02	5
	Katherine	21 Aug 02	11		Toowoomba	20–25 Nov 02	6
	Katherine	23 Aug 02	15		Toowoomba	20–25 Nov 02	5
Central Highlands	Emerald	26 Jun 02	24		Brookstead	20–25 Nov 02	5
	Emerald	27 Jun 02	20		Murgon	10 Jan 03	13
	Emerald	02 Jul 02	15		Murgon	16 Jan 03	60
	Emerald	11 Jul 02	8		Dalby	24 Jan 03	17
	Emerald	25 Jul 02	10		Kingaroy	29 Jan 03	5
	Emerald	09 Aug 02	11	Macintyre Valley	Goondiwindi	11 Oct 02	7
Dawson-Callide Valleys	Theodore	10 Apr 02	15		Goondiwindi	17 Oct 02	8
	Biloela	17 Apr 02	15		Goondiwindi	08 Jan 03	7
	Theodore	12 Sep 02	12		Goondiwindi	11 Jan 03	10
	Theodore	11 Oct 02	40		Goondiwindi	15 Jan 03	27
	Theodore	21 Nov 02	9	Murrumbidgee Valley	Griffith	08 Oct 02	30
	Theodore	28 Nov 02	8		Griffith	22 Oct 02	15
	Biloela	06 Dec 02	15		Griffith	22 Oct 02	12
Darling Downs	Dalby	03 Oct 02	7		Griffith	29 Oct 02	27
	Kingaroy	17 Oct 02	15		Griffith	21 Nov 02	14
	Kingaroy	18 Oct 02	10		Griffith	03 Dec 02	15
	Kingaroy	23 Oct 02	7		Griffith	03 Dec 02	38
	Murgon	23 Oct 02	6		Griffith	10 Dec 02	15
	Murgon	23 Oct 02	10		Griffith	10 Dec 02	53
	Murgon	23 Oct 02	14		Griffith	30 Dec 02	11
	Kingaroy	25 Oct 02	14	Victoria	Tatura	03–06 Jan 02	24
	Kingaroy	25 Oct 02	6		Tatura	16–20 Jan 03	26
	Toowoomba	25 Oct 02	15				

varied from year to year over the research period (tables 2, 3 and 4).

November 1999 to March 2000

AMOVA analysis for the Darling Downs and Central Highlands from November 1999 to March 2000 indicated that the genotypes present in these two regions differed significantly from each other (tables 2 and 3). For assignment test analysis, the assumptions of Paetkau *et al*. (2004) best fit the biology of *H. armigera*. Assignment test results for January to March indicated that approximately 80% of the *H. armigera* individuals found in the Central Highlands were of local origin and 96% of the individuals found in the Darling Downs were locals (table 4).

April 2000 to March 2001

In the period April 2000 to March 2001, the proportion of individuals that were local varied from 71.4% in the Lower Namoi Valley (October to December), to 96.1% in the Dawson-Callide Valleys (October–December). During this year the AMOVA results showed that each of the three regions were genetically distinct (tables 2 and 3). The genotypes present in the Darling Downs were most dissimilar to those in the Dawson-Callide Valleys growing region

Table 2. Analysis of molecular variance (AMOVA) for *Helicoverpa armigera* major crop growing regions in eastern Australia.

Period	Number of regions	% Between regions (*P* value)	% Between collections (*P* value)	% Within collections (*P* value)
November 1999–March 2000	2	5 (0.01)	12 (0.01)	83 (0.01)
April 2000–March 2001	3	6 (0.01)	17 (0.01)	77 (0.01)
April 2001–March 2002	5	1 (0.01)	19 (0.01)	80 (0.01)
April 2002–March 2003	7	15 (0.01)	24 (0.01)	61 (0.01)

Table 3. Analysis of molecular variance (AMOVA) for *Heliothis armigera* population in pairs of regions in eastern Australia.

Regions compared	Year	% Between regions (*P* value)	% Between collections (*P* value)	% Within collections (*P* value)
1999–2000				
Darling Downs to Central Highlands	Nov 99–Mar 00	5 (0.01)	12 (0.01)	83 (0.01)
2000–2001				
Darling Downs to Dawson-Callide Valleys	Apr 00–Mar 01	4 (0.01)	14 (0.01)	82 (0.01)
Darling Downs to Lower Namoi Valley	Apr 00–Mar 01	2 (0.01)	13 (0.01)	85 (0.01)
Dawson-Callide Valleys to Lower Namoi Valley	Apr 00–Mar 01	3 (0.01)	14 (0.01)	83 (0.01)
2001–2002				
Darling Downs to Dawson-Callide Valleys	Apr 01–Mar 02	0 (0.43)	13 (0.01)	87 (0.01)
Darling Downs to Central Highlands	Apr 01–Mar 02	4 (0.01)	13 (0.01)	83 (0.01)
Darling Downs to Lower Namoi Valley	Apr 01–Mar 02	0 (0.06)	13 (0.01)	87 (0.01)
Darling Downs to Murrumbidgee Valley	Apr 01–Mar 02	2 (0.01)	14 (0.01)	84 (0.01)
Dawson-Callide Valleys to Central Highlands	Apr 01–Mar 02	1 (0.01)	14 (0.01)	85 (0.01)
Dawson-Callide Valleys to Lower Namoi Valley	Apr 01–Mar 02	0 (0.02)	14 (0.01)	86 (0.01)
Dawson-Callide Valleys to Murrumbidgee Valley	Apr 01–Mar 02	1 (0.01)	14 (0.01)	85 (0.01)
Central Highlands to Lower Namoi Valley	Apr 01–Mar 02	0 (0.04)	15 (0.01)	85 (0.01)
Central Highlands to Murrumbidgee Valley	Apr 01–Mar 02	0 (0.13)	17 (0.01)	83 (0.01)
Lower Namoi Valley to Murrumbidgee Valley	Apr 01–Mar 02	0 (0.97)	15 (0.01)	85 (0.01)
2002–2003				
Darling Downs to Dawson-Callide Valleys	Apr 02–Mar 03	1 (0.01)	25 (0.01)	74 (0.01)
Darling Downs to Central Highlands	Apr 02–Mar 03	14 (0.01)	18 (0.01)	68 (0.01)
Darling Downs to Macintyre Valley	Apr 02–Mar 03	1 (0.03)	25 (0.01)	74 (0.01)
Darling Downs to Murrumbidgee Valley	Apr 02–Mar 03	9 (0.01)	20 (0.01)	71 (0.01)
Darling Downs to Northern Territory	Apr 02–Mar 03	13 (0.01)	19 (0.01)	68 (0.01)
Darling Downs to Victoria	Apr 02–Mar 03	2 (0.01)	23 (0.01)	75 (0.01)
Dawson-Callide Valleys to Central Highlands	Apr 02–Mar 03	10 (0.01)	16 (0.01)	74 (0.01)
Dawson-Callide Valleys to Macintyre Valley	Apr 02–Mar 03	5 (0.01)	22 (0.01)	73 (0.01)
Dawson-Callide Valleys to Murrumbidgee Valley	Apr 02–Mar 03	2 (0.01)	21 (0.01)	77 (0.01)
Dawson-Callide Valleys to Northern Territory	Apr 02–Mar 03	9 (0.01)	18 (0.01)	73 (0.01)
Dawson-Callide Valleys to Victoria	Apr 02–Mar 03	6 (0.01)	19 (0.01)	75 (0.01)
Central Highlands to Macintyre Valley	Apr 02–Mar 03	21 (0.01)	11 (0.01)	68 (0.01)
Central Highlands to Murrumbidgee Valley	Apr 02–Mar 03	19 (0.01)	14 (0.01)	67 (0.01)
Central Highlands to Northern Territory	Apr 02–Mar 03	1 (0.16)	10 (0.01)	89 (0.01)
Central Highlands to Victoria	Apr 02–Mar 03	22 (0.01)	8 (0.01)	70 (0.01)
Macintyre Valley to Murrumbidgee Valley	Apr 02–Mar 03	12 (0.01)	17 (0.01)	71 (0.01)
Macintyre Valley to Northern Territory	Apr 02–Mar 03	21 (0.01)	10 (0.01)	69 (0.01)
Macintyre Valley to Victoria	Apr 02–Mar 03	4 (0.01)	13 (0.01)	83 (0.01)
Murrumbidgee Valley to Northern Territory	Apr 02–Mar 03	20 (0.01)	15 (0.01)	65 (0.01)
Murrumbidgee Valley to Victoria	Apr 02–Mar 03	13 (0.01)	16 (0.01)	71 (0.01)
Northern Territory to Victoria	Apr 02–Mar 03	22 (0.01)	5 (0.01)	73 (0.01)

(AMOVA 4% $P = 0.01$) and were different from those of the Lower Namoi Valley by an AMOVA of 2% ($P = 0.01$). *Helicoverpa armigera* collected from the Dawson-Callide Valley were differentiated from those collected in the Lower Namoi Valley by an AMOVA of 3% ($P = 0.01$). The Mantel test revealed a small but significant correlation $r = 0.108$ ($P < 0.001$) of increasing geographical distance with increasing genetic distance.

April 2001 to March 2002

During the April 2001 to March 2002 sampling year AMOVA analysis (tables 2 and 3) identified limited differentiation between all five regions with several regions being genetically indistinguishable. Several of the AMOVA results were not statistically significant with *P*-values > 0.05. The highest AMOVA differential score was present between the Darling Downs and Central Highland regions (AMOVA 4% $P = 0.01$). The Mantel test again correlates increases in genetic distance with increases in geographic distance $r = 0.053$ ($P < 0.001$), although the correlation was lower than in the previous year. During this period, up to 40% of individuals sampled from a region were migrants into that region (Murrumbidgee Valley January–March 2002). The highest 'local' populations (98%) were for the Dawson-Callide Valleys in the July to September period. For most populations, 14–30% of the sampled individuals originated from outside the region where they were collected. There was some difficulty with the assignment test in this year, with a high proportion of individuals not being assigned at a significant level for several collections. This is likely to be a result of the lack of differentiation between some regions, and therefore the level of migration is probably underestimated.

April 2002 to March 2003

This growing season was characterized by high levels of migration early in the year, with lower levels of migration later in the year (table 4). Between 16 and 40% of sampled

Table 4. Assignment of *Helicoverpa armigera* migrants identified using Monte-Carlo resampling of gametes (Paetkau *et al.*, 2004) using GeneClass 2.3 (Piry *et al.*, 2004).

Collections				% Assigned							
Month (Year)	Region	*n*	*n* unassigned	Central Highlands	Dawson Callide	Darling Downs	Macintyre Valley	Namoi Valley	Murrumbidgee	Northern Territory	Source unknown
Jan–Mar (2000)	Central Highlands	25	5	80.0		20.0					
	Darling Downs	225	20	3.9		**96.1**					
Oct–Dec (2000)	Dawson-Callide Valleys	188	36		**96.1**	2.0		2.0			0.0
	Darling Downs	63	0		1.6	**87.3**		11.1			0.0
	Namoi Valley (Lower)	10*	3		14.3	14.3		**71.4**			0.0
Jan–Mar (2001)	Dawson-Callide Valleys	222	10		**88.7**	5.7		5.7			0.0
	Darling Downs	30	7		4.3	**87.0**		8.7			0.0
	Namoi Valley (Lower)	36	4		6.3	3.1		**90.6**			0.0
Apr–Jun (2001)	Dawson-Callide Valleys	20	3		**94.1**			5.9			
	Namoi Valley (Lower)	115	8		13.1			**86.9**			
Jul–Sep (2001)	Central Highlands	74	31	**86.0**	9.3	0.0	2.3				2.3
	Dawson-Callide Valleys	308	111	0.0	**98.0**	2.0	0.0				0.0
	Darling Downs	15	6	0.0	11.1	**77.8**	11.1				0.0
	Macintyre Valley	15	1	0.0	7.1	0.0	**92.9**				0.0
Oct–Dec (2001)	Central Highlands	5*	1	50.0	0.0	0.0	25.0	0.0	25.0		0.0
	Dawson-Callide Valleys	107	61	2.2	**80.4**	2.2	8.7	0.0	2.2		0.0
	Darling Downs	30	13	0.0	17.6	**70.6**	5.9	0.0	0.0		5.9
	Macintyre Valley	17	10	0.0	0.0	0.0	**71.4**	0.0	0.0		28.6
	Namoi Valley (Lower)	19	9	0.0	10.0	0.0	0.0	**90.0**	0.0		0.0
	Murrumbidgee Valley	23	13	0.0	0.0	0.0	10.0	0.0	**70.0**		20.0
Jan–Mar (2002)	Central Highlands	60	15	75.6	13.3	2.2		8.9	0.0		0.0
	Dawson-Callide Valleys	59	13	8.7	**76.1**	6.5		4.3	4.3		0.0
	Darling Downs	15	4	0.0	9.1	**72.7**		0.0	18.2		0.0
	Namoi Valley (Lower)	15	8	0.0	0.0	14.3		**85.7**	0.0		0.0
	Murrumbidgee Valley	40	10	10.0	3.3	6.7		16.7	**60.0**		3.3
Apr–Jun (2002)	Central Highlands	44	11	**81.8**	6.1		6.1			6.1	0.0
	Dawson-Callide Valleys	15	0	13.3	**60.0**		13.3			6.7	6.7
	Macintyre Valley	23	8	0.0	13.3		**80.0**			0.0	0.0
	Northern Territory	15	5	10.0	10.0		0.0			**80.0**	0.0
Jul–Sep (2002)	Central Highlands	44	7	**83.8**						16.2	
	Northern Territory	26	1	4.0						**84.0**	
Oct–Dec (2002)	Dawson-Callide Valleys	15	4		**72.7**	9.1	0.0		9.1		9.1
	Darling Downs	108	21		1.1	**94.3**	2.3		2.3		0.0
	Macintyre Valley	23	4		0.0	5.3	**94.7**		0.0		0.0
	Murrumbidgee Valley	230	29		1.0	0.0	1.0		**97.5**		0.5
Jan–Mar (2003)	Darling Downs	95	20			**89.3**	10.7				
	Macintyre Valley	44	4			10.0	**90.0**				

* Very small sample size may be subject to substantial overestimate of migrant.
Shaded areas indicate unavailability of samples.
Values in bold indicate % local.

individuals were identified as migrants in the April–September period. From October, less than 10% of individuals were identified as immigrants in all regions but the Dawson-Callide Valleys (October–December). High migration may have persisted longer in the Dawson-Callide Valleys, or the migration may have been overestimated due to the relatively small sample number ($n = 15$). The highest proportion of local *H. armigera* (97.5%) was in the Murrumbidgee Valley in October–December, and the highest level of immigration 40% in the Dawson-Callide Valleys in April–June. The differentiation between-collections and between-regions identified by AMOVA analysis in this year (tables 2 and 3) was the highest recorded over the period of study. With the exception of the Central Highlands and Northern Territory, all of the regions were significantly differentiated. The highest differentiation was between Northern Territory and Victoria (AMOVA 22% $P = 0.01$), and between Victoria and the Central Highlands (AMOVA 22% $P = 0.01$). The high differentiation resulted in the largest correlation between geographical distance and genetic distance (Mantel test $r = 0.233$, $P < 0.001$).

Discussion

The population dynamics and migratory behaviour of *H. armigera* is highly variable between years in Australia. Over the period of this study, broad panmictic migration was not extensive, with studies from three of the four seasons showing genetic distinctions between most regions (table 3). The use of assignment testing on *H. armigera* individuals has also provided insight into the direction of migration events between regions in Australia, and into the extent of immigration into regions over time. The direction and extent of migration (table 4), and the resulting regional differentiation of *H. armigera* have a significant impact on the management of the pest and are discussed on a seasonal basis.

Seasonal reviews

A schematic representation of the patterns of local recruitment and migration between regions is presented in fig. 2. In broad terms, 1999–2000 and 2000–2001 were characterized by moderate gene flow and significant differentiation between the regions. By contrast, 2001–2002 was characterized by very high levels of migration, and very little regional differentiation. Migration dropped substantially during 2002–2003, and the differentiation between regions was very high.

In the 1999–2000 period, the Darling Downs and Central Highlands were analysed using AMOVA and assignment tests. These data indicated that the populations of *H. armigera* in these two regions differed significantly from each other during this season (table 3). Although gene-flow was not free, there is still evidence of substantial migration late in the season into the Central Highlands.

The rate of gene-flow for the 2000–2001 season was similar to the previous year, with each of the three regions studied; Darling Downs, Dawson-Callide Valleys and the Lower Namoi Valley, been genetically distinct. There was a significant correlation between geographical distance and genetic distance, that is proximal collections were more genetically similar than distant collections, as indicated by the Mantel test ($r = 0.108$, $P < 0.001$). This is reflected in the AMOVA analysis where the Darling Downs populations were more similar to the Lower Namoi Valley (2% variance) than the more remote Dawson-Callide Valleys (4% variance). The assignment test (table 4) suggests that this result may be due to a much larger contribution of Namoi Valley immigrants into the population of *H. armigera* occurring in the Darling Downs (11.1% for October–December, and 8.7% for January–March) than from Dawson-Callide Valleys immigrants into the Darling Downs populations (1.6% for October–December, and 4.3% for January–March).

During the 2001–2002 season, *H. armigera* migration and population mixing across regions were very high. The AMOVA analysis (tables 2 and 3) identified low levels of variance between regions, with regions in Central Queensland and northern New South Wales being genetically indistinguishable. The assignment test data for this period indicates that the numbers of source population into a region varies. There are examples of multiple immigrant sources to a region, e.g. the Dawson-Callide Valleys in January–March had immigration from the Central Highlands, Darling Downs, Namoi Valley and the Murrumbidgee. Notably, the Dawson-Callide Valleys and the Central Highlands were genetically distinct despite their geographical proximity. Closer examination of the assignment data for this period indicates that there was very little gene-flow from the Central Highlands into the Dawson-Callide Valleys, with migrants being substantially derived from other regions.

The very high levels of migration continued into the first half of the 2002–2003 period, with a substantial reduction from October 2002 to March 2003. Despite the high gene flow for the first half of this year, there was a significant isolation by distance for the year as a whole (Mantel $r = 0.233$, $P < 0.001$), and the genetic differentiation between regions was the highest recorded during the study. The very high differentiation between the regions was established within two or three generations, despite moderate levels of migration still occurring. Genetic drift alone is unlikely to drive the accumulation of this amount of population differentiation over such a small number of generations, showing the significant contribution that selection and management may be having on the population dynamics of this species.

Isolation by distance

Significant isolation by distance results when there is higher gene flow and thereby limited genetic distance between nearby populations, and reduced gene flow resulting in increased genetic distance between distant populations. As a result, there is a positive correlation between geographical and genetic distance. Isolation by distance is not always expected in species with high mobility (Arguedas & Parker, 2000). The Mantel analyses conducted for *H. armigera* in Australia were consistent with an isolation by distance model. Significant isolation by distance effects have been reported for other Lepidoptera such as *Chazara briseis* Linnaeus (Lepidoptera: Satyridae) (Johannesen *et al.*, 1997), and *Hesperia dacotae* Skinner (Lepidoptera: Hesperiidae) (Britten & Glasford, 2002). The implications of isolation by distance are that immigrants are more likely to come from nearby regions than from afar. This contrasts with previous observations by Daly & Gregg (1985), that the differentiation between *H. armigera* populations does not have a geographic pattern.

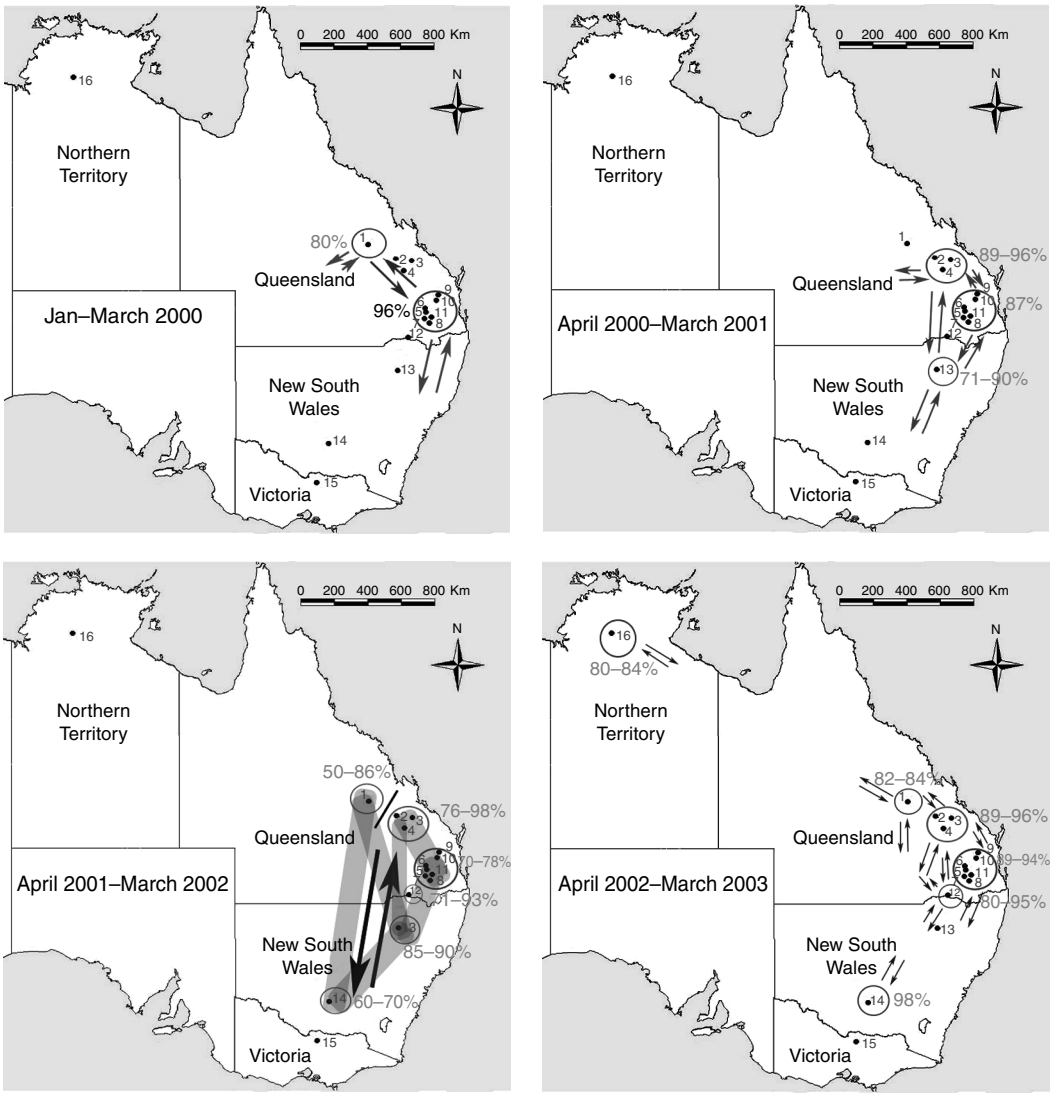


Fig. 2. Schematic representation of local recruitment and migration of *Helicoverpa armigera* in Australia based on AMOVA analysis and assignment testing. The proportion of local recruitment is indicated as a percentage for each region. The arrow width is indicative of the level of migration between regions characterized by assignment testing. Each region was significantly differentiated in the AMOVA analysis except in April 2001 to March 2002, where non-differentiated populations are indicated by grey zones. Locality names are listed in fig. 1, results of AMOVA analyses are summarized in tables 2 and 3 and assignment data are shown in table 4.

The data presented here demonstrate that the migration patterns vary from year to year, and it may be that the observations of Daly & Gregg (1985) reflect the migration patterns in that particular year, and conversely, our data reflect the migration patterns for 1999–2003. It is outside the scope of the current discussion to analyse the biological basis of such variation but it may be a result of host availability, meteorological conditions such as rainfall, wind direction and speed or severe storms, or other limiting factors (Rochester, 1999). For example, the data for Daly & Gregg (1985) were collected in 1983, when rainfall was very high in eastern Australia, with the very high rainfall (top decile) recorded in the Central Highlands, Dawson-Callide Valleys, Darling Downs, Macintyre Valley and the Lower Namoi Valley (Australian Bureau of Meteorology data). By contrast, during the period of the current study, most cropping regions suffered severe drought, with rainfall varying from average to very much below average (Australian Bureau of Meteorology data). Such extremes in rainfall patterns will have resulted in substantially different crop and native vegetation patterns, and are likely to have substantial effects on migratory behaviour.

Migration events in *H. armigera* may occur as single long distance events, probably under favourable environmental conditions, or as smaller stepping stone movements, perhaps even over several generations. In the years of low or moderate migration, there was substantial genetic differentiation between regions and a significant isolation by

distance effect. The assignment analysis suggested a tendency for immigrants to have originated substantially from nearby regions. It seems likely that gene-flow in these years may occur primarily in the stepping stone model. By contrast, in the 2001–2002 period there was a much reduced isolation by distance effect, and very little regional differentiation. During this period there was evidence of migratory events occurring over very large distances, for example in October–December 2001, immigrants into the Namoi Valley were derived from the Dawson-Callide Valleys. At the same time there were examples of genetic differentiation and limited gene-flow between nearby regions, e.g. the Central Highlands and the Dawson-Callide Valleys. It seems likely that long distance dispersal events played an important role in shaping the genetic structure of *H. armigera* in this season, despite most migration still occurring between nearby regions (therefore the still significant isolation by distance effect).

Implications for pest management

This study has characterized highly variable patterns of migration of *H. armigera* across cropping regions in Australia with some years having high migration between regions, and other years very little migration. Rapid population dynamics resulted in differentiation between regions being established in only a couple of generations. These patterns have significant implications for the management of insecticide resistance in *H. armigera*.

During periods of low migration there was clear differentiation between the regions, with regional populations of *H. armigera* being relatively independent. Therefore, problems that arise from management practices, such as insecticide resistance, are likely to be derived locally. The rapid establishment of genetic differentiation in 2002–2003 is consistent with the local management practices having substantial impact on the genetic texture of the pest populations. Development of resistance will be exacerbated in periods of low migration as there is no influx of susceptible individuals to dilute resistance.

The national coordination of insecticide resistance management strategies is critical, even in periods of low migration. Our data show that migration patterns are highly variable, and that periods of very high migration will occur. During these times, insecticide resistance may be transferred rapidly between cropping regions. This has been observed previously with the rapid spread of pyrethroid and endosulfan resistance in Australia (Forrester *et al*., 1993; Gunning & Easton, 1994).

Conclusions

Using microsatellite data collected over many of the cotton and grain regions of eastern Australia over the 38-month period from November 1999 through to January 2003, high, moderate and low gene-flow years have been detected and described. In 2001–2002, significant genetic mixing occurred across distant cropping regions in eastern Australia, indicating substantial gene-flow between these regions. In contrast, the genetic data in other years characterized significant genetic differentiation between regions, with little evidence of large-scale movement of *H. armigera* across cropping regions in Australia. In 2002–2003, significant differentiation of *H. armigera* populations between regions occurred within two or three generations following high migration. Pest management and selection can thus be assumed to be having a significant impact on the population dynamics of *H. armigera*, as this rapid differentiation cannot be accounted for by genetic drift alone.

These data provide evidence that the direction of moth movement differs from season to season, and even within some seasons, highlighting the importance of studies in groups such as the Lepidoptera extending over consecutive years, as short-term sampling may be misleading when population dynamics and migration change so significantly. This research demonstrates the importance of maintaining a nationally coordinated insecticide resistance management strategy. In some years, *H. armigera* populations may be independent within each region and thus significantly influenced by local management practices. However, periods with high migration across the cropping regions will occur and resistance may rapidly spread. Current research is incorporating the resistance/susceptible status and migratory origin of *H. armigera* to directly track the movement of resistance between growing regions.

Acknowledgements

The authors would like to thank all the growers and collectors involved in this study particularly David Murray, Melina Miles, Peter Gregg, Paul Grundy, Martin Dillon, Scott Hardwick, David Kelly, Hugh Brier, Carrie Hauxwell, Joanne Dawson, Cathy Mansfield, Iain Kay, Julie O'Halloran, Robert Dimsey, Ian Crosthwaite, Annie Spora, Annie Sullivan, Ingrid Christiansen, Macpherson Ag., Ag. Street Services, and Andrew Ward. We also appreciate the efforts of our collaborators, the editors and two reviewers for their significant contribution to the analysis and presentation of our research. Joanne McDiarmid, Joanne Kent and Mark Schutze provided technical assistance in this project. Trevor Wardill generously prepared the map presented. The Grains Research and Development Corporation, the Cotton Research and Development Corporation, and The University of Queensland provided financial support for this project.

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(Accepted 18 March 2005)