Chapter 2

Genetic relationships among populations of *Cosmopolites sordidus* based on AFLP analysis

Abstract

The banana weevil, *Cosmopolites sordidus*, is a serious pest of banana and plantain (*Musa*) and has been distributed to most areas where the crops are grown. Pest status is variable around the world, and may be influenced by genetically distinct populations of weevil. The limited mobility of banana weevils suggests restricted gene flow and the evolution of biotypes within areas. The aim of the study was to quantify the genetic relatedness within and among geographically separated populations of *C. sordidus*. Six populations from four countries were sampled: Australia, Costa Rica, South Africa (South Coast, North Coast and Tzaneen) and Uganda. DNA was isolated from 12 individuals per population and subjected to amplified fragment length polymorphism (AFLP) analysis. The AFLP analysis involved DNA restriction with *Eco*RI and *Pst*I enzymes, ligation of adapters, and a pre-selective and five selective PCR amplifications. Empirical analysis of the AFLP fingerprints showed that, within populations, genetic diversity varied from 16-53% (the proportion of polymorphic loci), with the South Coast and Tzaneen/Australian populations the least and most variable, respectively. The coefficient of gene differentiation showed that the Tzaneen population were the most differentiated from the South Coast population, while the South and North Coast populations were the most similar. All the populations showed statistically distinct marker frequencies, except for the Costa Rican and South and North Coast populations, which were similar. Based on the simple mismatch coefficient, a neighbour-joining tree showed the Australian, Ugandan and South African coastal populations produced monophyletic groups, while the South African Tzaneen population were removed from the other populations and presented an ancestral state.

Keywords: AFLP, insect, population genetics, *Cosmopolites*

2.1 Introduction

The banana weevil borer, *Cosmopolites sordidus* (Germar), has been recorded as the most important insect pest of banana and plantain in the world (Waterhouse & Norris 1987; Gold *et al*. 1999). The weevil is found in almost all banana growing areas, with only regions of North Africa and Israel apparently free of the pest (Cardenosa 1953, Cuillé & Vilardebó 1963, Simmonds 1966, Castrillon 1991, Gettman *et al*. 1992; Robinson 1996). Damage results from larvae tunnelling in the rhizome, thereby causing a reduction in yield and lodging of plants. Yield losses of between 20 and 100% have been associated with banana weevil infestations (Mitchell 1980, Anonymous 1986, Koppenhöfer & Schmutterer 1993, Peña *et al*. 1993; Rukazambuga *et al*. 1998). Dissemination most often takes place by means of infested plant material, but crawling adults also colonise nearby plantations (Feakin 1971, Franzmann 1972, Waterhouse & Norris 1987; Seshu Reddy *et al*. 1999). The cryptic nature of the banana weevil, and the fact that infestation symptoms of the weevil resemble nematode and bacterial head rot (rhizome rot) (*Erwinia* spp.) damage (Jones 2000) has caused the time of its introduction(s) to be underestimated, and allowed the pest to remain undetected in certain areas (Gold *et al*. 2003).

The weevil has been reported as a major production constraint in several tropical and subtropical localities (Froggatt 1926, Harris 1947, Braithwaite 1963, Sikora *et al*. 1989, Seshu Reddy 1993, Davide 1994; Maolin 1994), including the Indo-Malayian region, its presumed area of origin (Zimmerman 1968c, Stover & Simmonds 1987; Vittayaruk *et al*. 1994). Empirical pest status, however, is unsure (Ostmark 1974), and appears to be related to several factors, including weevil biotypes (Fogain & Price 1994; Gowen 1995). Biotypes have been defined as organisms that share a specified genotype or the genotype (or peculiarities) so shared (Anonymous 2005), and as a population within an insect species that differs in their ability to utilise a crop plant (Gallun & Khush 1980). Maxwell & Jennings (1980) described a biotype as an individual or a population that is distinguished from the rest of its species by criteria other than morphology, e.g. a difference in parasite ability. The latter definition should be applied with caution, as dimorphic biotypes have been reported (Starks & Burton 1972; Saxena & Rueda 1982).

The *Cosmopolites* genus (Coleoptera: Curculionidae: Rhynchophorinae) comprise only two species, the banana weevil, *C. sordidus* and *C. pruinosus* Heller

(Zimmerman 1968a, b, c). *Cosmopolites pruinosus* is morphologically very similar to *C. sordidus*, but differs externally in the nature of pruinosity on the dorsum and the character of the elytral striae (Zimmerman 1968a, c). The former is associated with bananas in Borneo, Philippines and the Caroline Islands (Zimmerman 1968a, b) and considered to be a secondary pest species (Masanza 2003). Zimmerman (1968c) provided taxonomic keys for the species. The limited mobility of banana weevils suggests the existence of isolated populations with restricted gene flow, and also the evolution of new biotypes (Gold *et al*. 2003). The occurrence of weevil biotypes has been postulated after pathogenicity of an entomopathogenic nematode strain varied between geographically different populations of *C. sordidus* (Parniski *et al*. 1990; Kermarrec *et al*. 1993). Traore *et al*. (1993) also suggested that weevil biotypes exist with different developmental temperature requirements. Studies on banana tolerance or resistance were cautioned to consider possible geographical differences between weevil populations (Fogain & Price 1994). Different biotypes could also have contributed to variable weevil responses to semiochemical trapping in different countries (De Graaf *et al*. 2005). Genetic research using random amplified polymorphic DNA (RAPD)-PCR produced variable results, but generally supported the existence of weevil biotypes (Ochieng 2001; Gold *et al*. 2003). The applicability of the method, however, is limited (Vos *et al*. 1995; Zhu-Salzman *et al*. 2003), because of extreme sensitivity to variations in experimental conditions (Ellsworth *et al*. 1993, Muralidharan & Wakeland 1993, Vos *et al*. 1995; Mueller & Wolfenbarger 1999).

Amplified fragment length polymorphism (AFLP) analysis is considered to be the ideal marker system for resolving genetic relatedness among individual organisms, populations and species (Mueller & Wolfenbarger 1999). The technique, developed by Vos *et al*. (1995), and originally known as selective restriction fragment amplification (SRFA) (Zabeau & Vos 1993), is a high-throughput, highly reproducible genome wide DNA fingerprinting technique. AFLP generates a large number of potential markers across the genome that may counteract the low information content of its dominant markers. The identity of same sized fragments is unknown, but the possibility that products of different loci have the same molecular weight is probably very small for closely related species (Yan *et al*. 1999; Kosman & Leonard 2005). It has proven to be a powerful method for characterising infraspecific polymorphism among insects (Reineke *et al*. 1999, Yan *et al*. 1999, Parsons & Shaw

2001, Ravel *et al*. 2001, Garcia *et al*. 2002; Carisio *et al*. 2004) and distinguishing known insect biotypes (Cervera *et al*. 2000; Zhu-Salzman *et al*. 2003). Restriction fragment length polymorphism (RFLP) and sequencing require more development time with greater costs, and the number of independent loci assayed is often low (Parsons & Shaw 2001).

The aim of the study was to determine the genetic relatedness within and among populations of *C. sordidus* from different geographic origins using AFLPs. The study will help to clarify the role of biotypes in host plant susceptibility, weevil development and behaviour. In future it might provide useful information before the implementation of integrated pest management strategies for countries affected by the pest.

2.2   Material and methods

2.2.1   Sample collection and DNA extraction

Banana weevils, identified as *C. sordidus* according to the key provided in Zimmerman (1968c), were collected from three geographical areas in South Africa during 2004, and also from Australia, Costa Rica and Uganda (Table 2.1). Dissection of residual pseudostems (Australian samples), split pseudostem traps (Ugandan and South African South Coast samples) and pheromone (Cosmolure®) trapping (Costa Rican and South African North Coast and Tzaneen samples) were used to collect weevils. Before DNA extraction, all the weevils were preserved in absolute ethanol. For molecular analysis, a total of 12 individuals (six females, six males) were randomly selected per locality. *Sitophilus orizae* (L.) (Coleoptera: Curculionidae: Rhynchophorinae), a weevil pest of stored grain, was used to serve as outgroup in this study (Table 2.1).

Total genomic DNA was isolated from beetles with the abdomen, elytra and wings removed. Weevils were first placed in a heat block at 55 °C for 10 minutes to evaporate the ethanol and then re-hydrated in distilled water for 10 minutes. Samples were frozen in liquid nitrogen and ground with Eppendorf micro-pestles in 1.5 ml Eppendorf tubes (Hamburg, Germany). DNA extractions followed a commercial protocol (High pure PCR template preparation kit, Roche Diagnostics, Mannheim, Germany) and were stored at -20 °C.

2.2.2 AFLP procedure

The method described by Vos *et al*. (1995) was followed with minor modifications. Isolated DNA (100 ng) was restricted with two six-base recognition restriction enzymes, *Eco*RI (Roche Molecular Biochemicals, Manheim, Germany) and *Pst*I (Fermentas International Inc., Ontario, Canada). The corresponding double stranded adapters (Table 2.2) (Inqaba Biotechnical Industries (Pty) Ltd.) were subsequently ligated to the sticky ends of fragments. *Eco*RI and *Pst*I primers (Inqaba Biotechnical Industries (Pty) Ltd.) with no selective nucleotides were used for preselective PCR amplification (Table 2.2). An initial screening using 12 selective primer pair combinations was performed on a randomly selected individual of each population. Combinations providing clear and reproducible electrophoretic patterns with the highest levels of polymorphisms between individuals were determined. Five *Eco*RI (Biolegio BV Nijmegen/ Malden, The Netherlands) and *Pst*I (Inqaba Biotechnical Industries (Pty) Ltd.) primer combinations were selected for further analysis (Table 2.2).

Selective amplification products were analysed with a LI-COR® model 4200S Automated DNA Analyser (LI-COR® Biotechnology Inc., Nebraska, USA). Fragments were scored with the automated programme AFLP-Quantar Pro 1.0 (Key Gene Products 2000) and confirmed by a visual check. Loci showing clear and unambiguous banding patterns were scored and uncertain fragments were considered as missing data. Band sizes were estimated with a standard size (50-700 bp) IRD-labelled marker (LI-COR® Biotechnology Inc.).

2.2.3 Statistical analysis

To estimate the genetic diversity in and among *C. sordidus* populations, the following assumptions were made: AFLP markers behave as diploid, dominant markers with alleles either present (amplified, dominant alleles) or absent (not amplified, recessive alleles), co-migrating fragments and fragments not amplified were identical among and within populations; AFLP fragments segregated according to Mendelian expectations, and genotypes at all AFLP loci were assumed to be in Hardy-Weinberg equilibrium (Yan *et al*. 1999; Despres *et al*. 2002).

All the loci obtained with the five primer combinations were used in the analyses. Genetic diversity within weevil populations was estimated from the percentage of polymorphic loci out of all polymorphic loci (*%PL*), Shannon's

Information Index (I) (Lewontin 1972) and Nei's (1973) gene diversity (h), using POPGENE version 1.31 (Yeh *et al*. 1997). Pair-wise Product-moment correlations of the different indices were conducted in STATISTICA version 7 (Statsoft Inc. 2004). To evaluate population structure in terms of among-population and among-group differentiation, total genetic diversity was partitioned among groups, among populations within groups, and within populations by conducting a hierarchical analysis of molecular variance (AMOVA) on (the required) squared Euclidian pair-wise distances (1000 permutations) (Excoffier *et al*. 1992, Huff *et al*. 1993, Peakall *et al*. 1995; Despres *et al*. 2002) using ARLEQUIN version 2.000 (Schneider *et al*. 2000). Genetic differentiation among populations was assessed by calculating Nei's coefficient of gene differentiation, G_{st} (equivalent to Wright's F_{st}) (Nei 1973) and estimating gene flow, N_m (Slatkin & Barton 1989) from G_{st} (POPGENE version 1.31). Interpopulation differentiation was scrutinised by using TFPGA version 1.3 (Miller 1997) to perform Monte Carlo approximations of Fisher's exact (*RxC*) test (Raymond & Rousset 1995) on marker frequencies at each locus between all pairs of populations. To determine the phylogenic relationships among individuals, a neighbour-joining dendrogram (Saitou & Nei 1987), based on the simple mismatch coefficient (squared Euclidian distance), was constructed with 5000 bootstrap (Felsenstein 1985) replications, using the program MEGA version 3.1 (Kumar *et al*. 2004) (Kosman & Leonard 2005). A correlation between Nei's unbiased genetic distance (Nei 1978) and simple mismatch coefficients and geographic distance (in km) among populations (Garcia *et al*. 2002; Carisio *et al*. 2004) was investigated with a Mantel test (Mantel 1967) using TFPGA version 1.3. The distance matrices were transformed (ln (x+1)) and 10 000 random permutations used in the analysis (TFPGA version 1.3).

2.3   Results

2.3.1   AFLP patterns

Each primer combination produced approximately 100 to 150 amplified fragments between 50-700 bp (Fig. 2.1), to give a total of 659 fragments, with 604 loci polymorphic for *C. sordidus*. Visual assessment of all the raw data suggested that, within banana weevil populations, the Tzaneen and Australian individuals showed relatively high marker variability (Fig. 2.1). Unique bands were identified most

frequently for these populations, especially for Tzaneen, where bands were often specific to individual level (Fig. 2.1). The Costa Rican and North and South Coast populations from South Africa appeared to share relatively high levels of marker homogeneity, with differences essentially based on band frequency. Similarities of the Australian and Ugandan populations were also evident with the Costa Rican and South African North and South Coast populations, while the South African samples from Tzaneen showed a more distinct fingerprint. The outgroup demonstrated little conformity with the other samples, and displayed the highest proportion of population specific bands (Fig. 2.1).

2.3.2 Intra population genetic diversity

Empirical analysis of the loci showed that the Tzaneen (South Africa) and Australian populations were the most variable, with 53.48% and 45.03% polymorphic loci, respectively (Table 2.3). The South African South Coast population was the most uniform (16.06 *%PL*), while the diversity of the remaining populations was close to the overall mean of 35.02% polymorphic loci. The indices of Shannon (*I*) and Nei (*h*) peaked at 0.169 and 0.101 for Tzaneen and 0.2 and 0.132 for Australia. The two indices also supported the South Coast (South Africa) population as the least variable (*I*=0.075, *h*=0.05). The three measures of intra population diversity were correlated (*I* vs. *h*: R^2=0.982; *%PL* vs. *I*: R^2=0.787; *%PL* vs. *h*: R^2=0.668, all *P*<0.001). Among all the South African populations the percentage polymorphic loci, Shannon (*I*) and Nei (*h*) diversity measures were (mean ± SD) 34.33 ± 18.73%, 0.126 ± 0.048 and 0.079 ± 0.026, respectively (data not shown).

2.3.3 Population structure

The AMOVA revealed that the genetic variation within *C. sordidus* was, in general, equally divided among and within the populations studied (Table 2.4). Genetic differences between populations were highly significant. Grouping of populations showed significant structure when the South African coastal populations and the Costa Rican population were combined and compared to the other populations. As a group, the three South African populations were also significantly differentiated from the other populations, but the proportion of variance contained in the former grouping was higher (10.85%) than the latter (3.19%). In both groupings, the most variation was contained within populations, while variation amongst populations was also high

72

(>40%) and showed significant differentiation. Amongst the South Coast, North Coast and Tzaneen populations in South Africa, significant differences existed ($P<0.001$), with 51.59% and 48.41% of genetic variation contained amongst and within the populations, respectively (Table 2.4).

The global G_{st} value among all populations and among South African populations was 0.4744 and 0.4316, respectively, while associated gene flow (N_m) for all the populations and for South African populations was 0.5540 and 0.6586 (data not shown). The coefficient of gene differentiation and gene flow calculated for pairwise population comparisons indicated that, among all the populations, the greatest differentiation occurred between the South African South Coast and Tzaneen populations, with about 47% difference between the populations, equating to a mean of 0.57 migrants per generation (Table 2.5). The populations sharing the most genetic similarity were the South and North Coast populations from South Africa, with a G_{st} and N_m value of 0.13 and 3.35 respectively (Table 2.5). Based on the Monte Carlo approximation of Fisher's exact test (through 1000 dememorisation steps, in 10 batches with 2000 permutations per batch), most population pairs were significantly different ($P<0.001$) (data not shown). Only the Costa Rican population was not significantly ($P>0.999$) differentiated from the two South African coastal populations, whom also showed no significant ($P=1.000$) among population differences (Table 2.5).

2.3.4 Phylogeny

The neighbour-joining phenogram showed high bootstrap support for the partitioning of banana weevil populations (Fig. 2.2). The outgroup provided an alternative root. The Tzaneen population from South Africa was separated (bootstrap value 99%), and the basal divergent population of the other *C. sordidus* individuals (Fig. 2.2). Monophyletic clusters of the South African South and North Coast, Australian and Ugandan populations were supported by high bootstrap values (≥90%) (Fig. 2.2). The node grouping the South African coastal populations with Costa Rican individuals was not very robust (bootstrap value 43%) (data not shown). A recent common ancestor between Australia and Uganda was supported by a 99% bootstrap value (Fig 2.2). The Australian and the Tzaneen populations showed relatively low levels of similarity between individuals, whilst the highest level of similarity between samples was found in the South Coast population from South Africa (Fig. 2.2).

2.3.5 Isolation by distance

The correlation between Nei's unbiased genetic distance and geographic distance for all pair-wise comparisons among the six banana weevil populations, indicated an overall non-significant pattern of isolation by distance ($R=0.053$, $P=0.849$). The Costa Rican and Tzaneen (South Africa) samples generally showed a negative relation between genetic and geographic distance (data not shown). Removal of these populations from the data matrix improved the fit of the isolation pattern, but not significantly so ($R=0.935$, $P=0.083$). Correlation between the simple mismatch coefficient and geographic distance supported a non significant pattern of isolation by distance ($R=-0.201$, $P=0.737$).

2.4 Discussion

Diversity in *C. sordidus*, collected from four countries in three continents revealed that, within all populations, the percentage of polymorphic loci ranged from 16-53%, with an average diversity of 35%. Among the South African populations, within-population diversity was slightly lower. These results are in contrast to a mean of 92% polymorphic loci (percentage of all loci), ranging from 78-98%, reported for the species following RAPD analysis of a worldwide population (Ochieng 2001). The diversity reported within a region (Uganda) ranged from 78-100%, with a mean of 94% polymorphic loci. RAPD analysis of *C. sordidus* (Ochieng 2001) was based on 46 loci and 15 worldwide populations, while 37 loci and 15 populations were studied in Uganda. The present study is based on 659 loci (91% polymorphic loci) and the information content is, therefore, more than 14 times higher. The proportion of polymorphic loci in the present study is comparable to recent AFLP studies in termites (8-39%) (Garcia *et al.* 2002), crickets (28-43%) (Parsons & Shaw 2001) and three species of dung beetles (44.2-79.7%) (Carisio *et al*. 2004).

The low within-population diversity of the South Coast population in South Africa suggests a founder effect, where the current population was introduced as a small number of genetically related individuals. Alternatively, or in combination with a founder effect, strong selection pressures (including chemical control) may have contributed to the lower levels of diversity. In turn, the higher genetic diversity observed for the Australian and Tzaneen (in South Africa) populations suggests a

relatively large establishment population and/or lower selection pressures. The unique bands observed within these populations suggested intra-populational sub-structuring, or pooling of populations that differed in genetic composition.

Genetic variation among banana weevil populations was significantly genealogically and spatially clumped, despite relatively high levels of variation within populations. Molecular variance analysis indicated that the South African coastal populations grouped more closely with the Costa Rican population than with the Tzaneen population. Nevertheless, even for the former grouping, 89% of genetic variance was still partitioned (almost equally) among and within the populations. Among all the populations and the South African populations, most variation was equally divided or between, and not within populations, as reported by Ochieng (2001).

The coefficient of gene differentiation (G_{st}) can be interpreted according to Wright's (1978) suggestions for F_{st} (= G_{st}): The range 0 to 0.05 may be considered as little, 0.05 to 0.15 as moderate, 0.15 to 0.25 as great and values above 0.25 as very great genetic differentiation. Similarly, gene flow (N_m) values of less than one can indicate little or no gene flow (Crow & Aoki 1984). The overall genetic differentiation of *C. sordidus* populations, and also the populations from South Africa was, therefore, very great, with migration between populations very rare. Pair-wise comparisons indicated a very great differentiation between most populations, with a great separation between the South African coastal and Costa Rican populations, and with moderate genetic differences between the North and South Coast population from South Africa. As expected, results on gene differentiation were supported by the dependent gene flow parameter. Some degree of gene flow was suggested between populations of great genetic differentiation i.e. the South African coastal populations and Costa Rica. According to the Monte Carlo approximation of Fisher's exact test, most populations were separate entities, except for the Costa Rican and South African South and North Coast populations, which were similar. The significance should be interpreted with caution, as the approximation for diploid dominant data sets can only be performed on marker frequencies (Miller 1997) and thus may lead to an overestimate of population differentiation (Arafeh *et al*. 2002). The neighbour-joining dendrogram of *C. sordidus* showed that the South African coastal, Australian and Ugandan populations were distinct groupings, while the South African Tzaneen population presented the

ancestral state of the banana weevils. Phylogenetically, a recent common ancestor between the Costa Rican and South African coastal populations was not strongly supported.

Most of the data suggested that, under similar ecological and agronomical conditions, the most robust comparisons can be made between local coastal populations and studies conducted in Costa Rica. All the data suggested that the South African coastal, Australian, Ugandan, and Tzaneen populations could be classified as separate taxonomic units. Especially the Tzaneen population had a relatively unique AFLP fingerprint, but then also showed high within population diversity. Results were relative to a low number of *C. sordidus* populations sampled and even though three continents were included, analysis of additional populations is required to test the hypothesis. Biotype status of the different *C. sordidus* populations should be quantified under controlled studies in relation to host plant susceptibility, development and behaviour.

Genetic inter-populational differences are assumed to depend on genetic drift and gene flow (Carisio *et al.* 2004). Based on a model of population structure among organisms whose dispersal ability is constrained by distance (Kimura 1953; Kimura & Weis 1964), a positive correlation between geographical and genetic distances suggests a basic equilibrium between drift and gene flow, while no correlation indicates drift prevalence (Hutchison & Templeton 1999; Despres *et al.* 2002). No correlation between genetic and geographical distance for *C. sordidus* was found in the present study. Genetic drift could, therefore, be most important in shaping present day genetic diversity patterns of the banana weevil. Nevertheless, the data indicated that *C. sordidus* probably does not strictly conform to the model. The relative close genetic relationship and geographic distance between the North and South Coast populations in South Africa suggests gene flow or recent separation. The genetic disparity between the coastal populations and the Tzaneen population, in turn, can support isolation and genetic drift. However, the underlying genetic data (high genetic differentiation, high within population diversity and unique bands) of the Tzaneen population suggested that it could be the result of random dissemination from a number of populations rather than extreme genetic drift from a common or local ancestor. The species was first reported in South Africa in the 1920s (Cuille 1950; Simmonds 1966), but the original timing and source of the introductions are unknown. The current and future population genetics of the species may, therefore,

be complicated by the past and future dissemination of infested plant material within and between areas, of which no reliable records exist.

2.5 Acknowledgements

2.6   References

ANONYMOUS. 1986. *Banana Research in East Africa. Proposal for a Regional Research and Development Network*. INIBAP, Montpellier, France.

ANONYMOUS. 2005. *WordNet, a lexical database for the English language. Cognitive science laboratory, Princeton University, NJ*. World Wide Web: http://www.cogsci.princeton.edu/cgi-bin/webwn?stage=1&word=biotype.

ARAFEH, R.M.H., SAPIR, Y., SHMIDA, A., IRAKI, N., FRAGMAN, O. & COMES, H.P. 2002. Patterns of genetic and phenotypic variation in *Iris haynei* and *I. atrofusca* (*Iris* sect. *Oncocyclus* = the royal irises) along an ecogeographical gradient in Israel and the West Bank. *Molecular Ecology* 11: 39-53.

BRAITHWAITE, B.M. 1963. The banana beetle borer. *The Agricultural Gazette* 7: 396-419.

CARDENOSA, R. 1953. El genero *Musa* en Columbia. *Notas Agronomia* 6: 1-368.

CARISIO, L., CERVELLA, P., PALESTRINI, C., DELPERO, M. & ROLANDO, A. 2004. Biogeographical patterns of genetic differentiation in dung beetles of the genus *Trypocopris* (Coleoptera, Geotrupidae) inferred from mtDNA and AFLP analysis. *Journal of Biogeography* 31: 1149-1162.

CASTRILLON, C. 1991. Manejo del picudo negro (*Cosmopolites sordidus* Germar) en plátano y banano de la zona cafetera de Colombia. In: *Memorias* IX *ACORBAT*. 349-362.

CERVERA, M.T., CABEZAS, J.A., SIMÓN, B., MARTINEZ-ZAPATER, J.M., BEITIA, F. & CENIS, J.L. 2000. Genetic relationships among biotypes of *Bemisia tabaci* (Hemiptera: Aleyrodidae) based on AFLP analysis. *Bulletin of Entomological Research* 90: 391-396.

CROW, J.F. & AOKI, K. 1984. Group selection for polygenic behavioral trait: Estimating the degree of population subdivision. *Proceedings of the National Academy of Sciences of the United States of America* 81: 6073-6077.

CUILLÉ, J. 1950. *Recherches sur le Charancon du Bananier*. Institut des Fruits Agrumes Coloniaux; Serie Technique No 4, Paris.

CUILLE, J. & VILARDEBO, A. 1963. Les calandrini nuisibles au bananier. In: Balachowsky (Ed.) *Entomologie Appliquee a l'Agriculture*. 1099-1114. Masson et Cie Ed., Paris.

DAVIDE, R.G. 1994. Status of nematode and weevil borer problems affecting banana in the Philippines. In: Valmayor, R.V., Davide, R.G, Stanton, J.M., Treverrow, N.L., & Roa, V.N. (Eds.) *Proceedings of Banana Nematode/Borer Weevil Conference in Los Banos, Philippines: INIBAP*. 79-88.

DE GRAAF, J., GOVENDER, P., SCHOEMAN, A.S. & VILJOEN, A. 2005. Efficacy of pseudostem and pheromone seasonal trapping of the banana weevil *Cosmopolites sordidus* in South Africa. *International Journal of Pest Management* (*In Press*).

DESPRES, L., LORIOT, S. & GAUDEUL, M. 2002. Geographic pattern of genetic variation in the European globeflower *Trollius europaeus* L. (Ranunculaceae) inferred from amplified fragment length polymorphism markers. *Molecular Ecology* 11: 2337-2347.

ELLSWORTH, D.L., RITTENHOUSE, K.D. & HONEYCUTT, R.L. 1993. Artifactual variation in randomly amplified polymorphic DNA banding-patterns. *Biotechniques* 14: 214-217.

EXCOFFIER, L., SMOUSE, P.E. & QUATTRO, J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction sites. *Genetics* 131: 479-491.

FEAKIN, S.D. 1971. *Pest Control in Bananas*. 2nd Edition. London, PANS.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783-791.

FOGAIN, R. & PRICE, N.S. 1994. Varietal screening of some *Musa* cultivars for susceptibility to the banana weevil, *Cosmopolites sordidus* (Coleoptera: Curculionidae). *Fruits* 49: 247-251.

FRANZMANN, B.A. 1972. Banana weevil borer in North Queensland. *Queensland Agricultural Journal* 98: 319-321.

FROGGATT, J.L. 1926. *The Banana Weevil Borer*. Bulletin No. 4. Queensland Department of Agriculture and Stock, Brisbane, Australia.

GALLUN, R.L. & KHUSH, G.S. 1980. Genetic factors effecting expression and stability of resistance. In: Maxwell, F.G. & Jennings, P.R. (Eds.) *Breeding Plants Resistant to Insects*. 64-85. Wiley, New York.

GARCIA, J., MAEKAWA, K., MIURA, T & MATSUMOTO, T. 2002. Population structure and genetic diversity in insular populations of *Nasutitermes*

takasagoensis (Ispotera: Termitidae) analysed by AFLP markers. *Zoological Science* 19: 1141-1146.

GETTMAN, A.D., MITCHELL, W.C., LI, P. & MAU, R.F.L. 1992. A hot water treatment for control of the banana root borer, *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) in banana planting stock. *Proceedings of the Hawaiian Entomological Society* 31: 59-63.

GOLD, C.S., PEÑA, J.E. & KARAMURA, E.B. 2003. Biology and integrated pest management for the banana weevil *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae). *Integrated Pest Management Reviews* 6: 79-155.

GOLD, C.S., RUKAZAMBUGA, N.D.T.R., KARAMURA, E.B., NEMEYE, P. & NIGHT, G. 1999. Recent advances in banana weevil biology, population dynamics and pest status with emphasis on East Africa. In: *Proceedings of a Workshop on Banana IPM, Nelspruit, South Africa*. November 1998: 33-50.

GOWEN, S.R. 1995. Pests. In: Gowen, S.R. (Ed.) *Bananas and Plantains*. 382-402. Chapman & Hall, London.

HARRIS, W.V. 1947. The banana borer. *East African Agricultural and Forestry Journal* 13: 15-18.

HUFF, D.R., PEAKALL, R. & SMOUSE, P.E. 1993. RAPD variation within and among natural populations of outcrossing buffalograss *Buchloe dactyloides* (Nutt) Engelm. *Theoretical and Applied Genetics* 86: 927-934.

HUTCHISON, D.W. & TEMPLETON, A.R. 1999. Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. *Evolution* 53: 1898-1914.

JONES, D.R. 2000. Introduction to banana, abacá and enset. In: Jones, D.R. (Ed.) *Diseases of Banana, Abacá and Enset*. 1-36. CAB International, Wallingford.

KERMARREC, A., SIRJUSINGH, C., MAULÉON, H. & SARAH, J.L. 1993. Biological control of weevils and whitegrubs on bananas in the Caribbean: A review. In: Gold, C.S. & Gemmil, B. (Eds.) *Biological and Integrated Control of Highland Banana and Plantain Pests and Diseases. Proceedings of a Research Coordination Meeting in Cotonou, Benin: IITA*. 155-170.

KEY GENE PRODUCTS. 2000. *AFLP-Quantar Pro 1.0.* Wageningen, The Netherlands.

KIMURA, M. 1953. Stepping-stone model of population. *Annual Report of the National Institute of Genetics* 3: 62-63.

KIMURA, M. & WEIS, G.H. 1964. The stepping-stone model of population structure and the decrease of genetic correlation with distance. *Genetics* 49: 561-576.

KOPPENHÖFER, A.M. & SCHMUTTERER, H. 1993. *Dactylosternum abdominale* (F.) (Coleoptera: Hydrophilidae): A predator of the banana weevil. *Biocontrol Science and Technology* 3: 141-147.

KOSMAN, E. & LEONARD, K.J. 2005. Similarity coefficients for molecular markers in studies of genetic relationships between individuals for haploid, diploid and polyploid species. *Molecular Ecology* 14: 415-424.

KUMAR, S., TAMURA, K. & NEI, M. 2004. MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefings in Bioinformatics* 5:150-163.

LEWONTIN, R.C. 1972. The apportionment of human diversity. *Evolutionary Biology* 6: 381-394.

MANTEL, N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Research* 27: 209-220.

MAOLIN, S. 1994. Status of nematode and weevil borer problems affecting banana in China. In: Valmayor, R.V., Davide, R.G, Stanton, J.M., Treverrow, N.L., & Roa, V.N. (Eds.) *Proceedings of Banana Nematode/Borer Weevil Conference in Los Banos, Philippines, INIBAP*. 57-62.

MASANZA, M. 2003. *Effect of Crop Sanitation on Banana Weevil* Cosmopolites sordidus *(Germar) Populations and Associated Damage*. Ph.D. Thesis. Wageningen University, Wageningen, The Netherlands.

MAXWELL, F.G. & JENNINGS, P.R. 1980. *Breeding Plants Resistant to Insects*. Wiley & Sons, New York.

MILLER, M.P. 1997. *Tools for population genetic analysis (TFPGA) 1.3: A Windows program for the analysis of allozyme and molecular population genetic data*. Northern Arizona University, U.S.A.

MITCHELL, G. 1980. *Banana Entomology in the Windward Islands. Final report 1974-1978*. Centre for Overseas Pest Research, London.

MUELLER, U.G. & WOLFENBARGER, L.L. 1999. AFLP genotyping and fingerprinting. *Trends in Ecology and Evolution* 14: 389-394.

MURALIDHARAN, K. & WAKELAND, E.K. 1993. Concentration of primer and template qualitatively affects products in random-amplified polymorphic DNA PCR. *Biotechniques* 14: 362-364.

NEI, M. 1973. Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Science of the United States of America* 70: 3321-3323.

NEI, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583-590.

OCHIENG, V.O. 2001. *Genetic Biodiversity in Banana Weevil* Cosmopolites sordidus *Populations in Banana Growing Regions of the World*. Ph.D. Thesis, University of Nairobi, Kenya.

OSTMARK, H.E. 1974. Economic insect pests of bananas. *Annual Review of Entomology* 19: 161-176.

PARNISKI, P.F., TREVERROW, N.L., BEDDING, R.A. & SIKORA, R.A. 1990. Susceptibility of adults of the banana weevil borer *Cosmopolites sordidus* to 32 species and strains of *Heterorhabditis* and *Neoaplectana*. In: *Abstracts from the Second International Nematology Congress*. 121-122.

PARSONS, Y.M. & SHAW, K.L. 2001. Species boundaries and genetic diversity among Hawaiian crickets of the genus *Laupala* identified using amplified fragment length polymorphism. *Molecular Ecology* 10: 1765-1772.

PEAKALL, R., SMOUSE, P.E. & HUFF, D.R. 1995. Evolutionary implications of allozyme and RAPD variation in diploid populations of dioecious buffalograss *Buchloe dactyloides*. *Molecular Ecology* 4: 135-147.

PEÑA, J.E., DUNCAN, R. & MARTIN, R. 1993. Biological control of *Cosmopolites sordidus* in Florida. In Gold, C.S. & Gemmil, B. (Eds.) *Biological and Integrated Control of Highland Banana and Plantain Pests and Diseases. Proceedings of a Research Coordination Meeting in Cotonou, Benin: IITA*. 124-139.

RAVEL, S., MONTENY, N., OLMOS, D.V., VERDUGO, J.E. & CUNY, G. 2001. A preliminary study of the population genetics of *Aedes aegypti* (Diptera: Culicidae) from Mexico using microsatellite and AFLP markers. *Acta Tropica* 78: 241-250.

RAYMOND, M.L. & ROUSSET, F. 1995. An exact test for population differentiation. *Evolution* 49: 1280-1283.

REINEKE, A., KARLOVSKI, P. & ZEBITZ, C.P.W. 1999. Amplified fragment length polymorphism analysis of different geographical populations of the gypsy

moth, *Lymantria dispar* (Lepidoptera: Lymantriidae). *Bulletin of Entomological Research* 89: 79-88.

ROBINSON, J.C. 1996. *Bananas and Plantains*. CAB International, Wallingford.

RUKAZAMBUGA, N.D.T.M., GOLD, C.S. & GOWEN, S.R. 1998. Yield loss in East African highland banana (*Musa* spp., AAA-EA group) caused by the banana weevil, *Cosmopolites sordidus* Germar. *Crop Protection* 17: 581-589.

SAITOU, N. & NEI, M. 1987. The neighbour-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4: 406-425.

SAXENA, R.C. & RUEDA, L.M. 1982. Morphological variations among three biotypes of the brown planthopper, *Nilaparvata lugens* in the Philippines. *Insect Science and its Application* 3: 193-210.

SCHNEIDER, S., ROESSLI, D. & EXCOFFIER, L. 2000. *Arlequin ver. 2.000. A software for population genetics data analysis*. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

SESHU REDDY, K.V. 1993. An overview of the ICIPE'S project on the development of integrated pest management strategies for the control of the banana weevil/nematode complex. In Gold, C.S. & Gemmil, B. (Eds.) *Biological and Integrated Control of Highland Banana and Plantain Pests and Diseases. Proceedings of a Research Coordination Meeting in Cotonou, Benin: IITA*. 350-353.

SESHU REDDY, K.V., GOLD, C.S. & NGODE, L. 1999. Cultural control strategies for the banana weevil, *Cosmopolites sordidus* Germar. In: *Proceedings of a Workshop on Banana IPM, Nelspruit, South Africa*. November 1998: 51-57.

SIKORA, R.A., BAFOKUZARA, N.D., MBWANA, A.S.S., OLOO, G.W., URONU, B. & SESHU-REDDY, K.V. 1989. Interrelationship between banana weevil, root lesion nematode and agronomic practises and their importance for banana decline in the United Republic of Tanzania. *FAO Plant Protection Bulletin* 37: 151-157.

SIMMONDS, N.W. 1966. *Bananas*. 2nd Edition. Longmans Press, London.

SLATKIN, M. & BARTON, N.H. 1989. A comparison of three indirect methods for estimating average levels of gene flow. *Evolution* 43: 1349-1368.

STARKS, K.J. & BURTON, R.L. 1972. *Greenbugs: Determining Biotypes, Culturing and Screening for Plant Resistance*. Technical Bulletin, USDA-ARS. No 1556, 12pp.

STATSOFT INCORPORATED. 2004. *Statistica, version 7*. Tulsa, U.S.A.

STOVER, R.H. & SIMMONDS, N.W. 1987. *Bananas*. Third Edition. 469. John Wiley and Sons, New York.

TRAORE, L., GOLD, C.S., PILON, J.G. & BOIVIN, G. 1993. Effects of temperature on embryonic development of banana weevil *Cosmopolites sordidus* Germar. *African Crop Science Journal* 1: 111-116.

VITTAYARUK, W., WATTANACHAIYINGCHAROEN, W., CHUAYCHAROEN, T. & WATTANACHAIYINGCHAROEN, D. 1994. Status of weevil borer problems affecting banana in Thailand. In: Valmayor, R.V., Davide, R.G, Stanton, J.M., Treverrow, N.L., & Roa, V.N. (Eds.) *Proceedings of Banana Nematode/Borer Weevil Conference in, Los Banos, Philippines: INIBAP*. 106-114.

VOS, P., HOGERS, R., BLEEKER, M., REIJANS, M. VAN DER LEE, T., HORNES, M., FRIJTERS, A., POT, J., PELEMAN, J., KUIPER, M. & ZABEAU, M. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* 23: 4407-4414.

WATERHOUSE, D.F. & NORRIS, K.R. 1987. *Cosmopolites sordidus* (Germar). In: Waterhouse, D.F. & Norris, K.R. (Eds.) *Biological Control: Pacific Prospects*. 152-158, 454. Inkata Press, Melbourne.

WRIGHT, S. 1978. *Evolution and the Genetics of Populations. Variability Within and Among Natural Populations*. University of Chicago Press, Chicago.

YAN, G., ROMERO-SEVERSON, J., WALTON, M., CHADEE, D.D. & SEVERSON, D.W. 1999. Population genetics of the yellow fewer mosquito in Trinidad: comparisons of amplified fragment length polymorphism (AFLP) and restriction fragment length polymorphism (RFLP) markers. *Molecular Ecology* 8: 951-963.

YEH, F.C., YANG, R. & BOYLE, T. 1997. *Popgene, version 1.31. Microsoft windows-based freeware for population genetic analysis*. Joint Project Development by University of Alberta and the Centre for International Forestry Research.

ZABEAU, M. & VOS, P. 1993. Selective restriction fragment amplification: a general method for DNA fingerprinting. *European patent application, publication no. EP 0534858-A1, No. 92402629.7.*

ZHU-SALZMAN, K., LI, H., KLEIN, P.E., GORENA, R.L. & SALZMAN, R.A. 2003. Using high-throughput amplified fragment length polymorphism to distinguish sorghum greenbug (Homoptera: Aphididae) biotypes. *Agricultural and Forest Entomology* 5: 311-315.

ZIMMERMAN, E.C. 1968a. *Cosmopolites pruinosus*, a new pest of banana. *Journal of Economic Entomology* 61: 870-871.

ZIMMERMAN, E.C. 1968b. Rhynchophorinae of southeastern Polynesia. *Pacific Insects* 10: 47-77.

ZIMMERMAN, E.C. 1968c. The *Cosmopolites* banana weevils (Coleoptera: Curculionidae: Rhynchophorinae). *Pacific Insects* 10: 295-299.

Table 2.1. The geographical origin and global positioning system (GPS) co-ordinates of *Cosmopolites sordidus* populations and *Sitophilus orizae* (outgroup) sampled in 2004 for genetic analysis.

Species	Geographic origin	GPS co-ordinates	
		Latitude	Longitude
C. sordidus	Australia (New South Wales, Tweed Shire)	28º22'15''S	153º29'15''E
C. sordidus	Costa Rica (Limón, Guapiles)	10º36'10''N	84º17'11''W
C. sordidus	South Africa (KwaZulu-Natal, North Coast)	29º28'52''S	31º07'18''E
C. sordidus	South Africa (KwaZulu-Natal, South Coast)	30º58'14''S	30º15'33''E
C. sordidus	South Africa (Limpopo Province, Tzaneen)	23º48'09''S	30º07'41''E
C. sordidus	Uganda (Busoga Province, Kawanda)	0º25'05''N	32º31'54''E
S. orizae	South Africa (Gauteng, Pretoria)	25º39'00''S	28º22'30''E

Table 2.2. The sequences of adapters, primers and primer combinations used for amplified fragment length polymorphism (AFLP) analysis of different *Cosmopolites sordidus* populations.

Description	Sequence
*Eco*RI adapter	5´ CTCGTAGACTGCGTACC CTGACGCATGGTTAA 5´
*Pst*I adapter	5´ TGTACGCAGTCTAC ACGTACATGCGTCAGATGCTC 5´
*Pst*I primer	5´ GACTGCGTACATGCAG
*Eco*RI primer	5´ GACTGCGTACCAATTC
Preselective amplification primers	*Pst*I (+0) and *Eco*RI (+0)
Selective amplification primers (combination 1)	*Pst*I (+ACA) and *Eco*RI (+AT) [1]
Selective amplification primers (combination 2)	*Pst*I (+ACC) and *Eco*RI (+AT) [1]
Selective amplification primers (combination 3)	*Pst*I (+AGG) and *Eco*RI (+AT) [1]
Selective amplification primers (combination 4)	*Pst*I (+AGG) and *Eco*RI (+TC) [1]
Selective amplification primers (combination 5)	*Pst*I (+ACC) and *Eco*RI (+CC) [2]

[1] IRD-labelled *Eco*RI primer (800 nm) (LI-COR® Biotechnology Inc., Nebraska, USA).

[2] IRD-labelled *Eco*RI primer (700 nm) (LI-COR® Biotechnology Inc., Nebraska, USA).

Table 2.3. Intra population genetic diversity of *Cosmopolites sordidus* expressed as the percentage of polymorphic loci (*%PL*), Shannon's Information Index (*I*) and Nei's gene diversity (*h*). Standard deviations are in parenthesis.

Geographical population	Polymorphic loci	Genetic diversity parameter		
		%PL	*I*	*H*
Australia	272	45.03	0.200 (0.2666)	0.132 (0.1848)
Costa Rica	190	31.46	0.122 (0.2209)	0.078 (0.1518)
SA (NC) [1]	202	33.44	0.134 (0.2307)	0.087 (0.1584)
SA (SC) [2]	97	16.06	0.075 (0.1942)	0.050 (0.1342)
SA (TZ) [3]	323	53.48	0.169 (0.2117)	0.101 (0.1424)
Uganda	185	30.63	0.142 (0.2476)	0.095 (0.1715)
Mean	**211.5 (78.05)**	**35.02 (12.92)**	**0.142 (0.0425)**	**0.091 (0.0270)**

[1] South Africa (North Coast).

[2] South African (South Coast).

[3] South Africa (Tzaneen).

Table 2.4. Analysis of molecular variance (AMOVA) of *Cosmopolites sordidus* populations from six geographical areas. Analysis is indicated for all banana weevil populations with no hierarchical structure and for groupings of different populations.

| Grouping
Source of variation | d.f. | AMOVA parameter | | | |
		Sum of squares	Variance components	Percentage of variation	P-value
Cosmopolites sordidus					
Among populations	5	748.792	11.50238	49.51	<0.001
Within populations	66	774.167	11.72980	50.49	
Total	71	1522.958	23.23218	100	
SA (SC), SA (NC), CR vs.					
AUS, UG, SA (TZ) [1]					
Among groups	1	225.625	2.63426	10.85	<0.001
Among populations within groups	4	523.167	9.92182	40.85	<0.001
Within populations	66	774.167	11.72980	48.30	
Total	71	1522.958	24.28588	100	
SA vs. AUS, CR, UG [2]					
Among groups	1	171.375	0.75058	3.19	<0.05
Among populations within groups	4	577.417	11.05203	46.97	<0.001
Within populations	66	774.167	11.72980	49.85	
Total	71	1522.958	23.53241	100	
South Africa [3]					
Among populations in S.A.	2	367.333	14.19571	51.60	<0.001
Within populations in S.A.	33	439.500	13.31818	48.41	
Total	35	806.833	27.51389	100	

[1] A grouping of the South African North Coast, South African South Coast and the Costa Rican populations compared to a grouping of the Australian, Ugandan and South African Tzaneen populations.

[2] A grouping of the three South African populations (North Coast, South Coast and Tzaneen) compared to a grouping of the Australian, Costa Rican and Ugandan populations.

[3] Considering only the South African populations with no grouping.

Table 2.5. The coefficient of gene differentiation (G_{st}) and gene flow (N_m) among *Cosmopolites sordidus* populations from six different geographical areas.

Population	AUS [1]	CR [2]	SA (NC) [3]	SA (SC) [4]	SA (TZ) [5]	UG [6]
				G_{st}		
AUS [1]	-	0.3553	0.3174	0.3856	0.3554	0.2969
CR [2]	0.9071	-	[a] 0.2311	[b] 0.2177	0.4004	0.3054
SA (NC) [3]	1.0755	[a] 1.6636	-	[c] 0.1299	0.4045	0.3163
SA (SC) [4]	0.7966	[b] 1.7970	[c] 3.3489	-	0.4675	0.3586
SA (TZ) [5]	0.9067	0.7488	0.7362	0.5696	-	0.4003
UG [6]	1.1841	1.1374	1.0807	0.8941	0.7491	-

(N_m is indicated along the left side of the lower triangle.)

[1] Australia.

[2] Costa Rica.

[3] South Africa (North Coast).

[4] South Africa (South Coast).

[5] South Africa (Tzaneen).

[6] Uganda.

[a] Exact test: X^2=1130.44, *P*=0.9999.

[b] Exact test: X^2=1030.81, *P*=1.0000.

[c] Exact test: X^2=579.60, *P*=1.0000.

Figure legends

Figure 2.1. Amplified fragment length polymorphism (AFLP) fingerprint of selectively amplified DNA fragments from different *Cosmopolites sordidus* populations and *Sitophilus orizae* (outgroup). Molecular weight markers (M) and their sizes (in bp) are indicated. The inverted gel image of the selective primers *Pst*I (+ACC) and *Eco*RI (+AT) is presented for 12 individuals per population (six females and six males, respectively) between approximately 800 and 50 bp. Arrows mark selected polymorphisms. Not all polymorphisms are marked. [1] Australia, [2] Costa Rica, [3] South Africa (South Coast), [4] South Africa (North Coast), [5] Outgroup, [6] South Africa (Tzaneen) and [7] Uganda.

Figure 2.2. Neighbour-joining phylogram of *Cosmopolites sordidus* individuals from six populations and the outgroup population (*Sitophilus orizae*), based on the simple mismatch coefficient. Bootstrap values (5000 replications) are indicated on the branch nodes (only >70%) and a scale bar at the bottom of the graph indicates branch lengths.
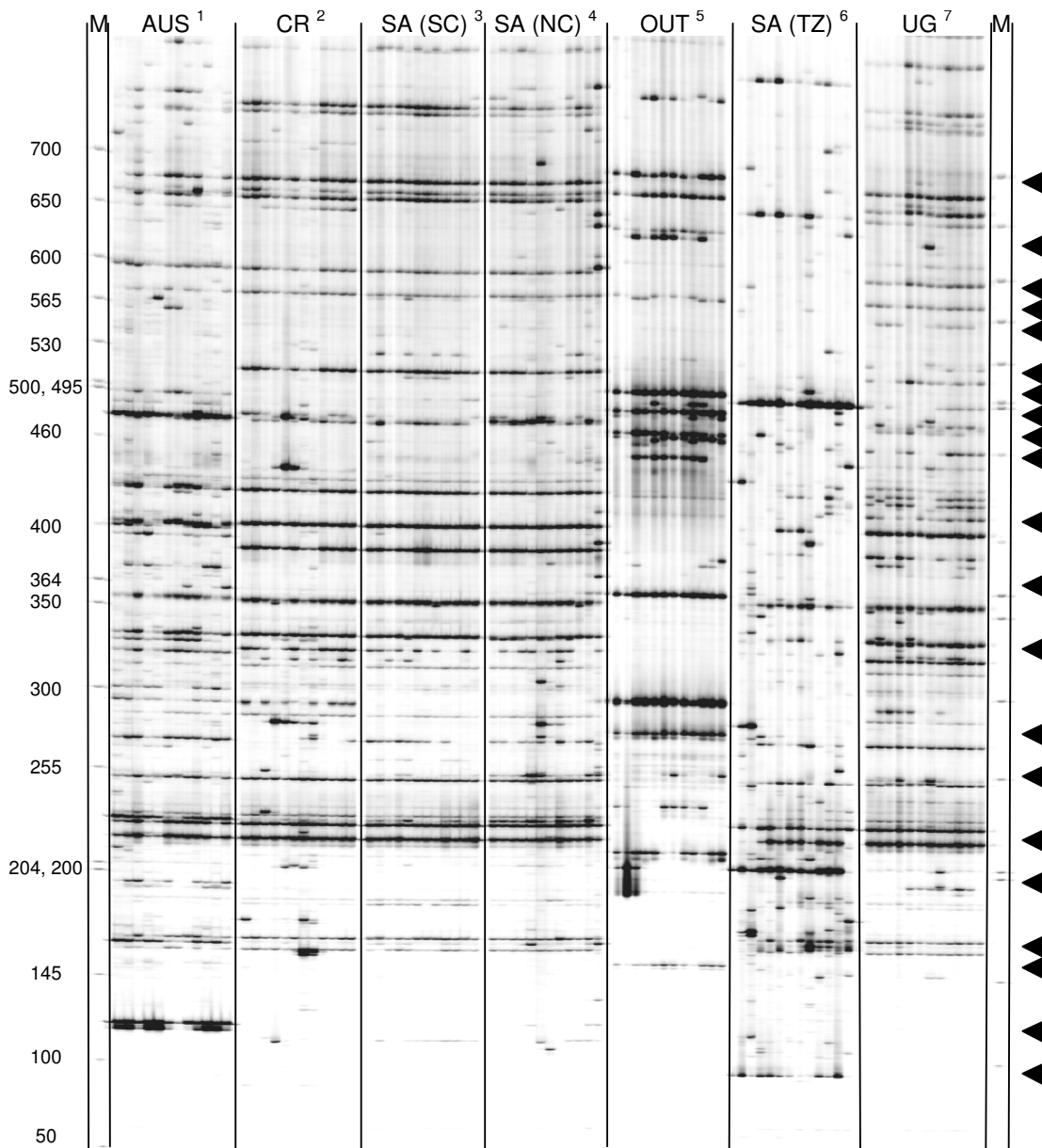
Figure 2.1

Figure 2.2