Cotton
RESEARCH & DEVELOPMENT

Annual, Progress and Final Reports

REPORTS

Part 1 - Summary Details

Please use your TAB key to complete Parts 1 & 2.

CRDC Project Number: **UQ32C**

Annual Report: ☐ Due 30-September

Progress Report: ☐ Due 31-January

Final Report: ☒ Due 30-September
(or within 3 months of completion of project)

Project Title: Population Genetics of Heliothis Migration, Recruitment and Origins

Project Commencement Date: 1/7/2000 **Project Completion Date:** 30/6/2003

Research Program: 3 Crop Protection

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Part 3.3 – Final Reports

1. Outline the background to the project.

Problems associated with pesticide application on the Darling Downs have not been solved by conventional strategies and alternate chemicals. Associated with these chemical use patterns is the development of resistance to insecticides in *H. armigera*. The use of molecular technologies for identification of Heliothis populations can provide answers to fundamental questions of population movement and development of insecticide resistance. These technologies will be applied in area wide management strategies presently under investigation in DAQ442 and in DAQ364 and from other recently developed Area Wide Management programs outside the Darling Downs. The proposed work will give precise information about local recruitment and migration of *H. armigera* and *H. punctigera* by providing an ability to track and study seasonal cohorts at a property management unit level. The microsatellite markers developed in UQ117 detect local population recruitment. It is now possible to measure local population recruitment and migration. The thrust of this project is to monitor local recruitment and the extent of migration over a further three growing seasons. The project will also begin the process of determining the source of migrating Heliothis populations. It is expected that this project will provide several major outputs.

The first is to provide further evidence of local recruitment and immigration. Some discussion of the UQ117 results demonstrated a need to continue the microsatellite monitoring into the 1999/2000 growing season to help strengthen the conclusions regarding the important finding that persistent genotypes of Heliothis over winter and contribute to locally recruited cohorts. The outcome of this research will help provide answers to proportional buildup of insecticide resistance between locally recruited and migratory cohorts. In addition defining the proportion of local recruitment and migration in other area wide management programs including central Queensland, Western Queensland, Northern New South Wales and Narrabri.

The second output of this project will commence the search for the source and extent of Heliothis migration. The outcome for industry providing support for this research will be the potential to show specifically where migrating populations come from and when migration occurs. During this project we will begin the development of an integrated information system for Heliothis management and deliver this in printed (published reports.

These will provide better, more specific information in the development of and investigation into area wide management strategies for invertebrate pest control in the grains industry. Ultimately the flow of benefits will come from a better-focused insecticide and bio-control usage. This could reduce the dependency on hard chemicals, preserving for longer time periods the efficacy of these existing chemicals. In addition it can provide the grains industry mechanisms for better environmental accountability to the Australian community. Finally at the completion of the proposed two-year project, the grains industry will have a fully operational facility in CID to continue monitoring recruitment and migration for as long as is deemed necessary.

There is currently strong support for this research and recent grower support is evidenced by the correspondences received by CID from the Heliothis Working Group, Dalby and the Darling Downs Cotton Grower Inc.. At present grower meetings have provided positive feedback on the project UQ117 and there is keen interest in the approach taken by UQ117 and this project.

In addition The University of Queensland has provided extra infrastructure support to CID to enhance the RDC investment. It is through these additional resources that this project can expand its commitments beyond the scope of the Darling Downs Area Wide Management program.

2. List the project objectives and the extent to which these have been achieved.

1. Further optimise and automate the microsatellite marker system for the management of Heliothis in grains and cotton, developed in research Project UQ117.

2. Provide evidence of local recruitment of Heliothis in grain and cotton crops and show the proportions of local recruitment and immigration over several crop sequences in the 2000/01 – 2002/03 growing seasons in a range of Area Wide Management programs in Queensland and New South Wales.

3. Attempt to define the source populations of Heliothis that migrate into the area wide management region of the Darling Downs under study in DAQ442, extending beyond this in the latter stages of this project.

3. Detail the methodology and justify the methodology used.

The project will focus on extending and optimising DNA based tools for analysis of population genetics of Heliothis. This will be done using the range of microsatellite molecular markers developed in project UQ117. There will be a focus on automating as much as possible the marker system to facilitate rapid data acquisition. These markers will be used to reveal the levels of local recruitment of Heliothis into populations found across the Darling Downs. In addition, the research program will examine how populations locally recruit and migrate across several crop sequences and seasons in established growing regions. Finally this project would seek to identify the origins of migratory Heliothis populations, which move into the established growing regions in each growing season.

Transgenic technologies are not the total answer to the problems of Heliothis control. Heliothis population dynamics have not been viewed on a regional scale and it is difficult to assess the impact of population movement and take pro-active measures to minimise crop damage. This program of research will provide genetic data to plan and present management strategies to grower groups for their inputs and advice. Establishment of population origins gives cotton and grains industries a pre-emptive ability to control Heliothis at its source before becoming a major problem.

The program of research will examine 2 sites in across the area Wide Management region of the Darling Downs. Material will be collected from single fields with 40 individuals collected form each field to provide the primary data sets over 4 time points in the Spring/Summer/Autumn growing period. In addition to this 20 individuals will be collected from a scattered range of fields (30 in total) over a 40 Km range (Figure 1). Microsatellite DNA markers will be used to measure the relatedness of these individuals to establish relative proportions of local recruitment and immigration. Data from these sites will be analysed in relation to the data collected from the field survey conducted by crop scouts as well as groups such as Dr Murray's in QDPI.

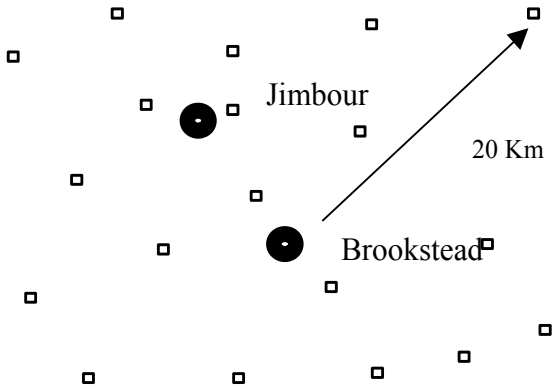

Figure 1. Collection Strategy
Jimbour and Brookstead will be the two intensive collection sites for the inter-field migration study. The 30 other sites will be within 20 Km of these two sites. These collections will study the inter-Area Wide Management migration.

Determination of migration of Heliothis is a large and complex problem. As a result of this we have broken the problem down into three distinct components in order to achieve satisfactory outcomes with reasonable resource constraints. The first component involves answering the question on a local scale, does the Heliothis found in one field come from an adjoining field? The second component involves answering the question in the Area Wide Management study region of the Darling Downs; does the Heliothis found in one field come from within 40 kilometres? The third component will answer the question, does the Heliothis found in a field come from outside the Darling Downs Area Wide Management region? All three components will be answered in this study. Subsequent years will use a similar strategy but expand the study in manageable increments to take the scope of collection beyond the Darling Downs region into other Heliothis affected areas. These would include the central and coastal Queensland, Northern and Southern New South Wales in years 2 and 3 respectively. This information will help underpin the development of strategic management plans for Heliothis and help control the build-up of insecticide resistance. If more data is added to these data over the longer-term information can be collected about resistance build-up to transgenic crops as well. This project will contribute to the overall development of a plan of actions that address the *Helicoverpa* problem. This plan will be developed in consultation with producers, consultants, researchers and extension personnel.

4. Detail and discuss the results including the statistical analysis of results.

Our data analysis has been divided into three major regions and is presented here in summary under the three sub headings.

Central Queensland Region
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 0.53-2.09 (with N = local population size, *m* = average rate of migration).

Nei's genetic distance 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.

Principle co-ordinate analysis 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 verses Biloela) within a region (between collections) was 14%, and within collections 86% (p=0.01).

Analysis of *H. armigera* using five microsatellite loci has shown significant differentiation between sampled collections in the Dawson/Callide Valleys (AMOVA 14% of variations is between collections) and low migration levels (N*m* = 0.53-2.09). This is consistent with the findings of Daly and Gregg (1985), where *H. armigera* was more restricted to cropping areas in comparison to the more mobile *H. punctigera*. However there was no significant differentiation between the collections from the Theodore area and those collections in the Biloela region in the 2000-2001 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 2000-2001 study of *H. armigera* in the Dawson/Callide Valleys, genetic structure was shown to vary seasonally, as genotypes were shifting as the months progressed. 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 between *H. armigera* in the months of September, October, November and December 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 small as well. The smaller genetic distances at this time may be due to larvae that are going into diapause in March being mostly the same moths which 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.

Darling Downs Region

The data analysis considered results on a yearly basis, with each year extending from the end of the summer growing season (April) through to March of the following year. Microsatellite analysis of *H. armigera* has shown high differentiation between collections over the 3 years of study, with AMOVA results showing between collection variations from 10% to 27%. Principle co-ordinate analysis of Nei genetic distance between collections indicated genotype shifts every few months, although in the April 2002 to March 2003 season this shift included a component of geographic differentiation. Sampling intensity increased with each year, permitting more detailed analysis each season. The genetic structures and profiles varied year to year over the research period.

Growing season from November 1999 - March 2000:

In the first data collection period from November 1999-March 2000 there was no significant differentiation between the two sampled regions of Jimbour and Brookstead, and a migration rate N*m* of 1.42 was recorded in February 2000 for Jimbour and Brookstead (with N = local population size, *m* = average rate of migration; Slatkin 1985, Slatkin and Barton 1989). An N*m* of 2.76 in March 2000 was recorded for the Brookstead and Jimbour localities.

Growing season from April 2000 - March 2001:

In the April 2000-March 2001 year, migration rates were slightly lower with N*m*=0.76 for October 2000 in Brookstead and Jimbour, and an N*m*=1.14 for March 2001 for the Cecil Plains, Kingsthorpe and Jimbour. During this period AMOVA results show that the Darling Downs was genetically distinct from both the Dawson-Callide Valleys growing regions (AMOVA 4% p=0.01) and the Lower Namoi (AMOVA 2% p=0.01).

Growing season from April 2001 - March 2002:

During the April 2001-March 2002 sampling year there was no significant difference between the collections of *H. armigera* from Jimbour to those from Brookstead. This is accompanied by a relatively high migration rate of N*m*=2.43 in October for the Brookstead and Gatton areas. The Darling Downs during this year had AMOVA differentiation from the McIntyre Valley of 1% (p=0.01) and of 2% from Griffith (p=0.01). There was no support

for a genetic differentiation of the Darling Downs collections from those of the Dawson-Callide Valleys or Lower Namoi at this time.

Growing season from April 2002 - March 2003:
Lastly, in the year extending from April 2002 to March 2003, very significant differentiation was evident in occurrences of *H. armigera* within the Darling Downs. Toowoomba, Kingaroy, Murgon and Dalby each formed distinct groupings with significant AMOVA supports. Migration rates at this time were also comparatively low (N*m* = 0.25-0.97). The Darling Downs in this year was genetically different from all other regions studied. Genotypes in the Darling Downs were most distantly related to those in Katherine (AMOVA 13% p=0.01) and the Dawson Callide Valleys (AMOVA 12% p=0.01), had levels of 9% differentiation from Griffith (p=0.01), and were most similar to the genotypes present in the Macintyre Valley (AMOVA 1% p=0.03).

Principle co-ordinate analysis (PCA) of Nei genetic distance between collections in the Darling Downs over 3 years, illustrates that the genotypes of *H. armigera* are shifting every few months. It should be noted however that for the collections which fall into the April 2002 to March 2003 period, the PCA analysis also reflects a component of geographic differentiation in its distributions, as in this period the geographic differentiation in the Darling Downs was statistically significant. This geographic differentiation in the April 2002 - March 2003 season is outlined further below. The genotype shifting which is seen in the remaining seasons is not a reflection of the geographic origin of the collections, as the AMOVA results demonstrate no statistically significant geographic structuring in the region at this time. This observed genotype shift is in concordance with observations made of *H. armigera* populations in the Dawson-Callide Valleys (Scott *et al.* 2003), and findings in Monarch butterflies (Eanes and Koehn 1978). This may reflect a 'founder-like effect' due to non-uniform distribution of the pest in both space and/or time, and as a result of fluctuating population sizes. Other likely contributors to shift in *H. armigera* genotypes are the high level of local selection applied to the species through insecticide usage, agricultural practices and seasonal variation. The principle co-ordinate analysis suggests there was a significant event in the Darling Downs between February of 2002 and October of 2002, as there is evidence of a large genotype shift at this time.

Genetic structuring in *H. armigera* has varied yearly over the 38-month collection period of this study. Over the course of this study sampling intensity also increased with each year providing more data, and allowing more detailed analyses in subsequent seasons.

Growing season from November 1999 - March 2000:
In the first year of study, November 1999-March 2000, AMOVA results comparing the Jimbour to the Brookstead area of the Darling Downs showed no significant differentiation of genotypes at the two localities. Estimates of migration as determined by the private alleles method (Slatkin 1985, Slatkin and Barton 1989) were N*m*=1.42 in February 2000 for Jimbour and Brookstead (with N = local population size, *m* = average rate of migration). In March 2000, an N*m* of 2.76 was obtained for the Brookstead and Jimbour localities. This data would suggest that in this initial study season, Jimbour and Brookstead was effectively a single management unit for *H. armigera,* with no statistically significant differentiation of genotypes between the regions. The migration rates in this year were relatively high in comparison to rates in subsequent years in the Darling Downs. Migrations rates of N*m*>1 is considered to be sufficient to make gene flow overcome genetic drift (Wright 1931). The migration rates in this study are smaller than those described in *H. armigera* by Korman *et al.* (1993), Nibouche *et al.* (1998) and Zhou *et al.* (2000). This may be partly due to a change towards microsatellite technologies, a reflection of the geographic scale analysed, or a reflection of the years in which this data was collected.

Growing season from April 2000 - March 2001:
In the April 2000-March 2001 year, migration rates were slightly lower than the previous year with N*m*=0.76 for October in Brookstead and Jimbour, and an N*m*=1.14 for March 2001 for Cecil Plains, Kingsthorpe and Jimbour. During this year, microsatellite analysis was also completed for *H. armigera* from 3 major crop-growing regions in Australia. This analysis showed that the Darling Downs was genetically distinct from both the Dawson-Callide Valleys growing region (AMOVA 4% p=0.01) and from the Lower Namoi region (AMOVA 2% p=0.01). The AMOVA values separating the Darling Downs from the Dawson-Callide Valleys and from the Lower Namoi, in combination with moderate migration rates, indicates that in this year there was a moderate level of *H. armigera* movement within the Darling Downs, although not high enough to remove all local structuring in the region (i.e. to overcome the effect of drift). If more intensive sampling had been available in this year the "effective populations" or "management units" may have been able to be described. Data from this year also suggests there was no massive movement of reproductively effective moths from either the Dawson-Callide Valleys or the Lower Namoi region into the Darling Downs.

Growing season from April 2001 - March 2002:
During the April 2001-March 2002 season there was again no significant difference between the collections of *H. armigera* from Jimbour to those from Brookstead. This was accompanied by a high migration rate in October for Brookstead and Gatton N*m* = 2.43. The Darling Downs during this year had AMOVA differentiation from the McIntyre Valley of 1% (p=0.01) and of 2% from Griffith (p=0.01). There was no genetic support for a differentiation of the Darling Downs collections from those of the Dawson-Callide Valleys or Lower Namoi at this time. In this year, the local AMOVA analysis between the Jimbour to Brookstead regions, the high migration rates and the AMOVA results between the Darling Downs and four other major growing regions through New South Wales and Queensland all support the suggestion that in this year, there was quite a significant and probably prolonged moth movement during the year. Further from this, it might be expected that the management of *H. armigera* in the Darling Downs may have been influenced by the occurrences of the pest in both the Dawson-Callide Valleys and the Lower Namoi regions. It is possible that any one of these three regions was the source of the *H. armigera* outbreaks during the year, or alternatively that the three regions all had a common source of *H. armigera* (which did not necessarily include any of the described regions). Ecological observations may bring more clarity to the probable source. It is interesting to note that in this year the Darling Downs was significantly different from the Macintyre Valley so the movement of *H. armigera* was not continuous from New South Wales through Queensland.

Growing season from April 2002 - March 2003:
In the final year of study, extending from April 2002 to March 2003, very significant differentiation was evident between occurrences of *H. armigera* within the Darling Downs. Toowoomba, Kingaroy, Murgon and Dalby each formed distinct groupings with significant AMOVA supports. Migration rates at this time were also comparatively very low (N*m* = 0.25-0.97). The low migration rates and the high levels of local differentiation are consistent with minimal *H. armigera* moth movement in this year. The intensive sampling undertaken in the Darling Downs in this year has highlighted the capacity of this microsatellite data to define potential management units for *H. armigera* even at the local scale. In addition to the local structure, the Darling Downs in this year was genetically different from all other regions studied at the time (Dawson-Callide Valleys, Macintyre Valley, Griffith and Katherine). Genotypes in the Darling Downs were most distantly related to those in Katherine (AMOVA 13% p=0.01) and the Dawson Callide Valleys (AMOVA 12% p=0.01). The Darling Downs had 9% (p=0.01) AMOVA differentiation from Griffith, and were most similar to the genotypes present in the Macintyre Valley (AMOVA 1% p=0.03). It is interesting to note that in this season *H. armigera* in the Darling Downs showed greatest similarity to the genotypes found in the Macintyre Valley. This is in contrast to the previous year where the Darling Downs had most similarity to collections in the Dawson Callide and Lower Namoi. Perhaps the small amount of moth movement that still occurs (even in low movement years) may have been in the direction from Macintyre valley to the Darling Downs, from the Darling Downs to the Macintyre Valley, or possibly that both these regions had some common origin of source moths in this year.

Narrabri Region
Low levels of gene flow were detected between the *H. armigera* collected from the Narrabri region and those from the Darling Downs region between March and June, 2000 (8% AMOVA variance, p<0.001). Significant local structure was also observed within each region at this time (15% AMOVA variance within both regions, p<0.001) while 77% of AMOVA variance (p<0.001) occurred within each collection of *H. armigera*. The *H. armigera* collected from the Narrabri, central Queensland and Darling Downs regions between March and June, 2001 were also compared. At this time, gene flow was more restricted between the regions than it was in 2000, with 14% AMOVA variance identified between regions (p<0.001). Increased genetic differentiation between regions in 2001 was not due to the addition of the third sampling region, central Queensland. When AMOVA analysis was conducted using only those individuals collected from Narrabri and Darling Downs in 2001, AMOVA variance between regions was 22% (p<0.001). Therefore, the analysis inclusive of central Queensland was a more conservative estimate of AMOVA variance between regions than when only the Narrabri and Darling Downs regions were compared. Local structure was also observed between collections within each of the three regions in 2001 (10% AMOVA variance, p<0.001) and was not as distinct as the genetic structure observed in 2000. A large proportion of the AMOVA variance was again identified between individuals from single collections (76% AMOVA variance, p<0.001).

To confirm the results of AMOVA, an estimate of the number of migrant *H. armigera* per generation (N*m*) that entered Narrabri in 2000 and 2001 was calculated using the private alleles method (Slatkin, 1985). The N*m* detected for Narrabri was 0.885 in April, 2000 and 1.787 in April, 2001. These low N*m* values support results

from the AMOVA that low levels of migrant *H. armigera* contributed to the genetic structure of *H. armigera* in the Narrabri region in autumn of 2000 and 2001.

Collections of *H. armigera* were examined from two area-wide management groups in the Narrabri region, Two Rivers and Myall Vale. Six out of eight collections from April 2000 in the Two Rivers area formed a genetically distinct cluster when a Principle Coordinates Analysis (PCA) plot was constructed based on Nei Genetic Distance. However, samples collected from the Two Rivers area in 2001 and those from the Myall Vale area in 2000-2001 did not form distinct genetic groupings. There was no genetic distinction between *H. armigera* collected from Two Rivers and those collected from Myall Vale (0% AMOVA variance was detected between these areas in 2000 and 2001), however the results from AMOVA were not significant ($p=0.365$ and $p=0.663$ in 2000 and 2001, respectively). In contrast, significant genetic structure was detected on a smaller geographic scale, with 16 and 12% of AMOVA variance partitioned between collections within each area in 2000 and 2001, respectively ($p<0.001$ in both years). Eighty-four and 88% AMOVA variance was also detected between individuals within each collection in 2000 and 2001, respectively ($p<0.001$ in both years).

A distinct genetic shift was observed over a period of one year when *H. armigera* collected from the Narrabri region in April 2000 were compared with those collected between March and June, 2001. This result was based on the PCA of Nei Genetic Distance.

This study examined *H. armigera* migration patterns on a regional scale using microsatellite DNA markers to detect gene flow between three cotton-growing regions, Narrabri, New South Wales, central Queensland and Darling Downs, Queensland. Only low levels of gene flow were detected between the three regions in autumn of 2000 and 2001. This low level of gene flow between regions over the study period indicated that the majority of *H. armigera* in each region originated locally. Further evidence for the low gene flow between collections of *H. armigera* was found in the high degree of genetic differentiation measured between collections within each region (15 and 10% AMOVA variance, $p<0.001$) and between individuals within each collection (77 and 76% AMOVA variance, $p<0.001$) in 2000-2001, respectively. If high levels of migration and successful breeding upon arrival occurred in 2000 and 2001, a more uniform genetic structure would be expected on both a regional and a local scale. Support for restricted migration of *H. armigera* in the Narrabri region has been provided in a mark recapture study when many *H. armigera* were recaptured within ten kilometres from the site of emergence (Fitt and Dillon 1993). However, when allozyme studies of *H. armigera* were carried out by Daly and Gregg (1985), they found no evidence of restricted gene flow. It is possible that migration of *H. armigera* occurred between the study regions over the time period analysed by Daly and Gregg (1985). Alternatively, the disparity in results between the allozyme study and this study could be due to differences in sensitivity of the molecular techniques used.

Although the *H. armigera* analysed in this study exhibited little evidence of migration, this does not imply that *H. armigera* are incapable of long-distance migration. For example, pollen studies have shown that *H. armigera* are capable of moving large distances (Gregg 1993). The low levels of gene flow detected in this study could have resulted from an abundance of crops such that local *H. armigera* were not forced to migrate into or out of the study area to obtain appropriate food or oviposition sites. A higher level of gene flow occurred between the major study regions in 2000 than in 2001 (8% AMOVA variance was measured between regions in 2000 in comparison with 14% AMOVA variance in 2001). This difference might be the result of a lower abundance of crops in 2000, thereby causing more *H. armigera* to migrate to obtain suitable food sources. Higher levels of gene flow in 2000 could also be influenced by meteorological conditions such as rainfall, temperature, and wind speed and/or direction.

Further support for the low levels of gene flow into Narrabri from other regions was found by calculating the values for Nm, or number of migrants per generation. Nm was low in both April 2000 (Nm=0.885) and in April 2001 (Nm=1.787). These rates were similar to those measured for *H. armigera* collected from the Dawson/Callide Valleys, central Queensland in March, 2001 (Nm = 2.09) (Scott *et al*., 2003). The number of migrants per generation entering Narrabri, NSW was low and therefore *H. armigera* collected from this region were mostly of local origin.

When genetic structure was examined between two area-wide management groups in the Narrabri region; Two Rivers and Myall Vale, *H. armigera* collected from Two Rivers were not found to be genetically distinct from those collected in Myall Vale (0% AMOVA variance between areas, $p=0.365$ and $p=0.663$ in 2000 and 2001, respectively). The lack of genetic structure between these two areas could be due to the small geographic distance (approximately 45 kilometres) separating these two areas or it could be season specific.

Collections of *H. armigera* from Narrabri made in autumn of 2000 were genetically distinct from *H. armigera* collected in autumn of 2001. This shift in genetic structure over time could be the result of many influences, including fluctuations in *H. armigera* abundance due to crop availability, climatic or seasonal variables such as rainfall and temperature, and reproductive factors. However, as *H. armigera* is a problematic pest, an important influence on its genetic structure may be management practices and the control of infestations using hard or soft chemistries. When chemistries are applied, this results in a bottleneck where the next generation will arise from a limited amount of genetic diversity. In addition, the next generation may arise from specially selected genotypes that were able to survive the application of chemistries due to the accumulation of resistance. Constant fluctuations in population size and genetic diversity would likely result in rapid changes in genetic structure over time as evidenced in this study. Changes in genetic structure have already been measured over one to three month intervals in *H. armigera* collected in central Queensland in 2001 (Scott *et al*., 2003). In Narrabri, *H. armigera* may be exposed to a number of pesticide applications over a growing season (M. Dillon, pers. comm.) and subsequently the observed change in genetic structure over one year was not unexpected.

This study has identified significant genetic variability between collections of *H. armigera*, not only on a regional scale (between three distinct cotton growing regions) but also within the Narrabri region and within individual collections. These results demonstrate that in autumn of 2000 and 2001, infestations of *H. armigera* in Narrabri were primarily the result of breeding by local populations. Although the majority of *H. armigera* in this study were found to originate locally, this species is known to be capable of migrating long distances (Gregg 1993). Monitoring *H. armigera* migration over a large scale will therefore identify if and when possible future long distance migration events occur. Future studies of *H. armigera* migration should ideally employ a combination of both tracking methods and molecular genetic techniques to detect and aid in predicting migration as well as to correlate migration with outbreaks of resistance. These studies will enhance our understanding of the migration patterns of this problematic pest and should allow for the design of more effective area-wide pest management strategies.

5. Provide a conclusion as to research outcomes compared with objectives. What are the "take home messages"?

Central Queensland
The results from this part of the study indicate that the management of *H. armigera* should be focused at the local/regional level in the Dawson/Callide Valleys, as the majority of the *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.

Darling Downs Region
The microsatellite data collected in the Darlings Downs over the 38-month period from November of 1999 through to January of 2003 has successfully described the level of *H. armigera* movement on local and regional scales. In the year from April 2001 through to March 2002 it showed genetic characteristics consistent with significant genetic mixing and thus adult movement across the Darling Downs. In contrast the genetic data finds, that in other years, such as April 2002 - March 2003, there was significant local structuring of populations within the Darling Downs, very low levels of migration, and no evidence of large-scale movement of *H. armigera* adults across cropping regions either within or between Australian states. This data also seems to provide some evidence for the direction in which movement may be occurring, and that the direction of movement may differ from year to year.

Narrabri Region
This study has identified significant genetic variability between collections of *H. armigera*, not only on a regional scale (between three distinct cotton growing regions) but also within the Narrabri region and within individual collections. These results demonstrate that infestations of *H. armigera* in Narrabri were primarily the result of breeding by local populations. Although the majority of *H. armigera* in this study were found to originate locally, this species is known to be capable of migrating long distances (Gregg 1993). Monitoring *H. armigera* migration over a large scale will therefore identify if and when possible future long distance migration events occur.

This microsatellite research supports the current area-wide management strategies for *H. armigera* by demonstrating that *H. armigera* can in some years be relatively 'local' and thus likely to be more significantly influenced by ecology and local management practices, however regional or Australia-wide approaches need to remain co-ordinated to manage the pest in years with high levels of migration.

6. Detail how your research has addressed the Corporation's three Outputs - Economic, Environmental and Social?

Output 1
The research project UQ32C is providing valuable information on population movement of Heliothis. With the current state of movement being very small the focus is on management at a local level though the Area Wide Management groups within all of the regions under study. Many AWM groups have meet with CID staff to discuss the present low level of migration and so the focus is turning to critical evaluation and implementation of strategies that will minimise the prevalence of Heliothis early in the season to ameliorate the locally recruited problems that have been arising in late Stage II and Stage III. Species composition has been examined across all regions and was assessed for its value to cotton and grain growers in the 2002-2003 season. Pesticide usage can be reduced or adjusted if high numbers of *H. punctigera* are present against the more problematic *H. armigera*. This information will be available every 12 weeks in the 2002-2003 season.

Output 2
CID has been developing a working relationship with Cotton Grower Services to deploy a trial diagnostic service to the cotton industry in the 2002-2003 season. A Memorandum of Understanding (MOU) is in place between CID and CGS. CID has previously developed diagnostics for information support to the cotton and grains industries. The diagnostics to be deployed this season include, Trichogramma parasitism, NPV infection and species diagnostic (*H. punctigera* vs. *H. armigera*). Additionally, CID has been discussing and progressing forward on developing further diagnostics to resistance gene targets currently available in the scientific literature. These are ready for use in the 2003-2004 season. The intent of this deployment is to allow growers and consultants to make management decisions about the need or otherwise for chemistry application should there be high levels of egg parasitism by Trichogramma wasps, changing species composition and infection rates of NPV. This will be done across time providing a simple and flexible way of assessing which, if any chemistries or biocontrols can be used, potentially minimising the use of hard chemistries over softer options. While softer options tend to more expensive "going soft early" allows the beneficial potential to be fully realised making numbers of applications fewer, less destructive and allows the retention of hard chemistries if needed in Stage III.

The technology developed has the capacity to be used as a secondary research tool by other researchers looking at ecological and behavioural aspects of Heliothis in the cotton cropping system. Some examples of these are:

- Genetic arcitecture of Heliothis within trap crops and cotton, within non-Bt cotton and Bt cotton cultivation.
- Fate of egg lay from indvidual female Heliothis.
- Mapping of resistance genes to control chemicals.

Output 3
It is anticipated that information from this research will allow more targeted (i.e. Minimal) application of damaging chemistries. The environmental aspects of chemical use and their exposure and affects on rural communities will be minimised.

7. Provide a summary of the project ensuring the following areas are addressed:

Five microsatellites loci were screened across twenty-eight localities from eight cotton and grain growing regions across Australia over a 38-month period (from November of 1999 through to January of 2003). The regions included the Central Highlands, the Dawson-Callide Valleys, the Darling Downs, Macintyre Valley, Namoi Valley, Murrumbidgee Valley, Tatura and Katherine. Using AMOVA analysis, mantel tests and migration rates calculated using private alleles, this study describes both high and low *H. armigera* movement. The April 2001 to March 2002 period showed genetic characteristics consistent with significant gene flow post moth migration occurring between regions during this year. However, the year from April 2002 to March 2003 demonstrated significant genetic structuring, indicating an absence of large-scale gene flow from *H. armigera* migration across cropping regions. These data indicate the importance of the current area-wide management strategies by demonstrating that in some years *H. armigera* is relatively sedentary and thereby influenced by local management practices. However, the coordination of regional and Australia-wide approaches needs to continue as significant migratory events can occur in some years.

The current microsatellite study has two facets that have given it particular value; the microsatellite technology and the sampling strategy.

Microsatellites are a robust technology that has been applied very widely to assess gene flow and population structuring. Microsatellites have several key advantages; they have a very high mutation rate (up to 1 in 1000 gametes in humans), are transferable and amenable to high throughput analysis (requiring only small amounts of crude DNA extraction). The allelic diversity of microsatellites make them the system of choice for assessing gene flow, for example, in this study 147 alleles were identified from 5 loci for samples collected in winter 2001 (Mar-Aug). This far exceeds the allelic diversity for any previous genetic analysis in *Helicoverpa* anywhere in the world.

This study has been undertaken to answer questions concerning the migration and recruitment (i.e. gene flow) of *H. armigera* on a broad range of scales; analysis between nearby fields (1-10km), within area wide units (10-

50km), within a region (50-250km) and between regions (250-1000+km). To attempt to answer questions over these scales it is critical that samples are appropriately stratified. Furthermore, the timescale within and between seasons adds another level of complexity. Even if analysis was to focus on a single scale, e.g. the level of gene flow between the regions, sampling within regions is still required to provide a comparative scale for assessing inter-regional variation.

A particular highlight of this project has been the intensive sampling across cotton and grain growing areas of Eastern Australia. To date more than 5000 *H. armigera* individuals have been analysed. This comprises the largest single study of insect population genetics we can identify. This has allowed us to relate the wealth of population information obtained to questions of broad migration and recruitment and to local questions of management. The large number of populations analysed have allowed us to empirically assess the effects of simpler sampling strategies. Unsurprisingly, if the number of populations is reduced, the incidence of rare alleles identified is significantly eroded and the allele frequencies potentially skewed. For example data demonstrates significantly reduced allele diversity in St George and the Darling Downs where fewer populations were available due to decreased pest pressure. If the number of populations analysed were reduced, a similar loss of allelic diversity occurs as in the central Qld collections.

(a). Technical advances achieved (eg commercially significant developments, patents applied for or granted licenses, etc.)

Diagnostic development capabilities and microsatellite technologies have been developed and refined. A number of facilities are present in the cotton growing regions of Australia and can be made commercially ready when the cotton industry comes back to full production following the drought.

(b). Other information developed from research (eg discoveries in methodology, equipment design, etc.)

This project has made significant advances in protocols of large sample coordination and sample processing. While the protocols are equipment intensive there is no new design but rather an optimal way in which to conduct work flow routines in a molecular laboratory that significantly streamlines sample processes and analysis. The Centre has developed significant expertise in dealing with large format systems and coordination of analysis and reporting.

(c). Are changes to the Intellectual Property register required?

There will be no changes required to the Intellectual Property register.

8. Detail a plan for the activities or other steps that may be taken:

(a). To further develop or to exploit the project technology.

This project is a continuing program of research and investigation. Clearly supporting the use of this equipment and process to develop molecular based diagnostics for decision support management would allow enhancement of the total pest picture in the agro-ecosystem of cotton. The technologies and expertise developed in this project could be very simply used to study the population structure and migration of any insect species, either pest or beneficial. Exploitation of these "know-how" only requires project planning and funding.

(b). For the future presentation and dissemination of the project outcomes.

At this time a number of publications are in preparation and a number in print. This project is ongoing and the presentation of results and findings will continue at grower field days, organised meetings such as the Toowoomba planning meeting, Entomology conferences and through written reports. Many of these are of an adhoc nature and are taken up as opportunities present themselves or when the research team is invited to attend (such as the CCA meetings).

(c). For future research.

The future research is currently underway in CRDC/GRDC Project UQ 35C. Future studies of *H. armigera* migration should ideally employ a combination of both tracking methods and molecular genetic techniques to detect and aid in predicting migration as well as to correlate migration with outbreaks of resistance. These studies will enhance our understanding of the migration patterns of this problematic pest and should allow for the design of more effective area-wide pest management strategies.

9. List the publications arising from the research project and/or a publication plan.

Scott KD, Wilkinson KS, Merritt MA, Scott LJ, Lange CL, Schutze MK, Kent JK, Merritt DJ, Grundy PR, Graham GC (2003) Genetic shifts in *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) over a year in the Dawson/Callide Valleys. *Australian Journal of Agricultural Research* 54, 739-744.

Scott, K.D., Lange,C.L., Scott,L.J, Graham,G.C. (submitted). Isolation and characterisation of microsatellite loci from *Helicoverpa armigera* Hübner and *Helicoverpa punctigera* Wallengren (Lepidoptera: Noctuidae). *Molecular Ecology Notes*.

Scott,K.D., Lawrence,N., Lange,C.L., Scott,L.J., Wilkinson,K.S., Merritt,M.A., Miles,M., Murray,D., Graham,G.C. (submitted). Seasonal gene-flow of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) in the Darling Downs from Novemeber 1999-March 2003. *Australian Journal of Agricultural Research*

M.A.Merritt, K.S.Wilkinson, K.D.Scott, M.Dillon, L.J.Scott, C.L.Lange, M.K.Schutze, J.K.Kent, D.J.Merritt and G.C.Graham (prepared) Gene flow in two consecutive collections of the Lepidopteran pest, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) from the Narrabri region, New South Wales. *Australian Journal of Agricultural Research*

Scott,K.D., Wilkinson,K.S., Lawrence,N., Lange,C.L., Scott,L.J., Merritt,M.A., Graham,G.C. (prepared). *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) population differentiation and movement across Australia from November 1999-March 2003. *Molecular Ecology*

Wilkinson, K.S., Merritt, M.A, Scott, K.D., Lange, C.L., Schutze, M.K., Scott, L.J., Kent, J.K., Merritt, D.J., and Graham, G.C. (prepared) The distribution of *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) and *Helicoverpa punctigera* Wallengren (Lepidoptera: Noctuidae) in Australia over a three-year period. *The Australian Journal of Entomology*.

Merritt,M.A., Scott,K.D., Wilkinson,K.S., Scott,L.J., Lange,C.L., Schutze,M.K., Kent,J.K., Merritt,D.J., Graham,G.C. (2003) Population genetics and migration of the Lepidopteran pest, *Helicoverpa armigera* over a two year period in Australia. Plant and Animal Genome Conference, San Diego, USA.

10. Provide an assessment of the likely impact of the results and conclusions of the research project for the cotton industry. Where possible include a statement of the costs and potential benefits to the Australian cotton industry or the Australian community.

The likely impacts of these outcomes is an increased ability to monitor and track populations of Heliothis in Cotton growing regions of Queensland and New South Wales. With the utility of these microsatellite markers in *H. punctigera* proven, it would also be possible to extend studies of *H. punctigera* in the southern states such as Victoria and South Australia if necessary or desirable.

Ultimately the sources of resistance build-up could be found and management strategies implemented for build-up avoidance and control.

The current project was a study to understand, using DNA marker systems the proportion of local recruitment to migrants in a defined study area. Cotton industry could employ this marker technology into area wide management strategies as decision support tools for control strategy selection. To do this a database system capable of online interrogation is needed to allow all researchers, crop consultants and growers working in area wide management strategies, access to the most recent source population information. Management recommendations arising from this data could be included in industry publications.

The research work to commence this year will begin the search for the origins and extent of Heliothis migration. The outcomes for the cotton industry in the second project will be to show specifically where migrating populations come from and when migration occurs. While this is an enormous task, the project seeks to commence the development of an integrated strategy that can continue to provide information to growers, crop consultants and managers of Heliothis problems to help sharpen decision processes in terms of chemical and bio-control usage in cooperative and structured area wide management. Ultimately the flow of benefits will come from a better-focused insecticide, GM crop deployment and bio-control usage. This should reduce the dependency on hard chemicals and preserve for longer time the efficacy of these chemicals.

Part 4 – Final Report Executive Summary

Provide a one page Summary of your research that is not commercial in confidence, and that can be published on the World Wide Web. Explain the main outcomes of the research and provide contact details for more information. It is important that the Executive Summary highlights concisely the key outputs from the project and, when they are adopted, what this will mean to the cotton industry.

Cotton bollworm (*Helicoverpa armigera* Hübner, Lepidoptera: Noctuidae) is an insect that often develops resistance to agricultural insecticides and is a significant pest on cotton, grains and horticultural crops due to its polyphagous nature and ability to rapidly develop large pest populations. *H. armigera* is widely distributed, occurring in Africa, the Middle East, India, Australia, and Asia. The effective management of *Helicoverpa* in crops is complicated by the variability in their infestation levels between regions and across seasons. The capacity *H. armigera* has for extensive movement greatly complicates the understanding of population dynamics, because local populations at any time may consist of elements of diverse origins. To improve the effectiveness of managing this significant pest, advancements in our understanding of the genetic structures and migratory behaviour of *H. armigera* will be critical. Diverse approaches, both traditional and molecular, have been employed to assess dispersal, migration and gene flow of *H. armigera* across agricultural growing regions in Australia. The results of these studies have been mixed, identifying either low levels of migration, high levels of long-range dispersal, or an intermediate status. Microsatellites loci were screened across twenty-eight localities from eight cotton and grain growing regions across Australia over a 38-month period (from November of 1999 through to January of 2003). The regions included the Central Highlands, the Dawson-Callide Valleys, the Darling Downs, Macintyre Valley, Namoi Valley, Murrumbidgee Valley, Tatura and Katherine. Using AMOVA analysis, mantel tests and migration rates calculated using private alleles, this study has revealed both high and low *H. armigera* movement.

It is clear that in most of the years of the study, the three major growing regions were mostly independent and so management of the pest could be achieved separately. Therefore in many years pest populations arise through local recruitment and are a direct consequence of local management practices. Despite genetic structuring being common during the study period there is still strong evidence that national coordination of management is important. For example in the high mixing year most regions were not distinguishable and could not be considered independent. Even on low or moderate genetic structuring years there were still small but potentially significant immigration events in many regions. The April 2001 to March 2002 period showed genetic characteristics consistent with significant gene flow post moth migration occurring between regions during this year. However, the year from April 2002 to March 2003 demonstrated significant genetic structuring, indicating an absence of large-scale gene flow from *H. armigera* migration across cropping regions.

This research supports the current area-wide management strategies for *H. armigera* by demonstrating that *H. armigera* can in some years be region specific and thus significantly influenced by local management practices. However regional or Australia-wide approaches need to remain co-ordinated to manage the pest in years were migration levels are high, as area-wide management strategies may be overcome in these years by more geographically significant events such as unseasonal weather patterns. Local population management in all regions may minimise the likelihood of large population migrations through continual pressure on pest numbers.

These data indicate the importance of the current area-wide management strategies by demonstrating that in some years *H. armigera* is relatively sedentary and thereby influenced by local management practices. However, the coordination of regional and Australia-wide approaches needs to continue as significant migratory events can occur in some years.

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