Final report - Assessment of genetic differentiation among Australian populations of *Helicoverpa* (ULA2C)

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This project examined genetic variation in Helicoverpa populations using variation in mitochondrial DNA (mtDNA) and variation in quantitative traits, and was supported by ARC as well as CRDC. In this project we developed the molecular techniques that allowed sampling of geographical variation using mtDNA. On theoretical grounds and on the basis of recent studies in other organisms this marker has the potential to be useful for detecting genetic differentiation among regions. Detection of genetic differences among populations of the two Helicoverpa pest species, the native budworm *H.punctigera* and the insecticide resistant bollworm *H.armigera*, may provide the ecologists with a useful tool for better understanding moth movement patterns. This would facilitate better prediction and control of infestations.

In *H.punctigera* 14 different genetic types were identified. One common type was at high frequency Australia-wide (seven regions were sampled) and rare unique types (single individuals) occurred in different regions. This pattern suggests extensive gene exchange and thus moth movement among the Australian population as a whole. Two samples, especially one from Mareeba in northern Queensland, had different unique variants at high local frequency, suggesting a degree of restriction on movement of moths into these areas. This finding is consistent with conclusions from earlier ecological work on the Mareeba population. The mtDNA marker may be valuable in such isolated areas; more extensive sampling over time may give valuable information on local movement patterns.

Results of sampling several laboratory cultures of *H.punctigera* that originated from different regions (cultures that originated from many field-captured individuals and that were maintained in large numbers for up to five generations) indicated surprising genetic differentiation and within culture homogeneity. The data suggest that characteristics measured on experimentally maintained native budworm cultures may not accurately reflect the field situation. After several laboratory generations strains become inbred, more quickly than anticipated (see below), and may yield spurious and unusual information.

Within Australian *H.armigera* a different pattern of mtDNA variation has been detected. In a similar overall sample size many more genetic types occur, 33 have been described to date. Further, unlike for the native budworm several types are found at relatively high frequency. Although most of the common types have been detected Australia-wide, from Kununurra through Biloela to Bairnsdale, the data suggest a north south difference in relative frequency of these types. This differentiation is particularly noticeable for two south Gippsland populations. MtDNA may be valuable for helping our understanding of north- south movement of moths in this species. More extensive sampling of geographical variation of mtDNA types is underway to substantiate and extend these observations so that we can properly evaluate the usefulness of mtDNA as a marker for detecting movement in bollworm moths.

A significant part of the DNA sequence of mtDNA from both pest species has been determined (including the entire control regions) and added to the international data banks (Genbank Accession Numbers L17343 for *H.punctigera* and UO2678 for *H.armigera*).

For the quantitative component of this project, *H. punctigera* populations from around Australia were characterized for life history traits and morphological traits. We wanted to see if quantitative differences could be detected between populations and related to climatic variation. This information is critical when attempting to extrapolate results obtained with one strain to other strains and to natural populations.

Replicate lines of *H. punctigera* were established from five locations and reared under the same laboratory conditions. Populations were compared for life history traits and morphological traits when reared at 35°C and 19°C. Populations reared at 35°C were similar except for the length of veins on the hindwings. Small differences were detected between populations reared at 19°C for development time, pupal weight, hindwing length and wing markings, as well as for the tendency of populations to enter diapause. Divergence among replicate lines was also evident for many traits even though lines were kept at a large census size. Crosses between populations from Western Australia and Queensland confirmed a genetic basis for differences in hindwing length. The adaptive significance of this geographic variation was not established.

As part of this work, inbreeding in *H. punctigera* was examined by crossing moths within and between families set up from field-collected females. There were large inbreeding effects for larval viability, and smaller effects for adult size and larval/pupal development time. Size and development time were compared in two recently-collected strains and two strains held in the laboratory for 8 or 22 generations. In both laboratory-adapted strains, the length of a hindwing vein was reduced and the time spent at the pupal stage was extended. There were also changes in pupal biomass but these were inconsistent. Means of F1s from crosses between the strains were intermediate for hindwing length and pupal biomass, while F1 means for development time were similar to those of recently-collected strains. This suggests that some changes in established stocks were due to laboratory adaptation rather than inbreeding. A comparison of the same lines held for one or four generations in the laboratory indicated changes in hindwing length and pupal development time in the same direction. Both laboratory adaptation and inbreeding can therefore influence *H. punctigera* phenotypes and these effects need to be considered when evaluating results obtained from unreplicated laboratory stocks.

Note:

The molecular genetic side of this project is continuing with CRDC support to Dr McKechnie at his Monash laboratory. A CRDC scholarship for Ph.D. studies to Ms Merrin Spackman (UMON2C) together with essential maintenance (UMON3C) has enabled adequate geographical sampling of *H.armigera* to proceed. Within the time frame projected (to mid-1995) we will complete the geographical sampling of *H.armigera*, and complete a preliminary survey of geographical variation in *H.armigera* of an entirely new molecular marker. The new marker, a microsatellite chromosomal gene, has potential to reveal regional genetic differentiation that is genetically independent of mtDNA variation. The work can be done relatively expediently since the geographical

samples to be used are those already prepared for the mtDNA study and are in our freezer. The rationale is that the use of multiple molecular markers, that each show some degree of regional differentiation, may provide more definitive information about movement of moths into cropping regions.

The quantitative work is also continuing with ARC support to Dr Hoffmann at La Trobe. New work on pre-reproductive period is considering its genetic basis and association with migratory ability in *H. punctigera*. We are also considering environmental factors influencing migration ability.

Publications:

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