Final Report to CRDC

Population Genetics of Heliothis Migration and Recruitment to Support a Regional Management Strategy

Glenn Graham

The University of Queensland

18 November 1999

Final Report to CRDC

Glenn Graham **8 November 1999**

Project Title: Population Genetics of Heliothis Migration and Recruitment to Support a Regional Management Strategy

Project No: UQ117 (98/GRDC003G)
CRDC Project No.: UQ25C

Organisation: Centre for Identification and Diagnostics of Insects and Weeds, The University of Queensland.

Administrative Contact: Ms Brenda Coate
E-Mail: bcoate@research.uq.edu.au **Phone:** (07) 3365 3519 **Fax:** (07) 3365 4455
Postal Address: Research Administration Office, Faculty of Biological and Chemical Sciences, The University of Queensland, St Lucia, 4072, Australia.

Project Supervisor: Mr Glenn Graham
E-Mail: G.Graham@cpitt.uq.edu.au **Phone:** (07) 3365 1863 **Fax:** (07) 3365 1861
Postal Address: Assistant Director, Centre for Identification and Diagnostics of Insects and Weeds, 4th Floor Gehrmann Laboratories, The University of Queensland, St Lucia, 4072, Australia.

Principle Researcher: Mr Glenn Graham
E-Mail: G.Graham@cpitt.uq.edu.au **Phone:** (07) 3365 1863 **Fax:** (07) 3365 1861
Postal Address: Assistant Director, Centre for Identification and Diagnostics of Insects and Weeds, 4th Floor Gehrmann Laboratories, The University of Queensland, St Lucia, 4072, Australia.

Summary:

The significant threat of Heliothis on the Darling Downs and elsewhere was demonstrated by the serious damage suffered by growers in the 1997/98/99 season. At present there is a need for a Management Strategy that considers the Heliothis problem at a population level across the entire Darling Downs.

The aim of this study was to construct a microsatellite library for the pest moth *Heliothis armigera* and to use this library to investigate temporal shifts in population structure. Specifically, the aim of the study was to determine whether 1998-1999 seasonal infestations of *H. armigera* in southwestern Queensland were the result of migration from an external source or were derived from previous populations. A secondary aim was to investigate the relationship between larval survivorship and source population.

Genotyping using microsatellite loci provides several advantages over other methods of measuring genetic variation in studies directed at determining relatedness between individuals and populations. These include high frequency of loci and occurrence in regions of low recombination; short length of sequences, which facilitates genotyping by automated techniques such as polymerase chain reaction (PCR); the ability to obtain results with small quantities of DNA; high levels of polymorphism which enables detection of small genetic variation; provides the highest probability of parentage determination and conservation of repeat sequences across related taxa, allowing use of primers developed for *H. armigera* to be used in *H. punctigera*.

Summary of Findings and Outcomes:

- Five microsatellite libraries were made of *H. armigera* and *H. punctigera*.
- 223 sequences performed, finding 40 microsatellite DNA repeats.
- 26 primer sets for microsatellites were developed for further analysis.
- At present 15 primer sets are used in analysis of *H. armigera* populations.
- Results from analysis:
  - In the spring population 35% were locally recruited (from only 7% of winter individuals), 65% immigrated.
  - Summer population contains 10% were locally recruited individuals (from only 5% of spring individuals), 90% immigrated.
  - Autumn population contained 35% were locally recruited individuals (from only 12% of summer individuals), and 65% immigrated.
- Migrations are highest during summer, presumably resulting from the general increase in insect numbers and dispersal associated with the warmer months.
- When each population experiences a bottleneck (eg after application of pesticides) some of the new migrant genotypes are lost, while some (perhaps including those which are resistant) are retained and carry through to the following generations.

- The persistence of original winter genotypes in all populations suggests linkage between microsatellites and factors that reduce mortality ie. Microsatellites are potential markers for pesticide resistance.
- These microsatellite markers could be exploited to track pesticide resistance after linkage to the markers is established.

The data illustrates the relative contribution of each population to those that follow. As the winter population represents only the first point in the time series, it is best interpreted as a tentative measure of contribution to following generations. Data from subsequent years will strengthen these data.

The primary points of note are that some genotypes from the original winter population persist throughout the season. All populations show a higher level of migration than local recruitment. The summer population contains the greatest proportion of migrants. Markers to reduced mortality factors may have been found and appears promising. While it is tempting to discuss origins of migrant populations these were not determined as part of this program of research and is the topic of subsequent work proposed in 2000/01.

Project Objectives:

This program of research is designed to achieve several things.

- Establish a DNA marker system using microsatellites to answer questions of population movement and recruitment. These markers are generic and may be used to answer other questions in population biology of Heliothis.

- Use these DNA markers to establish over 3 sites in the Brookstead/Cecil Plains region of the Darling Downs, what proportion of Heliothis is locally recruited and what proportion are migrants entering the regions of study.

Previous attempts to measure movements of Heliothis have had varying success. The current strategy has the advantage of identifying a single individual much like the work performed in human pedigrees. This gives us the advantage of greater accuracy of assigning individuals to preceding generations giving more precise measurements of recruitment and migration.

The project is an integral part of a series of studies designed to address the Heliothis problem and add a cooperative management strategy to controlling this very damaging pest through the Area Wide Management Strategy project conducted by QDPI on the Darling Downs.

Project Methodology:

Microsatellites are regions of nuclear DNA containing tandem repeats of a short (</=6 base pairs) sequence of nucleotides, flanked on each side by unique sequences (Weber & May 1989; Hughes & Queller 1993; Armour *et al.* 1994; DeWoody *et al.* 1995). The number of repeats of the base sequence at any given locus is generally determined in Mendelian fashion, with individuals receiving one allele from each parent (X repeat units from parent A and Y repeat units from parent B). Quantification of variation in repeat sequences can then be used to determine relatedness between individuals (Wall *et al.* 1993; Marklund *et al.* 1994; Craighead *et al.* 1995) and in population studies through comparison of allele freqnencies and generation of distance statistics (Nei 1978; Wall *et al.* 1993; Marklund *et al.* 1994; Roy *et al.* 1994).

Genotyping using microsatellite loci provides several advantages over other methods of measuring genetic variation in studies directed at determining relatedness between individuals and populations, including:

i. High frequency of loci (Valdes *et al.* 1993; Armour *et al.* 1994) and occurrence in regions of low recombination (Stephan & Cho 1994)

ii. Short length of sequences, which facilitates genotyping by automated techniques such as polymerase chain reaction (PCR) (Ellegren 1992; Menotti-Raymond & O'Brien 1995).

iii. The ability to obtain results with small quantities of DNA even when some degradation has occurred (Sherwin *et al.* 1991; Estoup *et al.* 1993;

Hughes & Queller 1993; Stephan & Cho 1994; DeWoody *et al.* 1995; Menotti-Raymond & O'Brien 1995)

iv. High levels of polymorphism which enables detection of variation not revealed using other methods (Hughes & Queller 1993) and provides the highest probability of parentage determination of all methods currently available (Marklund *et al.* 1994; Craighead *et al.* 1995).

v. Conservation of repeat sequences across related taxa, allowing use of primers developed for one species in studies of related species (Moore *et al.* 1991; Ellegren 1992; Estoup *et al.* 1993; Stephan & Cho 1994; DeWoody 1995; Menotti-Raymond & O'Brien 1995).

Increasing use of microsatellites in studies of population genetics and interrelatedness has resulted in refinement of methods and techniques (eg. Ostrander *et al.* 1992; Estoup *et al.* 1993; Valdes *et al.* 1993; Wall *et al.* 1993; Armour *et al.* 1994; Ferraris & Palumbi 1996; Strassman *et al.* 1996). Potential problems with techniques and analyses, such as non-amplification of alleles, (Pemberton *et al.* 1995; CSIRO 1996) and higher incidence of mutation in one sex (Weber & Wong 1993) are relatively well understood in comparison with other methods and measures can be taken to address these issues (Pemberton *et al.* 1995; Brownstein *et al.* 1996; CSIRO 1996).

Microsatellite techniques are now considered a standard method in studies of mating systems, relatedness and population genetics (Lessa & Applebaum 1993) and have been employed in a wide range of taxa (Amos *et al.* 1993; Brown *et al.* 1994, 1995; Hanotte *et al.* 1994; Marklund *et al.* 1994; Craighead *et al.* 1995; DeWoody *et al.* 1995; Gertsch *et al.* 1995; Mundy & Woodruff 1996).

1.0 Study Population

One thousand nine hundred and fifty (1950) *Heliothis* larvae and pupae were collected from 11 grain/crop farms in south western Queensland at four times, representing Winter 1998, Spring 1998, Summer 1998/1999 and Autumn 1999.

For the purposes of this study, a subset of 50 individuals from each location/time-point sample was selected using random-number tables (Watson 1992). As the larvae of *Heliothis armigera* and *Heliothis punctigera* cannot be reliably distinguished by morphological features alone, these individuals were then screened using an interspecific DNA assay, as described below. Once identified, *Heliothis punctigera* were removed from the data set.

An overall total of 665 *H. armigera* were genotyped in the course of the study. For the purposes of analysis however, individuals scored at less than 5 loci were eliminated, leaving a total of 570 individuals. A summary of sample sizes for each season is given in Table 1.

With respect to this study, no samples from the larger source population could be obtained. It should be noted that this imposes limitations upon the interpretation of results under the currently available statistical methods.

Table 1: Summary of Sample Sizes for Each Population

Population	Time Point	No of Individuals Genotyped
Winter 1998	T_0	124
Spring 1998	T_1	121
Summer 1998	T_2	156
Autumn 1999	T_3	169
	Total	**570**

2.0 Extraction of DNA

The hind prolegs of each larval specimen were removed using a sterile scalpel blade and DNA obtained using a standard Chelex extraction protocol (5% Chelex resin in TE buffer). The remaining tissue was then stored in 100% ethanol as a voucher specimen, for use in further studies if required.

Pupal specimens failed to yield DNA using either Chelex or MES-CTAB methods and so, are not included in this study.

3.0 Interspecific Assay

Identification to species level was achieved through amplification of the ITS region. One microlitre of Chelex-extracted DNA (~100pg) was added to master-mix containing 1 x PCR Buffer (1.5mM $MgCl_2$), 0.2mM dNTPs, 0.80μM of the primers ITSB (tcc tcc gct tat tga tat gc) and ITSN2 (ttc tcg cat cga tga aga acg) and 1.5 units of Taq polymerase. Amplification was carried out in a Corbett thermocycler (Corbett Research, Sydney) under the following conditions: One cycle of 94°C for 3 minutes, 50°C for 1 minute, 72°C for 2 minutes, followed by 39 cycles of 94°C for 30 seconds, 50°C for 30 seconds, 72°C for 90 seconds and 1 cycle of 72°C for 5 minutes.

PCR products were then subjected to electrophoresis on 2% agarose/1 x TBE gels at 130V for 60 minutes and visualised by staining with ethidium bromide. The size of the PCR product was then used to determine species (*H. armigera* produces a PCR product of 831 bp, while *H. punctigera* produces a PCR product 892 bp).

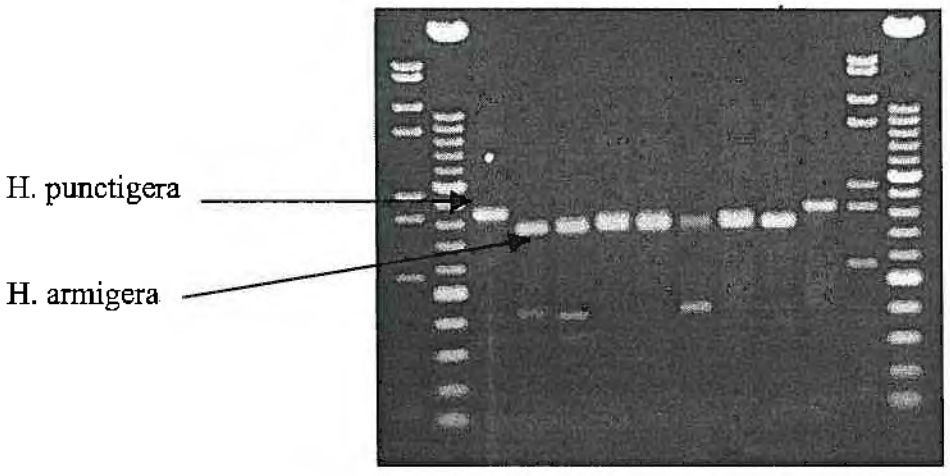


Figure 1 Interspecific assay identifies both *H. armigera* and *H. punctigera*

4.0 Construction of Genomic/Microsatellite Library

Microsatellite-containing clones were isolated from a *Heliothis armigera* genomic library, constructed by ligating 300-600bp fragments of microsatellite-enriched genomic DNA into a modified pUC19 vector. Clones were screened by direct automated sequencing and PCR primers designed for 26 loci.

5.0 Characterisation of Microsatellite Loci

Each locus was screened for polymorphism and PCR conditions optimised using a randomly selected subset of 12 field-collected *H. armigera* specimens. Twenty loci were identified as polymorphic and useful for population genetic studies according to the criteria proposed by Ferraris & Palumbi (1997).

The results presented here are based upon six loci (designated HaMs1, HaMs22, HaMs25, HaMs28, HaMs56 and HaMs171).

Amplification of microsatellite loci via polymerase chain reaction was undertaken in 25μL aliquots containing 0.10μM of each primer, 2mM $MgCl_2$, 1.24 x Qiagen PCR Buffer, 0.5 units of Taq polymerase and ~100pg of genomic DNA. Amplification was performed in a Corbett thermocycler (Corbett Research, Sydney) and consisted of an initial denaturation at 94°C for 30 seconds, followed by 50 cycles of 15 secs at 94°C, 30 secs at the appropriate annealing temperature for each primer pair (Table 1) and 30 secs at 72°C. PCR products were separated on 4 acrylamide gels using the Gel-Scan 2000 Real-Time Gel System (Corbett Research, Sydney) system. Loci with repeat units greater than or equal to 3 base pairs were stained using ethidium bromide and the GS 2000 laser system, while loci with smaller repeat units were scored through use of fluorescent labelling of the upper primer, again using the Gel-Scan 2000 laser-detection system. Allelic sizes were scored using One-Dscan 1.0 software.

Due to the large number of alleles at some loci, tests for Hardy-Weinberg equilibrium and linkage could not be conducted using standard analytical programs such as Genepop (Raymond & Rousset 1995), which are limited to consideration of only 99 alleles at each locus.

Expected and observed heterozygosity for each locus was therefore calculated using the parentage analysis program Cervus (Version 1.0) (Tristan Marshal, University of Edinburgh 1998; available from http://helios.bto.ed.ac.uk/evolgen/cervus) which computes these figures as the first stage in parentage analysis, using the algorithm of Nei (1987). Further statistical tests were not possible using currently available methods.

Cervus 1.0 was also used to calculate polymorphic information content (PIC) (Botstein *et al.* 1980; Hearne *et al.* 1992) and frequency of null alleles for each locus (Summers & Amos 1997).

6.0 Analysis of Population Structure & Genetic Distance

Microsatellite data were analysed using the "assignment test" and D_{LR} statistic of Paetkau *et al.* 1997 and Waser & Strobeck 1998. Originally developed to estimate the degree of differentiation between polar bear (*Ursinus maritimus*) populations, the

assignment test, and the associated genetic distance measure of D_{LR}, were preferentially selected over alternative methods for the following reasons:

1. The method utilises more of the information available in microsatellite data and is particularly effective when populations are clearly defined *a priori* (as in this study).
2. Unlike traditional measures (eg those based on Wright's F_{ST}) the Paetkau *et al.* method is based upon individual genotypes rather than population descriptors; making it possible to estimate dispersal rates between particular populations and to identify individual immigrants within the sample pool.

The assignment test assumes that: a) populations are in Hardy-Weinberg equilibrium and b) that there is no linkage disequilibrium. Population assignment is determined by genotyping individuals at multiple loci and calculating the expected frequency of each individual's genotype in each putative population – The individual is then assigned to the population in which it's (multilocus) genotype has the highest probability of occurrence.

Individuals displaying 'misassigned genotypes' (having lower likelihood in the population where it was sampled than in other sampled populations) are then viewed as immigrants that originated in their assigned population (Paetkau *et al.* 1998).

For the purposes of this study, the proportion of "unique" genotypes in each seasonal population (ie genotypes assigned to the nominal population) was interpreted as reflecting the number of migrants (from an external population). Genotypes shared with preceding populations (ie genotypes assigned to earlier populations) were interpreted as representing a line of descent while genotypes assigned to later populations were interpreted as representing the contribution to future populations.

The distance measure, D_{LR} is generated from assignment probabilities using the formula:

$$D_{LR} = (1/n_x \sum^{nx}_i \log L_{ixx}/L_{ixy} + 1/n_y \sum^{ny}_i \log L_{iyy}/L_{iyx})/2$$

Where: x and y are populations; n_x and n_y are sample sizes for each population; L_{ixx}, L_{ixy}, L_{iyy} and L_{iyx} are the likelihoods of the genotype of an individual from population x in population x and population y etc

(Paetkau *et al.* 1997, 1998)

The D_{LR} values generated in the analysis can also be used to calculate "probability of identity" (P_{ID}) of individuals, which provides information pertaining to

1. How useful the selected markers will be for both individualisation and parentage analyses.

2. The probability that two individuals drawn at random from a given population will have identical genotypes.

(Paetkau *et al.* 1997, 1998)

P_{ID} is calculated using the formula:

$$P_{ID} = \sum_i p_i^4 + \sum_i \sum_{j>I} (2p_I p_j)^2$$

Where p_I and p_j are the frequencies of the ith and jth alleles.

(Paetkau *et al.* 1997, 1998)

Genetic distance was generated using both Paetkau *et al.*'s distance likelihood ratio (D_{LR}) and Nei's genetic distance (D_s). While the D_{LR} statistic is likely to be the most applicable in this situation, the inclusion of D_s is intended to allow comparison with the more traditional methods and provide information which may be of use to others.

D_s was calculated using the formula:

$$D_s = -\ln[J_{xy}/\sqrt{J_x J_y}]$$

$$J_x = (\sum_j^r \sum^m i x_{ij}^2)/2$$

$$J_y = (\sum_j^r \sum^m i y_{ij}^2)/2$$

$$J_{xy} = (\sum_j^r \sum^m i x_{ij} y_{ij})/2$$

Where x and y are populations with r loci and m alleles at each locus, x_{ij} and y_{ij} are the frequencies of the ith allele at the jth locus in populations x and y respectively.

Nei (1972)

All assignment statistics and distance measures presented herein were generated using an online calculator maintained by the University of Alberta, Canada (http://www.biology.ualberta.ca/jbrzusto/Doh.html). Further analysis and exploration of the resultant data set was conducted using SPSS® Version 8.0 (Spss Inc., Chicago, Ill, U.S.A.) for Windows® (Microsoft Corporation, U.S.A.).

7.0 Relationship Between Larval Survival and Source Population

The relationship between survival of larvae and nominal and assigned population was investigated through the non-parametric correlation methods of Kendall (tau b) and Spearman (rho) (Coakes & Steed 1999).

Larval stage (first to sixth instar) was determined through reference to criteria established by Cotton Seed Distributors Australia, as outlined in Table 2

Table 2: Heliothis Stage Identification

Larval stages were identified through reference to the Heliothis stage identification criteria provided by Cotton Seed Distributors in the 1996/1997 Insect Management Plan for INGARD® Cotton Endorsed by TIMS.

Instar	Age (days)	Length (mm)
Hatching/first (neonate)	1	1.5
First	2	3
Second	3	7
Third	5	13
Fourth	8	23
Fifth	11	28
Sixth	14	30+

Instar stage was then scored as a discrete variable (range 1-6). Nominal and assigned populations were also scored as discrete variables ("1" = Winter 1998; "2" = Spring 1998; "3" = Summer 1998/99; "4" = Autumn 1999), such that a positive correlation between instar and assigned population would indicate increased survival rates for persistent genotypes.

Correlation analysis was performed using SPSS® Version 8.0 (SPSS Inc., Chicago, Illinios, U.S.A.) for Windows® (Microsoft Corporation, U.S.A.).

Project Results:

1.0 Allele Frequencies, Hardy-Weinberg Equilibrium and Polymorphic Information Content

The number of alleles at each given locus is summarised in Table 3.0. Results are shown for each separate population and for all populations combined.

P_{ID} values failed to be generated by the analysis program and so, are not presented here.

Table 3: Number of Alleles Identified at Each Locus in Each Population

Total number of alleles across all populations is given in the final row of the table.

Population	HaMs1	HaMs22	HaMs25	HaMs28	HaMs56	HaMs171
Winter 1998	111	107	63	84	93	106
Spring 1998	88	85	57	100	96	90
Summer 1998	117	121	50	115	121	111
Autumn 1999	110	148	54	107	108	129
Total	**170**	**240**	**98**	**159**	**159**	**154**

The very large allele numbers here have been of great concern. Following extensive rechecking of data and several randomly chosen markers all were confirmed as microsatellite markers. The large number of alleles at each locus may be either a product of the methodology employed (in which case more time will be required to refine the system) or may be a genuine reflection of microsatellite loci in *Heliothis*. The large number of alleles presented several difficulties in statistical analyses and if further

study shows that hyper-polymorphism is common, review and extension of current statistical methods will be required to enable any extension of the study.

Observed and expected heterozygosity frequencies suggest that HaMs22 and HaMs25 may not be in Hardy-Weinberg equilibrium and may have high frequency of null alleles. In the absence of further information, these loci were included in assignment analyses, with an aim to revise the information in a future study.

Table 4: Summary of Microsatellite Loci Characteristics

Table shows results of loci characterisation studies. Calculation of PIC and null allele frequencies follows the methods of Botstein *et al.* (1980) and Summers & Amos (1997) respectively. The large number of alleles at each locus prevented exact testing of the assumptions of the assignment test. Observed and expected heterozygosity frequencies suggest that HaMs22 and HaMs25 may not be in Hardy-Weinberg equilibrium and may have high frequency of null alleles. In the absence of further statistical tests however, full assessment could not be carried out.

Locus	n	H_e	H_o	PIC	Null Allele Frequency
HaMs1	570	0.992	0.933	0.991	0.0302
HaMs22	570	0.993	0.677	0.989	0.1874
HaMs25	567	0.979	0.832	0.978	0.0812
HaMs28	563	0.992	0.906	0.991	0.0448
HaMs56	550	0.992	0.929	0.991	0.0327
HaMs171	540	0.992	0.965	0.990	0.0130

2.0 Genetic Distance

Genetic distance between each pair of study populations is presented in Table 5.

Greatest genetic distances was recorded between the spring and summer populations. This may indicate that the summer population has a higher proportion of unique/new genotypes compared to other populations, which, in turn suggests that the summer population contained the highest level of immigrants. Shortest genetic distance was recorded between the winter and summer populations. Genetic distance between all other population pairs was approximately equidistant.

3.0 Structure of Populations with Reference to Migration & Descent

Figure 1 illustrates the proportion (in percentage format) of genotypes in each population, which are shared with/derived from the other populations in the data set.

As the winter population is the first point in the time series, the data this season is best interpreted as illustrating persistence of the original winter genotypes in future populations. Data for the spring population shows that ~18% of individuals are descended from the winter population, while ~52% are migrants. It also illustrates that little, if any, individuals present in the spring population contribute to the summer influx. The recurrence of spring genotypes in the autumn population then can be interpreted as reflecting either: a) a small number of individuals from the spring population contributing to the summer population and/or b) an influx of migrants into the summer population, some of which share genetic composition with the spring population (either by chance or by common ancestry).

Table 5: Genetic Distance between Study Populations

Data are presented in matrix format to allow inclusion of both D_{LR} (Paetkau *et al.* 1997) and D_s Nei 1972) statistics. The figures in the lower left half of the matrix are D_{LR} values, while those in the upper right half of the matrix are D_s values. Higher distance measures indicate greater differentiation between populations. These two distance measures are highly inter-correlated (Paetkau *et al.* 1997).

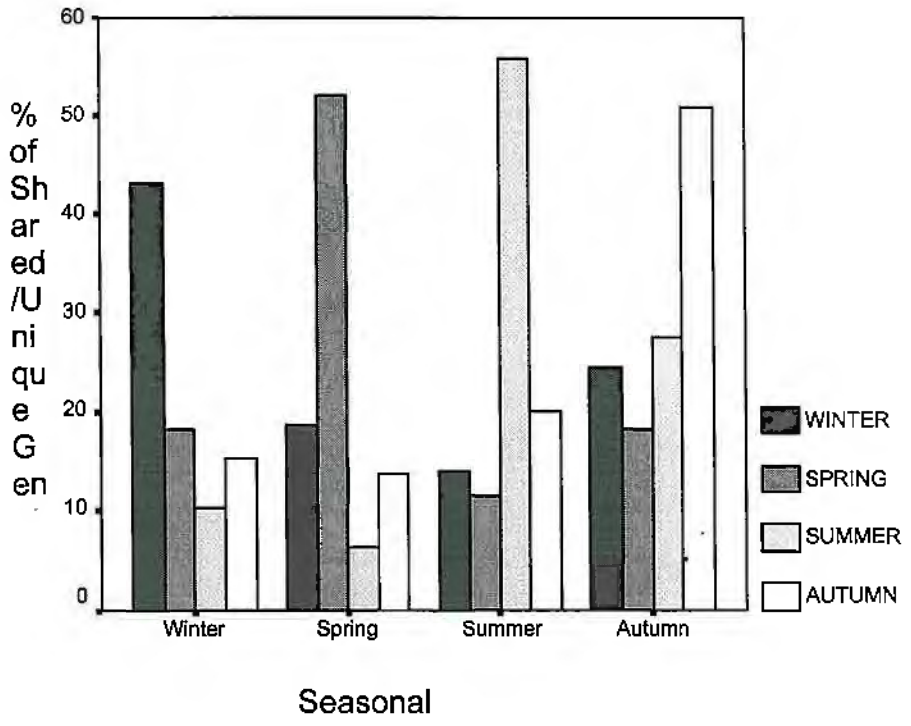
Population	Winter	Spring	Summer	Autumn
Winter	0.00	3.28	3.26	2.83
Spring	3.05	0.00	4.16	3.07
Summer	2.18	4.39	0.00	1.97
Autumn	2.78	3.10	3.15	0.00

Data for the autumn population indicates that this group is descended from a small number of individuals from the summer population plus a larger proportion of migrants.

The data for the summer population shows the same domination of the gene pool by immigrants, with smaller contributions from previous populations. Overall, the data indicate that the majority of individuals in any given population at any given time are migrants, but that a smaller number are descendants of preceding generations are always present.

Figure 1: Proportion of Inter-population Assignments

Data presented are percentages of cross-population assignment of genotypes (proportion of shared and unique genotypes in each population). A higher level of unique genotypes in each population (higher bar in own population column) indicates greater independence. Conversely, a higher level of genotypes shared with preceding populations (higher bars in other populations) indicates a higher level of derivation from those populations. In all cases, the greatest proportion of individuals are migrants (new/unique genotypes), while a smaller number are descendants of previous generations. Some genotypes from the original winter population persist throughout all time points.



The persistence of the original winter genotypes is worthy of further comment as it is suggestive of linkage between microsatellites and chemical resistance (ie microsatellites are potential markers for resistance). Two possible causes for this phenomenon are: a) a founder effect, diluted by the influx of migrants into each new seasonal population and/or b) correlation of microsatellite genotype with chemical resistance.

Logic suggests that each new influx of migrants into a population includes some individuals, which (either by chance or by preselection in the population of origin) carry resistance. If subsequent application of pesticides then removes non-resistant genotypes from the population, the remaining individuals (exhibiting the same microsatellite genotype as the resistant individuals from the original population) would be indistinguishable from the descendants of the original population. This could, in the long-term, produce a super-resistant population.

4.0 Relationship Between Larval Survival and Source Population

While both Spearman's rho and Kendall's tau b revealed a significant relationship between larval survival and source (assigned) population (tau b=0.109, p=0.002; rho=0.130, p=0.002; n=570) a stronger relationship was found to exist between larval stage and nominal population (tau b=0.167, p=0.000; rho=0.200, p=0.000; n=570).

This indicates that larval stage is more strongly correlated with season than source population, but as this study was not designed to investigate this *per se*, results should be interpreted with caution. There are many other factors (such as exact sampling date within a season and environmental/climatic factor) which have not been included and further investigation is required before definitive conclusions could be justified.

Research Outcomes Compared to Objectives:

- As set down in the first objective a series of microsatellite libraries have been developed for *Heliothis* species yielding twenty-six highly polymorphic loci, suitable for use in both population and parentage studies. Other microsatellite clones are available but have not been characterised.
- Due to the difficulty of identifying alcohol preserved *H. armigera* from *H. punctigera* by morphological means, a diagnostic assay has been developed for differentiating between the two species. While this was not an objective of the project it considered necessary in order to avoid problems of confusion in identity between the species which can occur symmetrically.
- The second objective of this study was to examine the utility of these markers in assessing the proportions of local recruitment and immigration of Heliothis. Results of microsatellite studies were used to investigate temporal shifts in population structure in *Heliothis armigera*. Results show that:
    - In each (seasonal) population, 5-50% (varying according to time of year) of individuals are descended from preceding populations (ie are survivors of pesticide applications). This provides measures of local recruitment.

- The remainder of the population is then comprised of migrant individuals, which may or may not share common ancestry (and therefore genetic composition) with those individuals already present.
- The contribution of migrants is maximal during summer, presumably resulting from the general increase in insect numbers and dispersal associated with the warmer months.
- When each population experiences a bottleneck (eg after application of pesticides) some of the new migrant genotypes are lost, while some (those which are resistant) are retained and carry through to future generations.

Impact on the Cotton Industry:

The likely impacts of these outcomes are as follows:

- 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* into the southern states such as Victoria and South Australia if necessary or desirable.
- Several microsatellite markers revealed progeny from a few individuals persisted in the populations across the entire growing season. While it has not been conclusively established, these markers may be linked to resistance to insecticides and could be exploited as markers for measuring resistance build-up in Heliothis populations. The advantage of these markers (should they be proven useful) is in their ability to show where resistance is present and what proportions of the population, both local and migrant, carry potential insecticide resistance. Ultimately when source population data is gathered, hot spots of resistance could be identified and managed in the appropriate ways.

Recommendations for Future Activities:

The current project was a pilot study to demonstrate whether a DNA marker system could be developed to provide information on the proportion of local recruitment to migrants in a defined area. Having achieved this, the next phase of development will be to use these markers to assess whether origins or source populations of migrating Heliothis can be identified. To achieve this, the following is proposed.

- The marker system developed here will require further refinement (allele number).
- Extended sampling period, widen sampling regime, extended time frame and test several new statistical methods (or extension of existing methods) to verify these and subsequent results in the studies.
- Further investigation into the potential markers for the identification of chemical-resistant genotypes.

- With the availability of new and potent pesticides and bio-pesticides currently under investigation, cotton growers 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 and growers working in Area Wide Management Strategies access to the most recent source population data. Management recommendations arising from this data could be included in the current Heliothis newsletter published by the QDPI, but should ultimately take the form of a World Wide Web site capable of delivering information to the farm gate.

Recommendations for further work are outlined in the attached document. This document is before the CRDC Board for funding consideration dependant on this report.

Publications:

Currently there are no publications of these findings due to the potential commercial nature of the resulting DNA marker system. Agreement on mode of publication is yet to be finalised by participating organisations.

References

- Amos B., Schlotterer C. & Tautz D. (1993). Social *Structure of Pilot Whales Revealed by Analytical DNA Profiling.* Scieoce 260: 570-573.
- Armour J.A.L., Neumann R., Gobert S. & Jeffreys J. (1994). *Isolation of Human Simple Repeat Loci by Hybridization Selection.* Human Molecular Genetics 3(4): 599-605.
- Brown M.R., Corkeron P.J., Hale P.T., Schultz K.W. & Bryden M.M. (1994). *Behavioural Responses of East Australian Humpback Whales Megaptera novaeangliae to Biopsy Sampling.* Marine Mammal Science 10(4): 391-400.
- Brown M.R., Corkeron P.J., Hale P.T., Schultz K.W. & Brydeo M.M. (1995). *Evidence for a Sex-Segregated Migration in the Humpback Whale (Megaptera noveangliae).* Proceedings of the Royal Zoological Society of London B:
- Brownstein M.J., Carpten J.D. & Smith J.R. (1996). *Modulation of Non-Templated Nucleotide Addition by Taq DNA polymerase: Primer Modifications that Facilitate Genotyping.* Biotechniques 20: 1004-1010.
- Coakes S.J. & Steed L.G. (1999). *SPSS Analysis without Anguish Versions 7.0, 7.5, 8.0 for Windows.* John Wiley & Sons, Brisbane, Queensland.
- C.S.I.R.O. (1996). *Microsatellite STS Null Alleles.* {Internet address: http://cgswww.adl.hort.CSIRO.au/cgs_genetics/gene2.html}, C.S.I.R.O. Australia, Division of Horticulture, Grapevine DNA Profile Database.
- Craighead L., Paetkau D., Reynolds H.V., Vyse E.R. & Strobeck C. (1995). *Microsatellite Analysis of Paternity and Reproduction in Arctic Grizzly Bears.* Journal of Heredity 86: 255-261.
- DeWoody J.A., Honeycutt R.L. & Skow L.C. (1995). *Microsatellite Markers in White-Tailed Deer.* Journal of Heredity 86(4): 317-319.
- Ellegren H. (1992). *Polymerase-Chain-Reaction (PCR) Analysis of Microsatellites - A New Approach to Studies of Genetic Relationships in Birds.* The Auk 109(4): 886-895.
- Estoup A., Solignac M., Harry M. & Cornuet J. (1993). *Characterisation of $(GT)_n$ and $(CT)_n$ Microsatellites in Two Insect Species: Apis mellifera and Bombus terrestris.* Nucleic Acids Research 21(6): 1427-1431.
- Ferraris J.D. & Palumbi S.R. (1996). *Molecular Zoology: Advances, Strategies & Protocols.* Wiley & Sons (Wiley-Liss) Incorporated, Brisbane.

- Gertsch P., Pamilo P. & Varvio S.L. (1995). *Microsatellites Reveal High Genetic Diversity Within Colonies of Camponotus Ants.* Molecular Ecology 4: 257-260.
- Hanotte O., Zanon C., Pugh A., Greig C., Dixon A. & Burke T. (1994). *Isolation and Charactrization of Microsatellite Loci in a Passerine Bird: The Reed Bunting Emberiza schoeniclus.* Molecular Ecology 3: 529-530.
- Hughes C.R. & Queller D.C. (1993). *Detection of Highly Polymorphic Microsatellite Loci in a Species with Little Allozyme Polymorphism.* Molecular Ecology 2: 131-137.
- Lesser E.P. & Applebaum G. (1993). *Screening Techniques for Detecting Allelic Variatian in DNA Sequences.* Molecular Ecology 2: 119-129.
- Marklund S., Ellegren H., Erikson S., Sandberg K. & Anderson L. (1994). *Parentage Testing and Linkage Analysis in the Horse Using a Set of Highly Polymorphic Microsatellites.* Animal Genetics 25: 19-23.
- Menotti-Raymond M.A. & O'Brien S.J. (1996). *Evolutionary Conservation of Ten Microsatellite Loci in Four Species of Felidae.* Journal of Heredity 86(4): 319-320.
- Moore S.S., Sargeant L.L., King T.J., Mattick J.S., Georges M. & Hetzel D.J.S. (1991). *The Conservation of Dinucleotide Microsatellites Among Mammalian Genomes Allows the Use of Heterologous PCR Primer Pairs in Closely Related Species.* Genomics 10: 654-660.
- Mundy N.I. & Woodruff D.S. (1996). *Polymorphic Microsatellite Markers in the Loggerhead Shrike Lanius ludovicianus Isolated from a Library Enriched for CA Repeats.* Molecular Ecology 5: 811-813.
- Nei M. (1978). *Estimation of Heterozygosity and Genetic Distance from a Small Number of Individuals.* Genetics 89: 583-590.
- Ostrander E.A., Jong P.M., Rine J. & Duyk G. (1992). *Construction of Small-Insert Genomic DNA Libraries Highly Enriched for Microsatellite Repeat Sequences.* Proceedings of the National Academy of Sciences U.S.A. 89: 3419-3423.
- Paetkau D. & Strobeck C. (1994). *Microsatellite Analysis of Genetic Variation in Block Bear Populations.* Molecular Ecology 3: 489-495.
- Paetkau D. & Strobeck C. (1995). *The Molecular Basis and Evolutionary History of a Microsatellite Null Allele in Bears.* Molecular Ecology 4: 519-520.
- Paetkau D., Calvert W., Stirling I. & Strobeck C. (1995). *Microsatellite Analysis of Population Structure in Canadian Polar Bears.* Molecular Ecology 4: 347-354.
- Paetkau D., Waits L.P., Clarkson P.L., Craighead L. & Strobeck C. (1997). *An Empirical Evaluation of Genetic Distance Statistics Using Microsatellite Data from Bear (Ursidae) Populations.* Genetics 147: 1943-1957.
- Pemberton J.M., Slate J., Bancroft D.R. & Barret J.A. (1995). *Nonamplifying Alleles at Microsatellite Loci: A Caution for Parentage and Population Studies.* Molecular Ecology 4: 249-252.
- Raymond M. & Rousset F. (1995). *GENEPOP (Version 1.2): Population Genetics Software for Exact Tests ond Ecumenicism.* Journal of Heredity 86: 248-249.
- Roy M.S., Geffen E., Smith D., Ostrander E.A. & Wayne R.K. (1994). *Patterns of Differentiation and Hybridisation in North American Wolflike Canids, Revealed by Analysis of Microsatellite Loci.* Molecular Biology & Evolution 11(4): 553-570.
- Sherwin W.B., Murray N.D., Marshall Graves J.A. & Brown P.R. (1991). *Measurement of Genetic Variation in Endangered Populations: Bandicoots (Marsupialia: Peramelidae) as an Example.* Conservation Biology 5(1): 103-108.
- Stephan W. & Cho S. (1994). *Possible Role of Natural Selection in the Formotion of Tandem-Repetitive Noncoding DNA.* Genetics 136: 333-341.
- Strassman J.E., Solis C.R., Peters J.M. & Queller D.C. (1996). *Strategies for Finding and Using Highly Polymorphic DNA Microsatellite Loci for Studies of Genetic Relatedness and Pedigrees.* Chapter 8, pages 163-180 in J.D. Ferraris & S.R. Palumbi (Editors), *'Molecular Zoology Advances Strotegies and Protocols'*, Wiley-Liss Publishers, Brisbane, QLD.
- Valdes A.M., Slatkin M. & Freimer N. (1993). *Allele Frequencies at Microsatellite Loci: The Stepwise Mutation Model Revisited.* Genetics 133: 737-749.
- Wall W.J., Williamson R., Petrou M., Papaioannou D. & Parkin (1993). *Variation of Short Tandem Repeats Within and Between Populations.* Human Molecular Genetics 2(7): 1023-1029.

- Waser S.K. & Barash D.P. (1983). *Reproductive Suppression Among Female Mammals: Implications for Biomedicine and Sexual Selection Theory.* Quarterly Review of Biology 58: 513-537.
- Watson R.K. (1992). *Statistical Tables for Students.* University of Melbourne, Parkville, Victoria.
- Weber J.L. & May P.E. (1989). *Abundant Class of Juman DNA Polymorphisms which can be Typed Using the Polymerase Chanin Reaction.* American Journal of Human Genetics 44: 388-396.
- Weber J.L. & Wong C. (1993). *Mutation of Human Short Tandem Repeats.* Human Molecular Genetics 2(8): 1123-1128.
- Botstein D, White R.L., Skolnick M. & Davis R.W. (1980). *Construction of a Genetic Linkage Map in Man Using Restriction Fragment Length Polymorphisms.* American Journal of Human Gnetics 32: 314-331.
- Hearne C.M., Ghosh S. & Todd J.A. (1992). *Microsatellites for Linkage Analysis of Genetic Traits.* Trends in Ecology & Evolution 8: 288-294.
- Nei M. (1987). *Molecular Evolutionary Genetics.* Columbia University Press, New York, U.S.A.
- Summers K. & Amos W. (1997). *Behavioural, Ecological and Molecular Genetic Analyses of Reproductive Strategies in the Amazonian Dart-Poison Frog, Dendrobates ventrimaculatus.* Behavioural Ecology8: 260-267.