Additional Info by Paul Benhaim 1/4/15

Southern Cross University
It's all about U

Southern Cross University

Industrial hemp research at Southern Cross University

One of Southern Cross Plant Science's exciting projects, aimed at enhancing regional sustainability, involves exploring the potential of industrial hemp, by identifying and characterising genetic variation that can contribute to economically sustainable production. This pre-breeding research is carried out under licences from the NSW government, is based on extensive genetic resources, and will draw on a range of expertise at SCU.

SCPS and Ecofibre Lifesciences (ELS), based in Maleny, Queensland, have established a partnership to establish, curate and characterise the unique Ecofibre germplasm collection to underpin global development of industrial hemp. The original base collection of hemp genetic resources includes a wide range of material from all continents of the world, including the centre of genetic diversity in Asia, and from China in particular.

A key aim of current projects is to exploit the wide genetic base of hemp to:

- Extend the latitudinal range over which hemp may effectively be grown
- Identify and characterise genetic variation that can contribute to added value end-use products
- Maximise the use of the internal 'hurd' fibre, and value of seed, oils and outer fibre
- Work with other research institutions to identify unique characteristics in hemp that are technically useful to general manufacturing and industry.

Although hemp is an ancient crop, and was a key part of the economy of many nations worldwide, it has missed out on the 'Green Revolution' in crop science that massively increased yields since WWII. SCPS is now working with Ecofibre and researchers elsewhere to redress this.

Approaches being taken include:

- Development of specialised facilities for regeneration of germplasm that affords a high level of pollen "contraception" between chambers. This is a proprietary, parallel, closed-loop filtered system developed by Ecofibre
- Establishment of a database to bring together data from all sources about hemp germplasm collections here and elsewhere
- Characterisation of flowering time and morphological differences (habit, vigour, male/female proportions) between accessions
- Genetic analysis of chemotypes and development of DNA markers for pre-breeding

This provides an exciting opportunity to explore the various adaptations within the *Cannabis* genepool for industrial hemp, particularly in terms of flowering requirements and latitude adaptation, but also for many other phenotypic traits. The eastern states of Australia provide a particular advantage compared with other countries in the world for field trialling and evaluation of germplasm, with latitudes ranging from Tasmania (42 S) to Northern Queensland (17 S). The collection represents extensive genetic variation fibre, seed composition and physical phenotypes.

- SCPS operates a dedicated Analytical Research Laboratory (ARL) that carries out routine screening of commercially generated hemp plant tissue to meet the requirements and obligations of licensed growers' for sampling and analysis of low THC hemp under the Hemp Industry Act 2008.
- The ARL also has the expertise and experience to characterise many other plant components, including fatty acids and essential oils
- Development of DNA markers to accelerate the breeding process (enabling early seedling selection

of key characteristics)

- SCPS is well placed to carry out additional research on marker assisted selection, genome wide selection (GWAS) and study of genotype by environment (GxE) interactions. SCPS has state of the art genomics and bioinformatics facilities including our Illumina DNA sequencing platform.
- Subsequent field testing at different latitudes to identify variation in flowering time – this variation is under genetic control, and much information from other plants and crops can now be transferred to hemp in order to optimise flowering time.
- Alongside its analytical chemistry capability, SCU has new Engineering laboratories and equipment for characterising physical properties, which will be used to identify new end uses.
- Modern breeding will allow a shuffling of the genes between drug types and hemp types, and DNA markers will enable introduction of beneficial characteristics from the drug varieties whilst guaranteeing Industrial Hemp THC levels.

SCPS initially received funding of $95,000 for this project from a 'Researchers in Business' grant through the Australian government's Enterprise Connect initiative, co-funded by Ecofibre. Ecofibre Life Science has also invested in sponsorship of a PhD project from 2013 and an Honours scholarship in 2014.

Scientists at SCU have world-leading expertise in crop genetics and genomics, and are in contact with researchers elsewhere (UK, Europe, USA) to apply the latest technologies to accelerate the development of modern hemp cultivars able to be cultivated in different latitudes.

The industrial hemp development program fits well with the wider SCPS interests and expertise in pre-breeding genetics, quantitative genetics, genomics, data management, agronomy and raw materials quality. Other programs from which SCPS draws on experience and expertise include those for Brassica crops (mustard, canola), rice and some recently domesticated sub-tropical crops such as Macadamia and tea-tree

All facilities and operations at SCPS are managed under strict operating procedures in line with licences granted for handling and storage of plant material, chemical extracts and standards.
THC = Tetrahydrocannabinol

Media

ABC Landline - broadcast 20th July 2014 (http://www.abc.net.au/landline/content/2014/s4049599.htm)

Discover SCU - Sept 2014 (http://discover.scu.edu.au/2014-08-august/hemp-bank-at-scu/)

Contact: Prof Graham King

Updated: 10 March 2015

ABN: 41 995 651 524
CRICOS Provider: 01241G

Southern Cross University

Plant Science
Analytical Research Laboratory

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CERTIFICATE OF ANALYSIS

SAMPLE	Hemp seed
CUSTOMER	Hemp Foods Australia
CERTIFICATION DATE	23 February 2015
CUSTOMER LOT BATCH No.	Sample 1
LABORATORY REFERENCE	ARL150067
JOB No.	A150031

TEST	RESULTS		TEST METHOD
	% w/w	ppm	
Δ9-THC*	nd†	-	ARL-TM164
Δ9-THC-acid**	1.2 e^{-5}	0.1	

Assay by HPLC (274 nm detection), *calculated as Δ9-THC, **calculated as Δ9-tetrahydrocannabinolic acid (Δ9-THC-acid)

† not detected

.....

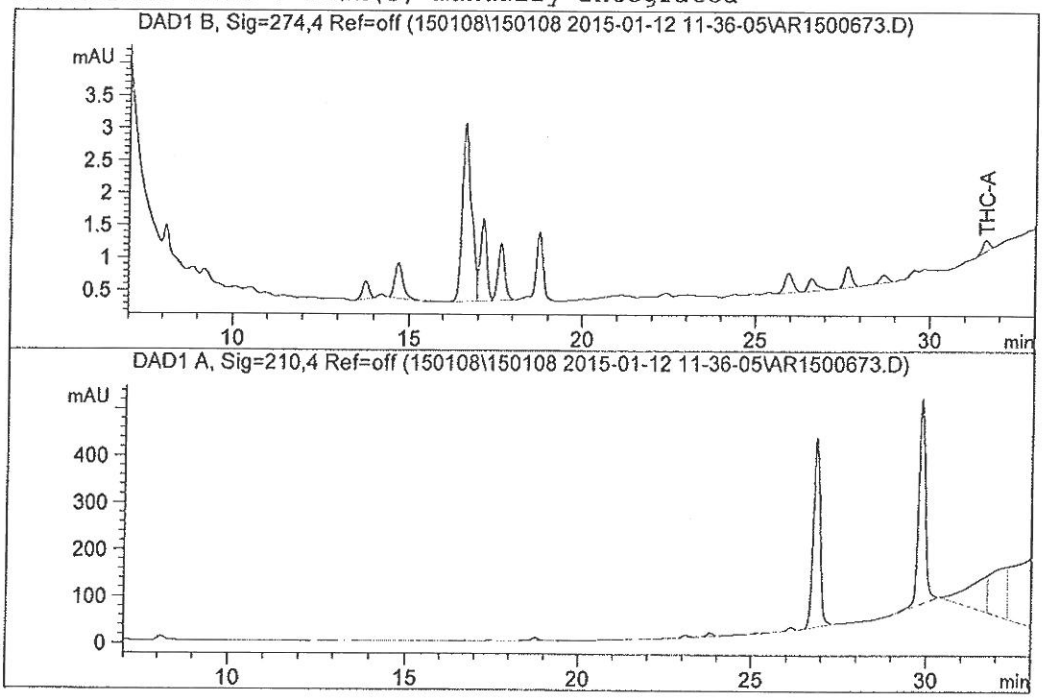
MR PETER MOUATT
SENIOR ANALYTICAL OFFICER

.....

MR ASHLEY DOWELL
MANAGER - ARL

Acq. Operator : DAS
Seq. Line : 17
Acq. Instrument : CHROMO-5
Location : Vial 63
Injection Date : 1/13/2015 6:08:12 PM
Inj : 1
Inj Volume : 5.000 µl
Acq. Method : D:\DATA\150108\150108 2015-01-12 11-36-05\QACANNA2.M
Last changed : 11/19/2014 9:32:47 AM by JB
Analysis Method : D:\METHODS\QACANNA2-MV274-210.M
Last changed : 2/5/2015 4:48:30 PM by DAS
(modified after loading)
Method Info : QTA cannabinoids - water/0.05% TFA - methanol - 250mm column - 0.5 mL/min

Additional Info : Peak(s) manually integrated



ESTD Percent Report

Sorted By : Retention Time
Calib. Data Modified : 2/5/2015 4:32:16 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Sample Amount: : 1045.00000 [mg/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 B, Sig=274,4 Ref=off
Signal 2: DAD1 A, Sig=210,4 Ref=off

RetTime [min]	Sig	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
17.000	2		-	-	-		CBD
18.900	1		-	-	-		CBD-A
25.000	1		-	-	-		THC
31.608	1	BB	2.37881	5.55596e-5	1.26474e-5		THC-A

Totals : 1.26474e-5

1 Warnings or Errors :

NDLERF

Application of new DNA markers for forensic examination of *Cannabis sativa* seizures – Developmental validation of protocols and a genetic database

Monograph Series No. 29

Funded by the National Drug Law Enforcement Research Fund
An Initiative of the National Drug Strategy

Application of new DNA markers for forensic examination of *Cannabis sativa* seizures – Developmental validation of protocols and a genetic database

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Funded by the National Drug Law Enforcement Research Fund, an initiative of the National Drug Strategy

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Abbreviations

ACT	Australian Capital Territory
bp	Base pair
BSA	Bovine Serum Albumin
CODIS	Combined DNA Index System
°C	Degrees Celsius
Δ	delta
dNTP	deoxynucleotide triphosphate
DNA	deoxyribonucleic acid
He	Expected Heterozygosity
F	field-grown
FI	Fixation Index
g	gram
H	hydroponic-grown
L	litre
µ	micro
m	milli
min	minute
M	molar
n	nano
Na	Number of Alleles
Ne	Number of Effective Alleles
Ho	Observed Heterozygosity
PCR	Polymerase Chain Reaction
P	pot-grown
PI	Probability of Identity
PIsibs	Probability of Identity between siblings
RMP	Random Match Probability
SWGDAM	Scientific Working Group on DNA Analysis Methods
s	second
STR	short tandem repeat
SA	South Australia
TAS	Tasmania
THC	tetrahydrocannabinolic acid
VIC	Victoria
WA	Western Australia

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- Dennis Pianca, Dr Julieanne Dougherty and Daniel Andres of the ACT Government Analytical Laboratory, Toxicology and Forensic Chemistry Unit, who assisted with sampling drug seizures from within the Australian Capital Territory, and provided laboratory access for DNA extractions from these samples.
- Tanya McKew of Forensic Science South Australia, for providing us with DNA from drug seizures within South Australia.
- Colin Priddis, Hannah Crisp, and Dr Kevin Ho of the Forensic Science Laboratory, Chemistry Centre Western Australia, for the supply of DNA from drug seizures within Western Australia.
- Dr Michael Manthey and Carl Grosser of the Forensic Science Service Tasmania, for the supply of DNA from seizures within Tasmania.
- the management of EcoFibre Industries Ltd, Queensland, Australia, for the supply of fibre varieties, and the AFP for laboratory access to extract DNA from these samples.

Nicotiana tabacum DNA was donated by Dr Spencer Whitney, Research School of Biological Sciences, and *Homo sapiens* DNA was supplied by an anonymous donor from the School of Botany and Zoology, the ANU. All other plant species were obtained from commercial nurseries in the ACT.

We thank Elizabeth McKeown and Meg Malaika for providing technical assistance in the laboratory and Christine Hayes and Dr Daniel Ebert for technical advice. Finally, we dedicate this report to the memory of Meg Malaika, who started this project but tragically was unable to see its completion.

Abstract

While *Cannabis sativa* has many industrial and therapeutic uses, drug varieties of *C. sativa* remain Australia's most frequently used illicit drug. It is widely presumed that organised crime groups largely supply the domestic black market for *C. sativa*. However, law enforcement agencies are often unable to link producers operating in suspected syndicates or to determine whether crops of legalised fibre varieties are being used for the covert production of drug varieties of the plant. Our specific objectives were to enable the transfer of DNA typing of *C. sativa* to the forensic community by: 1) validating a set of 10 Short Tandem Repeat (STR) markers for the forensic analysis of *C. sativa* seizures; and 2) establishing a database of genotypes across the 10 validated STR loci for approximately 500 *C. sativa* samples.

Our developmental validation based on recommendations of the Scientific Working Group on DNA Analysis Methods (SWGDAM) was conducted on a multilocus system of ten *C. sativa* STR loci. Amplification of the loci in four multiplex reactions was tested across DNA from dried root, stem and leaf sources, and DNA from fresh, frozen and dried leaf tissue with a template DNA range of 10.0 to 0.01 ng. The loci were amplified and scored consistently for all DNA sources when DNA template was in the range of 10.0 ng to 1.0 ng. Some allelic dropout and PCR failure occurred in reactions with lower template DNA amounts. Overall, amplification was best using 10.0 ng of template DNA from dried leaf tissue, indicating this is the optimal source material. Cross-species amplification was observed in *Humulus lupulus* for three loci but there was no allelic overlap. This was the first study following SWGDAM guidelines to confirm the feasibility of using STR markers for forensic analysis of *C. sativa*.

The database we established contains multilocus genotype data across the 10 validated STR loci for approximately 500 *C. sativa* plants representing drug seizures from five Australian states and territories and a selection of fibre samples. From the genotype data we were able to assess the number of alleles, allele frequency and degree of multilocus genotype sharing. Overall, we detected 106 alleles across 314 different multilocus genotypes. Fibre varieties were genetically more diverse than drug varieties of *C. sativa*. For example, while fibre samples represented only 11% of the total number of samples tested, these samples contained 86% of the total allelic diversity. Furthermore, 28% of the total of 106 alleles were only found in fibre samples. Moreover, all of the fibre samples tested had a unique multilocus genotype. Despite the lower genetic diversity of drug versus fibre samples, of the total of 106 alleles, 13% of the alleles detected were unique to the drug samples. Additionally, despite some genotype sharing, particularly within seizures, a high proportion of drug samples in our database did exhibit a unique multilocus genotype. These genetically distinct samples were found among field-, hydroponic- and pot-grown drug samples, but were most frequent in field-grown samples.

The finding of some genotype sharing within the drug samples is of interest. We evaluated two possibilities for this genotype sharing: 1) lack of sufficient resolution at the set of 10 STR loci used in the study; or 2) genotype sharing due to clonal propagation of the samples. Statistical analysis suggested that the 10 STR loci provided more than adequate resolution and on the weight of evidence we concluded that the genotype sharing was predominantly, if not exclusively, a consequence of clonal propagation. Consequently the finding of shared genotypes among seizures is likely due to either a common supplier, or direct links among seizures. If this genetic knowledge reinforces suspected linkages from other evidence, this combined knowledge may aid in prosecution.

Notwithstanding the potential intelligence information provided by genetic analysis of *C. sativa* drug seizures, our genetic database also highlights some present limitations of genetic analysis. As minimal overlap occurred between the drug and fibre sample populations in our database, we were more often than not able to distinguish between fibre and drug samples by population assignment procedures. However, assignment tests were not definitive for all samples. A DNA register of hemp/fibre varieties may alleviate this problem. Presently, it also appears unlikely that it will be possible to categorically assign a state of origin to an Australia seizure due to some sharing of genotypes among states. *Cannabis sativa* drug seizures from outside Australia may exhibit more informative differences. Therefore, future expansion of the current database may help to alleviate these limitations.

In conclusion, we have achieved our objectives to establish the accuracy and reliability of this technology through developmental validation, and compiled a genetic database for a substantial number of *C. sativa* samples. The next step in the implementation of *C. sativa* DNA typing can now be handed to established forensic laboratories. The final step will be realised when this technology is evaluated in the courtroom.

Chapter one: General Background

Both fibre and drug varieties of *Cannabis sativa* L. have a long association with humans. *Cannabis sativa* is thought to have originated in the central Asia region, and has since been distributed worldwide by humans who have cultivated the plant as a source of fibre, fodder, oils, medicines, and intoxicants for thousands of years (Small & Cronquist, 1976; Abel, 1980; Grispoon & Bakalar, 1993; Mercuri et al., 2002). Leaves and inflorescences contain psychoactive compounds collectively deemed cannabinoids, with Δ^9-tetrahydrocannabinolic acid (THC) being the most common (de Zeeuw et al., 1972). Drug varieties are typically characterised by elevated levels of THC (Pacifico et al., 2006). Despite the wide range of possible uses for *C. sativa*, due to its intoxicant properties, the cultivation and possession of the plant is prohibited by law in many countries.

Notwithstanding its prohibition in many jurisdictions, *C. sativa* is the most used illicit drug worldwide (Anderson, 2006). In Australia, as elsewhere, organised crime syndicates are often involved in large-scale production of *C. sativa*, with the commission of other offences related to the process of production-such as theft of electricity for hydroponics crops, firearms offences, money laundering, and violence to enforce debts or settle disputes-being common (Sherman, 1995; ACC, 2007).

In some jurisdictions licensing arrangements are available and advanced breeding schemes are actively cultivating low-THC varieties for fibre and seed oil industries (van der Werf et al., 1996; Struik et al., 2000; Ranalli, 2004). However, from a law enforcement perspective, the full-scale agriculture of *C. sativa* for fibre and seed oil poses a security problem, with the possibility of licensed crops being used as a cover for illegal drug crops and the potential for theft and subsequent fraudulent distribution of agricultural types as drug types. Also, there is the possibility of contamination of fibre crops with pollen of drug varieties as long distance dispersal of *C. sativa* pollen has been documented (Cabezudo et al., 1997). From an agricultural perspective, the inability to readily distinguish between fibre and drug *C. sativa* varieties based on morphology poses a major impediment to further development of the crop.

The ability to identify and/or link syndicates by determining the likely origin of seized drugs and to distinguish between legalised fibre crops and drug crops is highly sought by the international forensic community. In recent studies the geographical origin of seized *C. sativa* samples has been elucidated by the analysis of isotopic ratios combined with knowledge of the elemental makeup from geographical regions (Shibuya et al., 2006; Shibuya et al., 2007). While this method enabled *C. sativa* grown in the different local regions to be distinguished, it did not provide information that could link growers. Approaches utilising DNA information may provide even finer resolution than isotopic analysis and as such DNA-based tools for *C. sativa* identification and population studies are being developed by multiple research groups around the world. For example, DNA markers for distinguishing *C. sativa* from other plant species have been developed (Siniscalco Gigliano et al., 1997; Linacre & Thorpe, 1998) and population genetic surveys of genetic variation within *C. sativa* have been conducted using Polymerase Chain Reaction (PCR) based multilocus DNA fingerprinting methods (Gillan et al., 1995; Faeti et al., 1996; Jagadish et al., 1996; Kojoma et al., 2002; Datwyler & Weiblen, 2006). However, the dominant nature of these multilocus markers, and the potential for non *C. sativa* DNA amplification, limits their application for routine forensic analysis.

Codominant short tandem repeat (STR) markers, now the standard marker in human, animal, and most recently plant forensic investigations (Menotti-Raymond et al., 1997; Eichmann et al., 2005; Halverson & Basten, 2005; Menotti-Raymond et al., 2005; Butler, 2006; Craft et al., 2007), have recently been developed for *C. sativa* (Alghanim & Almirall, 2003; Gilmore & Peakall, 2003; Gilmore et al., 2003; Hsieh et al., 2003). STRs consist of tandemly repeated units of short nucleotide motifs, one to six base pairs (bp) long, with these regions occurring frequently throughout the genomes of plants and animals. STRs are widely considered the genetic marker of choice for population and identity studies within species due to their multiallelic nature and ease of transferability among laboratories (Jarne & Lagoda, 1996; Parker et al., 1998).

The first comprehensive study employing a subset of these STR markers provided information on *C. sativa* agronomic type, and the geographical origin of *C. sativa* drug seizures (Gilmore et al., 2003). This report builds on this earlier work and describes the development of an Australian national genotype database for the forensic investigation of *Cannabis sativa*.

Our specific objectives were to enable the transfer of DNA typing of *C. sativa* to the forensic community by: 1) validating a set of 10 STR markers for the forensic analysis of *C. sativa* seizures; and 2) establishing a database of genotypes across the 10 validated STR loci for approximately 500 *C. sativa* samples. Our sampling for the database included drug seizures from five states and territories of Australia and fibre varieties currently being evaluated for the hemp industry in Australia.

In this report we first present the outcome of our validation study. Our validation confirmed the reproducibility and reliability of the 10 STR loci that subsequently formed the basis of the genetic database that we describe and analyse in the second section of the report. We conclude our report with a general discussion on the forensic implications of our findings.

Chapter two: Marker Choice and Validation Requirements

2.1 Introduction

Codominant short tandem repeat (STR) markers, now the standard marker in human forensic investigations (Butler, 2006), have recently been developed for *Cannabis sativa* (Alghanim & Almirall, 2003; Gilmore & Peakall, 2003; Gilmore et al., 2003; Hsieh et al., 2003). The first study employing a subset of these STR markers provided information on *C. sativa* agronomic type, and the geographical origin of *C. sativa* drug seizures (Gilmore et al., 2003). However, in order to enable the use of *C. sativa* STR markers for routine forensic analysis, they need to be validated using standards that match those developed for human forensic DNA profiling (Miller Coyle et al., 2003a). Once validated, these methods may provide a powerful new investigative tool for intelligence analysis of organised and commercially motivated criminal activity involving *C. sativa*.

This section describes the developmental validation of a set of *C. sativa* STR markers based on the applicable guidelines established by the Scientific Working Group on DNA Analysis Methods (SWGDAM) (SWGDAM, 2004). Developmental validation is a critical first step in the transfer of new research tools to the forensic laboratory. The purpose of such validation is to provide detailed assessments of the sensitivity, accuracy and reproducibility of the DNA profiles generated by the genetic markers. Examination of the stability of various sources of DNA, including casework type samples, with respect to the production of reliable profiles, also forms an important component of developmental validation. Additionally, examination of species specificity and knowledge of population variation is required. To our knowledge this is the first investigation following SWGDAM validation guidelines to validate STR markers for forensic use in plants.

2.2 Methods

2.2.1 Loci and Multiplex Amplification Conditions

A subset of STR loci were chosen from the set of publicly available STRs for *C. sativa* (Alghanim & Almirall, 2003; Gilmore & Peakall, 2003; Gilmore et al., 2003). In this initial validation study we avoided loci with dinucleotide repeats as their DNA profiles can be more complicated to score. Consequently only tri- or penta-nucleotide repeat loci were chosen (with the exception of a combined di- and tri-nucleotide repeat unit).

Due to fragment size overlap and fluorescent dye constraints, the loci were divided into four separate groups for multiplex amplification. Multiplex amplification was carried out according to the conditions described in Table 2.1. Prior to finalizing the PCR conditions, the effect of magnesium concentration on each PCR multiplex was examined by amplifying 10.0 ng of a *C. sativa* control DNA sample with final $MgCl_2$ concentrations of 1.5, 2.0, 2.5, 3.0, and 4.0 mM. There was a trend for reduced PCR artefacts and more uniform heterozygote balance at the higher $MgCl_2$ concentrations (data not shown). Consequently, final $MgCl_2$ concentrations (3.0 – 4.0 mM) were adopted for subsequent multiplex PCR (Table 2.1).

A touchdown PCR thermal profile was employed. This allowed us to multiplex loci effectively, eliminating the need to PCR amplify each locus individually with differing cycling conditions (Don et al., 1991). Thermal cycling conditions were 95°C for 3 min, followed by ten cycles of 95°C 30 s, 66°C 30 s (reducing by 3°C every second cycle down to 54°C), 72°C 45 s, followed by 30

cycles of 95°C 30 s, 50°C 30 s, 72°C 45 s, with a subsequent final extension at 72°C for 30 min. Reactions were held at 10°C prior to further manipulation.

2.2.2 Tissue Source and DNA Extraction

Cannabis sativa samples were obtained from drug seizures from within the Australian Capital Territory (ACT). DNA from different tissue sources, tissue storage methods and the effect of DNA concentration on multiplex PCR were examined as follows.

Tissue source (air-dried leaf, stem and root) and storage method of leaf tissue (fresh, frozen at -80°C, and air-dried) were examined separately in triplicate using three independent samples for each category. Plant DNA was extracted from a selection of tissues using the DNeasy® Plant Kit (QIAGEN, Hilden, Germany). This extraction method has previously been validated for forensic DNA extraction of *C. sativa* by Miller Coyle et al. (2003b). DNA concentration for these validation experiments was standardised by precipitation with 0.3M Sodium Acetate with subsequent resuspension following standard protocols (Sambrook et al., 1989). DNA samples were electrophoresed along with known DNA concentration standards in 1.5% agarose gel containing ethidium bromide. Gels were recorded using a GelDoc XR Gel Documentation System (BIO-RAD, Hercules, CA, USA) and DNA concentration was estimated using Quantity One V5.6.2 software (BIO-RAD).

2.2.3 Sensitivity Study

To examine the appropriate range and limit of DNA template required for successful amplification, 10.0 ng, 1.0 ng, 0.1 ng, 0.01 ng of DNA from each tissue type and tissue storage condition were assessed. Each PCR batch contained two types of negative control; DNA storage buffer (Buffer AE, QIAGEN) and sterile distilled H_2O. An additional *C. sativa* positive DNA control (approx 1.0 ng) was also included. We subsequently recommend 1.0 - 10.0 ng of *C. sativa* DNA template as optimal, however, this was not known at this study's onset and therefore the amount of our control throughout was 1.0 ng.

2.2.4 Species Specificity

To assess their specificity the chosen *C. sativa* STR loci were tested for amplification across a range of non *C. sativa* DNA sources. This examination included species widely considered to be the most closely related to the *Cannabis* genus, *Humulus lupulus* (Hops), *Celtis australis* (Hackberry) and *Trema tomentosa* (Poison Peach). Also included were *Nicotiana tabacum* (Tobacco), a species known to be associated with *Cannabis* drug use (ACC, 2007), and *Homo sapiens* DNA, obtained using a BuccalAmp™ DNA Extraction Kit (EPICENTRE, Madison, WI, USA). For this test, 10.0 ng of each DNA sample was added in duplicate to multiplex PCRs (Table 2.1).

2.2.5 Fragment Detection and Genotype Analysis

In order to size and score the STR fragments, the amplification reactions were diluted (see Table 1) with sterile deionised water and one microlitre of each diluted reaction was added to a 19 µL mix consisting of 18.95 µL HiDi™ Formamide and 0.05 µL GeneScan™ - 500 LIZ™ Size Standard (Applied Biosystems, Foster City, CA, USA). Fragments were separated in Performance Optimised Polymer 4 (Applied Biosystems) and detected on an ABI PRISM® 3100 Genetic Analyser using the default sample injection settings.

To enable ease of transferability among laboratories, non overlapping bin size ranges were designed to match the tri- or penta-nucleotide repeat units with integer designations for fragment sizes and even left and right offsets.

Table 2.1. PCR components for each multiplex group. Concentrations indicated are for the final reaction volume.

	Loci	Forward Primer 5′ Label	Final concentration (forward and reverse primers)	Standard PCR components	Multiplex specific PCR components	Final Reaction Volume and Dilution factor *
Multiplex group 1	ANUCS501 C11-CANN1 ANUCS302	FAM VIC NED	0.1 µM 0.1 µM 0.1 µM	1 x PCR Buffer (QIAGEN) 0.2 mM dNTPs	4.0 µg BSA† 3.0 mM $MgCl_2$ 1 unit Taq DNA polymerase (QIAGEN)	Reaction volume: 40 µL Dilution Factor: 1:20
Multiplex group 2	ANUCS303 ANUCS305 B02-CANN2 ANUCS308	FAM VIC NED PET	0.1 µM 0.1 µM 0.1 µM 0.15 µM	1 x PCR Buffer (QIAGEN) 0.2 mM dNTPs	4.0 µg BSA 3.0 mM $MgCl_2$ 1 unit Taq DNA polymerase (QIAGEN)	Reaction volume: 40 µL Dilution Factor: 1:20
Multiplex group ‡ 3	ANUCS304 ANUCS301	PET VIC	0.2 µM 0.4 µM	1 x PCR Buffer (QIAGEN) 0.2 mM dNTPs	2.0 µg BSA 4.0 mM $MgCl_2$ 0.5 unit Taq DNA polymerase (QIAGEN)	Reaction volume: 20 µL Dilution Factor: 1:5
Multiplex group ‡ 4	B05-CANN1 B01-CANN1	NED PET	0.05 µM 0.2 µM	1 x PCR Buffer (QIAGEN) 0.2 mM dNTPs	2.0 µg BSA 3.0 mM $MgCl_2$ 0.5 unit Taq DNA polymerase (QIAGEN)	Reaction volume: 20 µL Dilution Factor: 1:10

*Post-PCR dilution factor prior to analysis on ABI PRISM® 3100 Genetic Analyzer

† Bovine Serum Albumin

‡Multiplex groups were combined with dilution following PCR

Fragment sizes, were determined using GENEMAPPER® Software 3.7 (Applied Biosystems). To ensure reliability, the genotype scoring process proceeded in two steps. First, genotype scoring was achieved by initially running the automatic scoring feature of GENEMAPPER® with default settings. Second, the automatic genotype scoring was manually checked. Any fragments not automatically scored but occurring within designated bins were manually scored if overall peak height was above 200 relative fluorescence units (rfu) if homozygous and 100 rfu if heterozygous.

The amount of amplification product for each allele was estimated from peak area values determined by the GENEMAPPER®. Additionally, allelic stutter proportion and heterozygote balance were measured from fragment peak height determined by the GENEMAPPER®. Allelic stutter proportion was calculated as the height of the stutter peak divided by height of the associated allelic peak. Stutter peaks were only considered in either homozygous samples or heterozygous samples where the stutter pattern was not obscured by an allelic peak. Additionally, stutter peaks were only considered if peak height exceeded 100 rfu. Heterozygote balance was calculated as the height of the smaller allelic peak divided by height of the larger allelic peak.

2.3 Results

2.3.1 Loci Characterisation

As anticipated for STR loci, the putative allele sizes only differed by the expected repeat unit length. Codominance was confirmed by the detection of no more than 2 alleles per sample. In most cases alleles were detected in both homozygous and heterozygous states.

As is common for STR loci (Gill et al., 2000a; Whitaker et al., 2001), there was some variation in heterozygote balance among the loci. For most heterozygous allele combinations at each locus, either PCR amplification marginally favoured the shorter allele or there was very little difference in the level of amplification for each allele (Figs 2.1a, 2.1b and 2.2a, 2.2b, 2.2c, and Table 2.1). However, there were several exceptions across the loci. In a number of particular heterozygous allelic combinations, heterozygote balance was lower than other allelic combinations for the same locus (Table 2.2). In addition, some heterozygous allele combinations at the loci B02-CANN2 and C11-CANN1 exhibited PCR amplification favouring the longer allele and also lower heterozygote balance. However, at these loci, not all heterozygous allelic combinations showed this amplification pattern (Table 2.2).

Typical STR stutter peaks (Walsh et al., 1996) were apparent at most loci (Figs 2.2b and 2.2c). Stutter peaks were identified without ambiguity from allelic peaks by their repetitive and substantially smaller height compared to the one or two major allelic peaks (Table 2.2). Allelic stutter proportions showed some variation among loci, and among alleles at the same locus (Table 2.2). The automatic scoring by GENEMAPPER® sometimes included these stutter peaks which required manual removal of these false allele calls. We note that there is further scope to modify the GENEMAPPER® analysis parameters to improve automatic scoring; however, manual checking of automatic scoring will always be essential.

2.3.2 Sensitivity and Stability

For all DNA sources and tissue storage methods, genotypes were amplified and scored consistently for DNA template amounts of 10.0 and 1.0 ng for all but locus ANUCS308. Within the 10.0 to 1.0 ng DNA template range, multiplex amplification of locus ANUCS308 was inconsistent, with

amplification failure occurring in approximately 33% of samples in this DNA amount range. For the accompanying loci in Multiplex Group 2, amplification failure was not observed at the 10.0 to 1.0 ng template DNA range, indicating that DNA quality was not responsible *per se*. Given this inconsistency of amplification despite adequate DNA quality, and that preliminary data indicated low allelic variation for this locus, it was removed from further validation analysis.

For all 10 remaining loci some amplification failure and allelic dropouts were detected with the lower DNA template amounts of 0.1 ng and 0.01 ng (Fig. 2.2a). For DNA template amounts of 0.1 ng and 0.01 ng, approximately 9% and 18% of samples respectively failed to amplify, and of the amplifiable samples, 1% and 5% of samples respectively showed an allelic dropout. Additionally a decrease in PCR amplification product was observed with decreasing amounts of template DNA across the different DNA sources and different tissue storage methods (Figs 2.1, 2.2). Generally there was little difference between the amount of amplification product when the PCR was initiated with 10.0 ng or 1.0 ng of template DNA for both tissue source and tissue storage method (Figs 2.1a, 2.1b). However DNA amplification from dried tissue was notably greater with the highest amount of template DNA (Fig. 2.1b). Multilocus genotypes were fully reproduced across the 10 loci. No unexpected genotypes were detected in the three replicates of each tissue type and tissue storage method when DNA template ranged from 10.0 ng to 1.0 ng.

2.3.3 Species Specificity

Three of the 10 loci-ANUCS303, ANUCS305 and B05-CANN1-produced discernable amplification products from *Humulus lupulus* DNA (Fig. 2.3). However, the level of amplification in *H. lupulus* was considerably lower than for *C. sativa* DNA and all putative alleles were smaller than the range of allele sizes known for *C. sativa*. Additionally, for the loci ANUCS303 and ANUCS305, the amplified *H. lupulus* fragments were not consistent with the repeat unit length of known *C. sativa* alleles. No other amplification products were detected for the non *C. sativa* species tested.

Table 2.2. Average allelic stutter proportion and average heterozygote balance for each locus.

Locus	Allele (bp)	Average Allelic Stutter Proportion *	Replicates	Heterozygous Allelic Condition	Average Heterozygote Balance †	Replicates
ANUCS501	88	0%	18	88/93	86%	3
	93	0%	3	88/98	73%	3
	98	0%	3			
C11-CANN1	152	11%	3	‡158/152	33%	3
	155	9%	12	‡158/155	47%	9
	158	13%	3	158/176	70%	3
	176	5%	3			
ANUCS302	139	8%	9	139/145	95%	3
	145	9%	6	139/154	97%	3
	151	6%	3	145/154	94%	3
	154	12%	6			
ANUCS303	145	5%	9	145/151	55%	6
	151	8%	15			
ANUCS305	142	1%	9	142/154	77%	9
	154	8%	18			
B02-CANN2	164	2%	3	‡167/164	30%	3
	167	3%	11	164/173	87%	3
	173	5%	5	‡173/167	84%	3
ANUCS304	171	20%	3	171/192	73%	3
	189	16%	3	189/207	88%	3
	192	29%	3	207/210	82%	3
	204	23%	3			
	207	25%	12			
ANUCS301	226	26%	6	226/232	14%	3
	232	24%	3	241/247	66%	3
	241	19%	3	244/265	32%	3
	244	22%	6			
	247	25%	3			
	265	31%	3			
B05-CANN1	236	3%	3	239/242	84%	6
	239	5%	9	239/245	96%	3
	242	5%	6			
	245	7%	3			
B01-CANN1	317	5%	3	326/329	79%	3
	326	9%	9	329/332	27%	3
	329	13%	6			

* Measured as height of the stutter peak divided by height of the associated allelic peak from profiles generated with 10.0 ng of template DNA added to multiplex PCR

† Measured as height of the smaller allelic peak divided by height of the larger allelic peak from profiles generated with 10.0 ng of template DNA added to multiplex PCR

‡ Heterozygotes displayed a greater level of amplification for the second allele

Figure 2.1. (a) Relative amounts of PCR amplification for all loci and all DNA sources combined over differing starting DNA template amounts. (b) Level of PCR amplification for differing DNA template amount and DNA source. Error bars represent standard error of the means.

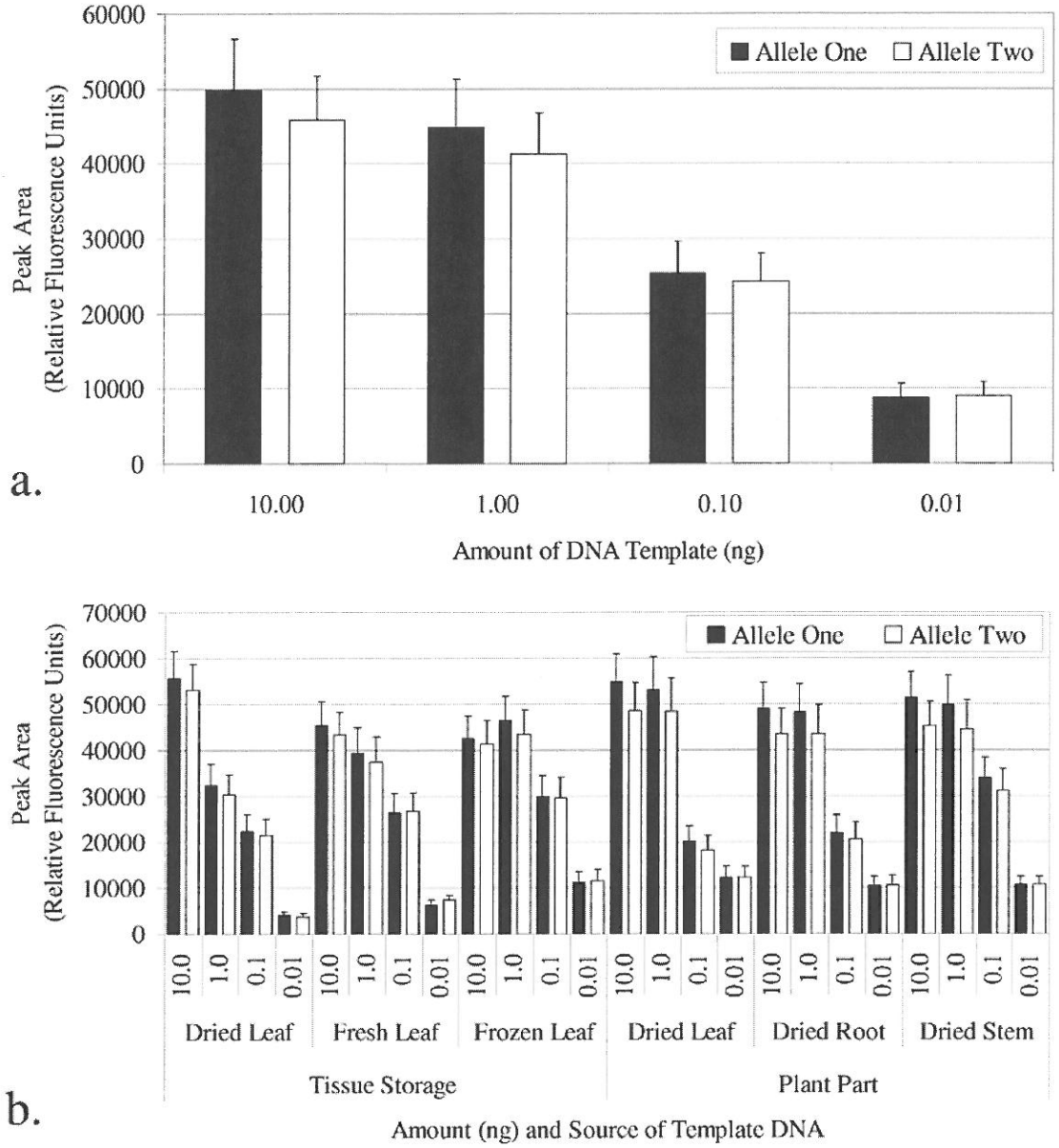


Figure 2.2. Electropherograms of three loci, a) ANUCS305, b) ANUCS304, c) ANUCS302, showing levels of amplification for DNA template amounts of 10 ng (top), 1 ng, 0.1 ng, 0.01 ng (bottom). An allelic dropout is evident for ANUCS305 at the 0.01 ng DNA template level.

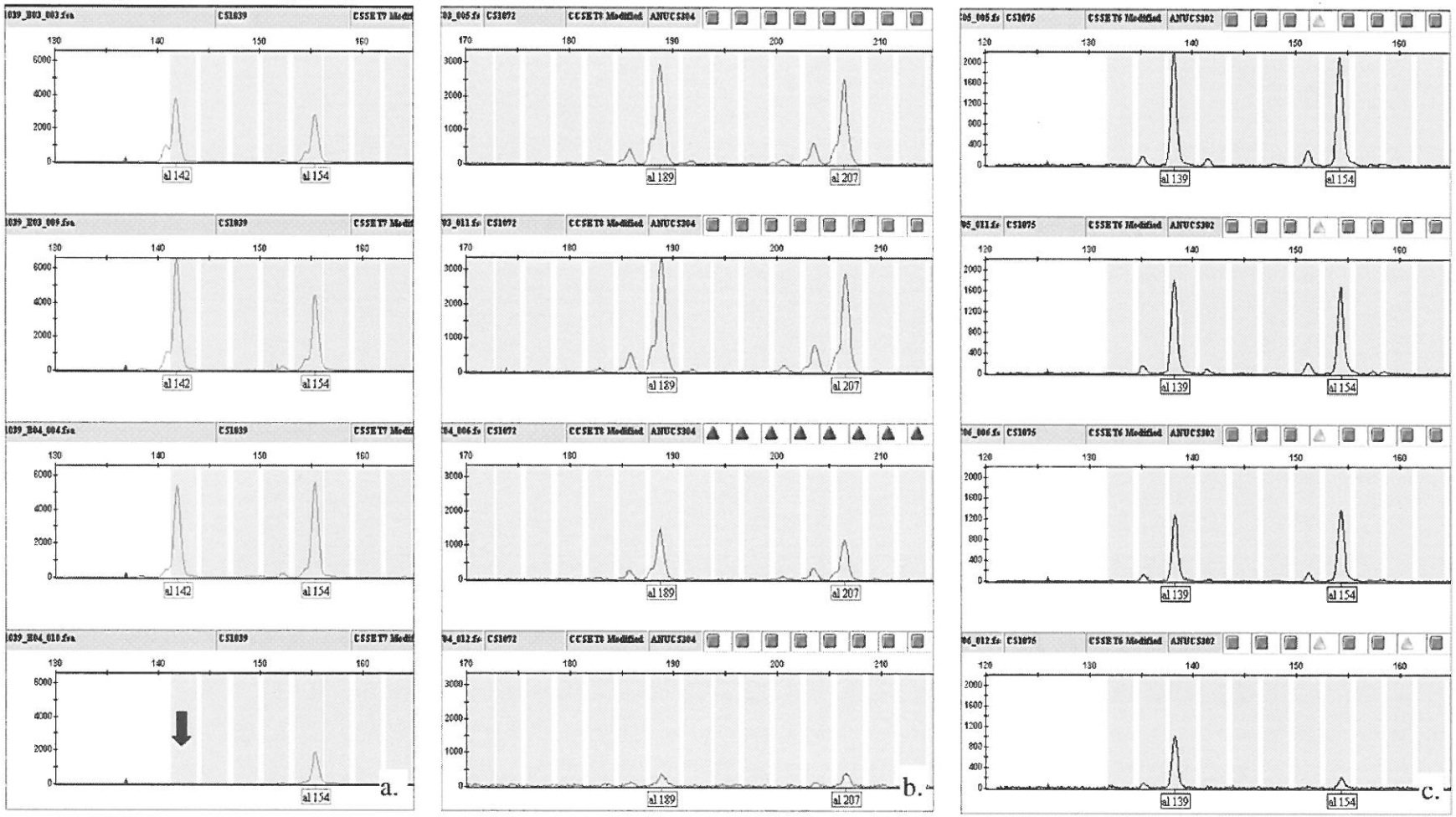
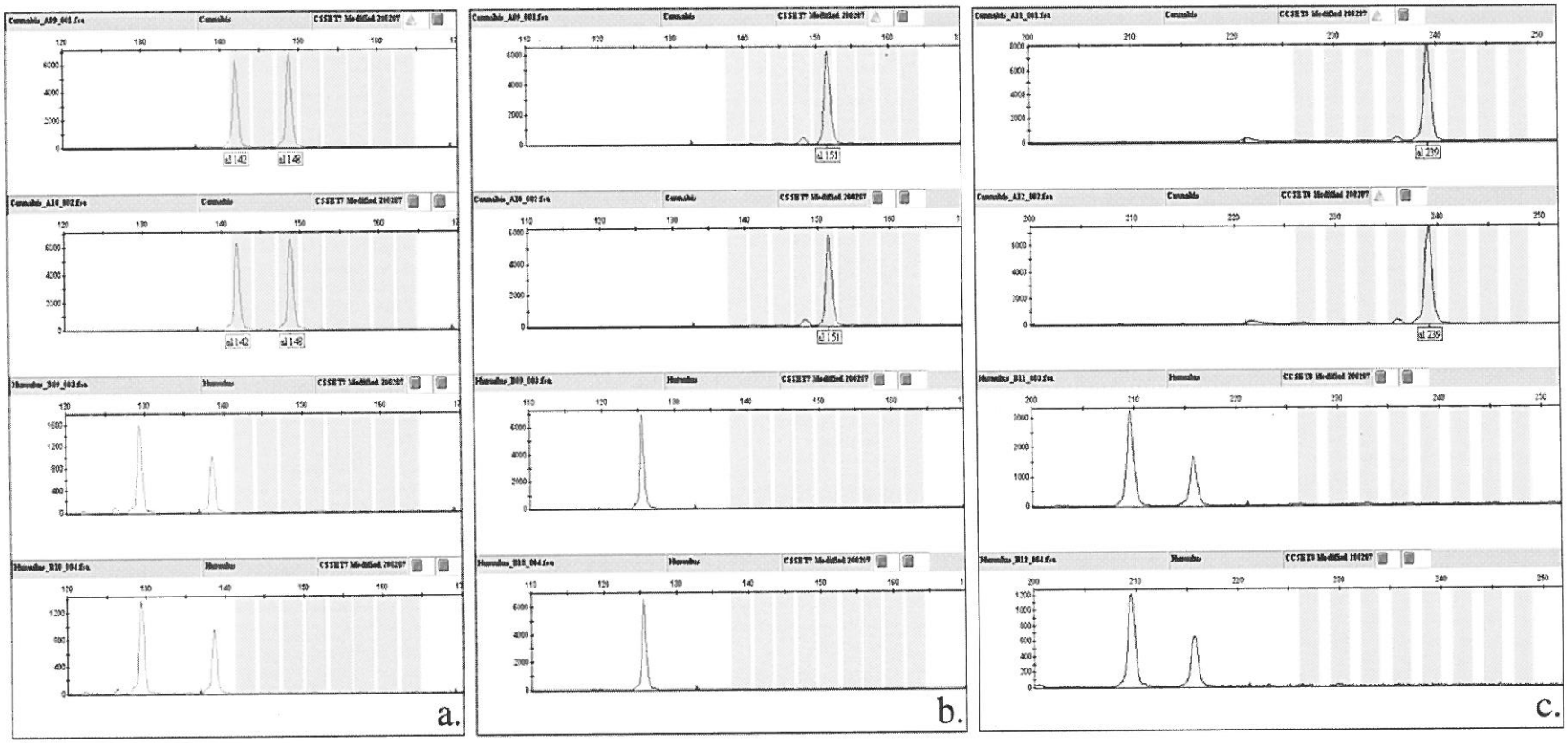


Figure 2.3. Electropherograms of three loci, (a) ANUCS305, (b) ANUCS303, (c) B05-CANN1, showing duplicate amplification products for *Cannabis sativa* (top two profiles) and *Humulus lupulus* (lower two profiles). Amplification products for *H. lupulus* fall outside the known allelic range of *C. sativa*.



2.4 Discussion

Following applicable SWGDAM guidelines, this developmental validation has shown that the set of 10 codominant *C. sativa* STR loci examined in this study can be routinely and reliably amplified and scored for the multiplex PCR conditions tested. This study now opens the way for internal validation studies within operational forensic laboratories. Given the expectation of some inter laboratory variation in optimal PCR conditions (Krenke et al., 2005), some minor modifications of the protocols tested here may be useful in subsequent internal validation studies. In the discussion that follows we offer recommendations for forensic laboratories planning to adopt these STR markers for forensic analysis of *C sativa*. We also highlight some of the issues encountered when applying SWGDAM validation guidelines to plants.

In our study, consistent genotypes were obtained from DNA templates in the range of 10.0 ng to 1.0 ng, from leaf, root and stem tissue of *C. sativa*. Despite success with root and stem tissue as a DNA source, where possible we recommend that DNA be obtained from either fresh or air-dried leaf as this tissue yielded the most consistent results. Leaf tissue is easily sampled and it is the most reliable source for morphological identification (Nakamura, 1969) if required.

As anticipated, where DNA is limited there is a risk of allelic dropout or overall amplification failure. We recommended that where possible 1.0–10.0 ng of DNA template be used for casework analysis of *C. sativa* with this multiplex system. We note that this is a larger amount of DNA that can be used in human forensics studies (Gill et al., 2000b). Additionally, while there were some variations in heterozygote balance and stutter proportions among alleles and heterozygote allelic combinations across the loci, allele scoring was never compromised by this variation.

Cross-species amplification of STRs in plants is common, but typically this is restricted to only a subset of loci in closely related species (Peakall et al., 1998). Cross-species amplification occurred between *C. sativa* and its close relative, *Humulus lupulus* for 3 of the 10 STR loci examined. As amplification in *H. lupulus* was poor and there was no allelic overlap between the two species, any contamination or misidentification can be easily detected. Furthermore there are obvious macroscopic morphological differences between *C. sativa* and *H. lupulus*. We anticipate that more likely sources of DNA contamination of casework samples will be from human or tobacco DNA. Crucially, neither of these DNA types amplify under these multiplex conditions.

The high sensitivity of these validated PCR protocols demonstrates the importance of minimising contamination from unknown sources of *C. sativa* DNA, with amplification occurring from as little as 0.01 ng of template DNA, albeit with some inconsistencies. Therefore standard forensic procedures such as isolating PCR preparation from template DNA extraction, use of sterile disposable plasticware, and avoiding aerosols carryover from pipettes (Higuchi & Kwok, 1989) is recommended.

This study indicated that inter-sample amplification failure of some loci can occur. While we eliminated one locus due to its high frequency of amplification failure, some sample-specific amplification failure may occur at the remaining loci in casework samples. This may be overcome by repeating the sample in a singleplex reaction (Gill et al., 1997).

The SWGDAM guidelines were specifically developed for human DNA forensic analysis (SWGDAM, 2004). Due to some differences between humans and *C. sativa* it was not possible to meet all of the SWGDAM guidelines. For example, SWGDAM guidelines recommend that inheritance and chromosomal mapping studies are completed. However, due to legal restrictions it was not possible to conduct breeding experiments with *C. sativa* in this study. Therefore inheritance characteristics (linkage or non Mendelian segregation) and chromosomal locations of these markers were not directly assessed. Measures of linkage disequilibrium (LD) in plants,

especially species which have been domesticated, often prove unreliable for inferring linkage given that the targeted selection of some phenotypic characters often impose a bias (Flint-Garcia et al., 2003). We also note that, unlike humans, *C. sativa* can be clonally propagated which avoids Mendelain segregation and results in identical genotypes between plants of clonal origin. Clonal reproduction has been shown to further bias LD estimates (Flint-Garcia et al., 2003).

The SWGDAM guidelines also specify that the ability to obtain reliable results from mixed source samples should be determined (SWGDAM, 2004). At least in initial forensic applications, we assume that an analysis of *C. sativa* DNA mixtures will prove to be both unnecessarily complex and likely to be of limited value to the law enforcement community. *Cannabis sativa* is commonly seized both as whole plants or highly homogenised dried fragments with the latter being possibly mixtures from several unknown and/or unlinked sources. Detecting a genotype mixture will show that the *C. sativa* sample was mixed at some point after production; it will not provide unequivocal evidence for when it was mixed, and by whom. We propose that analysis using this marker system will be most effective when seizures provide samples from which a single piece of intact tissue is easy to obtain. DNA mixtures of genetically distinct *C. sativa* individuals were not assessed in this study since genotype mixing at the time of seizure can be minimised in this way.

The present successful developmental validation of this set of 10 STR markers will allow for their conversion to an operational technology for routine forensic DNA analysis of *C. sativa* drug seizures.

Chapter three: Genotype Database for *Cannabis sativa*

3.1 Introduction

Cannabis sativa is an easily obtainable and highly exploited drug. While the plant has many industrial and therapeutic uses (Grispoon & Bakalar, 1993; Ranalli & Venturi, 2004), drug varieties of *C. sativa* remain Australia's most frequently used illicit drug (Anderson, 2006; ACC, 2007). It is widely presumed that organised crime groups largely supply the domestic black market for *C. sativa*. However, law enforcement agencies are often limited by their inability to link producers operating in suspected syndicates or to determine whether crops of legalised fibre varieties are being used for the covert production of drug varieties of the plant.

A wide range of botanical evidence is being increasingly used in forensic investigations. Historically this has centred on the use of distinctive morphological characters of seeds and pollen (Miller Coyle et al., 2001). More recently, genetic techniques are increasingly being adopted (Ward et al., 2005; Craft et al., 2007). The most commonly used genetic markers in human forensic investigations, short tandem repeat markers, have recently been developed for *C. sativa* (Alghanim & Almirall, 2003; Gilmore & Peakall, 2003; Gilmore et al., 2003; Hsieh et al., 2003), and a subset validated for use in forensic applications (see Section 2). These markers promise to assist forensic investigations of *C. sativa* drug seizures and to aid fibre variety breeding programs (Mandolino & Carboni, 2004; Ranalli, 2004).

With validated STR markers in hand for *C. sativa*, the next step before these genetic markers can be meaningful employed in forensic analysis is to develop a genetic database (Foreman et al., 2003). The purpose of such a database is to provide insight into the patterns of genotype and allelic variation within and among seizures, states or other sample groups. This knowledge is critical for understanding the capability and limitations of genetic analysis of *C. sativa* for forensic applications.

The aim of this section is to document the genetic diversity found at our 10 validated STR loci across a range of *C. sativa* samples representing both fibre and drug varieties. To our knowledge, this is the first genetic database in the world to be produced for validated STR profiles of *C. sativa*. We conclude this section by exploring the forensic insights provided by the database.

3.2 Methods

3.2.1 Sample Collection, DNA Extraction, and STR Genotype Scoring

We analysed a total of 510 individual *Cannabis sativa* samples, consisting of 440 known drug samples from 100 independent seizures and 57 known hemp/fibre samples from 12 independent groups (Table 1). *Cannabis sativa* drug samples were obtained from seizures from the following states and territories of Australia: the Australian Capital Territory (ACT); South Australia (SA); Western Australia (WA); and Tasmania (TAS). Samples of hemp/fibre varieties of *Cannabis sativa* were obtained from EcoFibre Industries (Toowoomba, Queensland, Australia). Drug samples consisted of plants that were grown using three different known methods: 'field', refers to samples grown in the ground and/or in fields; 'pot', refers to samples grown in pots or containers using artificial media or soil; 'hydroponic', refers to samples grown using hydroponic equipment. Among

the drug samples, hydroponically-grown samples were most numerous (41%), followed by field-grown (30%) and pot-grown (25%).

In addition to the above samples for which cultivar type, Australian state of origin, and growth type was known, two sets (listed below as Set 1 and Set 2) of *C. sativa* samples were obtained. Set 1: consisted of a set of drug samples from multiple seizures from within the ACT for which the growing conditions were unknown. The seizures from which these samples originated were subsequently denoted by '?'. Set 2: consisted of a further 13 *C. sativa* samples of uncertain cultivar type and origin, belonging to a single group of germinated seedlings, which were obtained from the Australian Federal Police (AFP). We included these ambiguous samples in analyses of total *C. sativa* only, but excluded then from calculations where cultivar type or state of origin was required. The *C. sativa* samples in Set 2 provided the opportunity to explore population assignment procedures described below.

Plant DNA was extracted as per Section 2.2.2. STR loci were PCR amplified for all samples following procedures outlined in Section 2.2.1 and multilocus genotypes were scored as described in Section 2.2.5.

Table 3.1. Summary of the state of origin and nature of *Cannabis sativa* samples used in this study. Samples were obtained from both drug seizures and licensed fibre varieties.

Region	Cultivar type	Growing Type	Number of samples	Number of Seizures
Australian Capital Territory	Drug	Hydroponic[1]	36	4
		Field[2]	46	13
		Pot[3]	73	7
		Unknown[4]	15	12
South Australia	Drug	Hydroponic[1]	82	13
		Field[2]	25	4
Victoria	Drug	Hydroponic[1]	29	15
		Field[2]	34	4
Western Australia	Drug	Hydroponic[1]	34	12
		Field[2]	28	3
		Pot[3]	29	12
Tasmania	Drug	Pot[3]	9	1
Unknown	Uncertain[5]	Unknown[4]	13	1
-	Fibre		57	12
		Total	510	113

[1] Refers to samples grown using hydroponic equipment
[2] Refers to samples grown in the ground and or in fields
[3] Refers to samples grown in pots or containers using artificial media or soil
[4] Growing conditions unknown (subsequently denoted by '?')
[5] Cultivar type uncertain

3.2.2 Allele Sequencing

A selection of alleles for each locus were directly sequenced to confirm the presence of the target STR and to assess whether alleles were the result of STR variation or other forms of genetic variation. Homozygous samples representing alleles of interest were chosen for sequencing and loci were PCR amplified in singleplex reactions using unlabelled forward primers following modified procedures found in Section 2.2.1. Amplification products were precipitated and sequenced in both directions following Porter et al. (2006).

3.2.3 Statistical Analysis of Genetic Data

The first step in our statistical analysis was to determine the number of multilocus genotypes present and whether any multilocus genotype sharing was evident among samples. Some sharing of multilocus genotypes was revealed by this analysis. This sharing may be attributed to either insufficient resolution of the genetic markers or clonal propagation of plants such that shared genotypes reflect a common clonal source. For the statistical analysis that follows we assumed that sharing of multilocus genotypes within a seizure most likely reflects a common clonal source, given the high frequency of clonal propagation of *C. sativa* (ACC, 2007). In this case only one representative of the genotype per seizure was included in subsequent allele frequency-based analyses. We further assumed that any sharing of multilocus genotypes among seizures was independent and unrelated, such that replicated shared multilocus genotypes were retained among seizures.

3.2.4 Allele Frequency-Based Statistical Analyses

The allele frequency-based statistical analyses were performed at multiple hierarchical levels. Analyses based on these levels included: a) the total data set of all *C. sativa* samples; b) all drug and fibre samples; c) drug samples divided into field- (F), hydroponic- (H) and pot-grown (P) groups; d) drug samples divided into Australian state of origin groups; and e) drug samples divided into individual seizure groups. For each analysis level we calculated a range of standard population genetic statistics including: the Number of Alleles (*Na*), the Number of Effective Alleles (*Ne*), Observed Heterozygosity (*Ho*), Expected Heterozygosity (*He*) and the Fixation Index (*FI*) for all 10 STR loci. These allele frequency-based statistics provide estimates of genetic diversity that can be compared among loci, among groups and among species and were calculated using the software GENALEX (Peakall & Smouse, 2006)

Hardy-Weinberg Equilibrium (HWE), and Linkage Disequilibrium (LD) tests were performed for each locus on all of the population groups listed above (except 'e') using the software GENEPOP (Raymond & Rousset, 1995). As noted in section 2, unlike human forensic DNA analysis where the assumption of random mating is closely approximated, we cannot assume this will be the case for *C. sativa* due to the ability to clonally propagate plants. Consequently, Mendelian segregation is avoided, resulting in identical genotypes between plants of clonal origin. Furthermore, measures of LD in domesticated plants often prove unreliable for inferring linkage given that the targeted selection of some phenotypic characters often impose a bias (Flint-Garcia et al., 2003). Clonal reproduction has been shown to further bias LD estimates (Flint-Garcia et al., 2003).

Following Gilmore et al. (2003), an Analysis of Molecular Variance (AMOVA) was performed using the population genetic analysis software, GENALEX (Peakall & Smouse, 2006), to separately estimate the degree of genetic differentiation among fibre and drug samples, among state of origin of drug samples, and among growth-type groups of drug samples.

3.2.5 Population Assignment

In order to test our ability to correctly assign a sample to a given *C. sativa* type (drug or fibre), following the recommendation of Paetkau et al. (2004) for predicting the statistical power of assignment tests, we plotted genotype log likelihood [*Log (L)*] biplots for the drug and fibre sample groups. In such biplots, a strong indication of sufficient statistical power to correctly assign a population to a sample is indicated when the two populations form discrete non-overlapping clusters (Paetkau et al., 2004). Genotype likelihood biplots were also generated for *C. sativa* drug samples between drug growth-type (hydroponically-, field- or pot-grown) and the Australian state of origin of the drug samples. Generation of these plots and standard population assignment tests were performed using GENALEX (Peakall & Smouse, 2006).

Subsequently, we performed simulation testing, using GENECLASS 2 (Piry et al., 2004), via the method of Paetkau et al. (1995) in which a novel Monte Carlo re-sampling method to test the null hypothesis that an individual sample originated in the population in which it was sampled. Population assignment based on *Log (L)* values, and the simulation based assignment tests were performed the on two sets of data. The first data set was generated by removing a random sub-sample of each of the known drug and fibre groups approximately equal to 10% of the original group's size, and placing these in a hypothetical unknown group. Specifically, twenty four random drug samples, and five random fibre samples were removed from the total and placed into an unknown group. With these samples excluded from frequency calculations, we then determined whether these hypothetically unknown samples were correctly assigned as drug or fibre types based on the estimated *Log (L)* values and the outcomes of simulation testing. This was repeated for a total of 5 replicate randomly produced data sets (145 samples in total). The second data set that was tested for population assignment consisted of the 13 *C. sativa* samples of uncertain cultivar type and origin was obtained from the Australian Federal Police.

3.2.6 Match Probabilities

In addition to the genotypic and allelic diversity measures, Random Match Probability (*RMP*) estimates for each given genotype/DNA profile, were calculated for each multilocus genotype. The *RMP* provides an estimate of the probability of encountering each specific multilocus genotype a second time within the population, assuming random mating (National Research Council, 1996; Samuels & Asplen, 2000). Additionally, we calculated: the overall Probability of Identity (*PI*), being the probability that two individuals drawn at random will have the same multilocus genotype; and the Probability of Identity between siblings (*PIsibs*), which considers potential relatedness of samples (Waits et al., 2001; Buckleton & Triggs, 2005). Despite violation of the random mating assumption, the *RMP*, *PI*, and *PIsibs* estimates may still provide useful comparative statistics among *C. sativa* genotypes and the seizures to which they are found. The *RMP*, *PI* and *PIsibs* estimates were calculated with GENALEX using the formulae below:

$$RMP = \prod p_i^2 \times \prod 2p_i p_j$$

Where Π indicates chain multiplication across each locus, p_i is the frequency of the *i*-th allele at homozygous loci, p_i and p_j are the frequencies of alleles at heterozygous loci for alleles represented in the specific multilocus genotype in question.

$$PI = 2(\sum p_i^2)^2 - \sum p_i^4$$

Where p_i is the frequency of the *i*-th allele at each locus for the particular population in question. The *PI* over multiple loci is calculated as the product of the individual locus *PI*'s. *PI* represents the average probability of a match for any genotype, rather than for a specific genotype, as in the case of the *RMP*.

$$PIsibs = 0.25 + (0.5\sum p_i^2) + (0.5(\sum p_i^2)^2) - (0.25\sum p_i^4)$$

Where p_i is the frequency of the *i*-th allele at a locus. The *PIsibs* over multiple loci is calculated as the product of the individual locus *PIsibs*.

3.2.7 Source of Analysis Software

All of the software used in our analyses, including supporting documentation, is freely available from the following internet based sources:
GENALEX: http://www.anu.edu.au/BoZo/GenAlEx/
GENEPOP: http://genepop.curtin.edu.au/
GENECLASS: http://www.montpellier.inra.fr/URLB/geneclass/geneclass.html

3.3 Results

3.3.1 DNA Sequencing of Common Alleles

DNA sequencing of a selection of alleles for 9 of the 10 STR loci confirmed that the STR loci originally described (Alghanim & Almirall, 2003; Gilmore & Peakall, 2003) was the basis of allele length variants. Optimal full length sequence data could not be generated for the locus ANUCS501 due to the short length of the amlicon. However, a 5 bp length difference between every allele found in this study for locus ANUCS501 indicated that the STR region was in fact amplified and that the alleles were generated by variation within the 5 bp STR region. At the remaining nine loci, sequencing revealed that alleles were generated by the expansion or contraction of the repeat unit of the STR, with one exception (C11-CANN1). Some alleles of the locus C11-CANN1 were the result of a 15 bp insertion 44 bp upstream of the STR unit in conjunction with an expansion or contraction of the core STR unit. However, despite this insertion, allelic size variation remained in multiples of the core STR repeat size (3 bp).

3.3.2 Multilocus Genotype Recovery

A total of 314 genotypes were detected over the 10 STR loci examined for all *C. sativa* samples. Of the 314 genotypes, all 57 fibre samples had a unique genotype. Amongst the 440 known drug samples, 197 genotypes were unique, with 47 genotypes being shared across the remaining 243 samples (i.e. 440 - 197) (Fig. 3.1a). The drug seizures from within the ACT from which growth-type was unknown (Set 1) included mostly unique multilocus genotypes but also some that were shared between these ACT seizures and among seizures from different states (see below). The 13 ambiguous samples belonging to a single group of germinated seedlings (Set 2) contained 13 unique multilocus genotypes

Figure 3.2 shows the number of different genotypes resolved for increasing combinations of loci, ordered from most to least informative. For fibre samples, all 57 genotypes were resolved with the combination of only three loci. For all drug samples, including genotype matches within seizures, the number of unique genotypes that were resolved started to plateau with the combination of 7 loci. There was little change in the number of unique genotypes recovered with the addition of the remaining 3 STR loci and all unique multilocus genotypes were resolved with the combination of the 8th and 9th loci (Fig. 3.2). The same pattern was found when all but one replicate of matching genotypes within independent seizures was excluded. Within this latter dataset, approximately 86% (235/271) of the samples could be resolved to a unique multilocus genotype using the 10 STR loci. The remaining 36 unresolved samples corresponded to the samples with matching genotypes found among seizures. All multilocus genotypes are reported in Table 6.1.

Figure 3.1. Patterns of genotype sharing among *Cannabis sativa* samples. The proportion of samples with unique versus shared genotypes for both *C. sativa* variety and drug growth-type are shown.

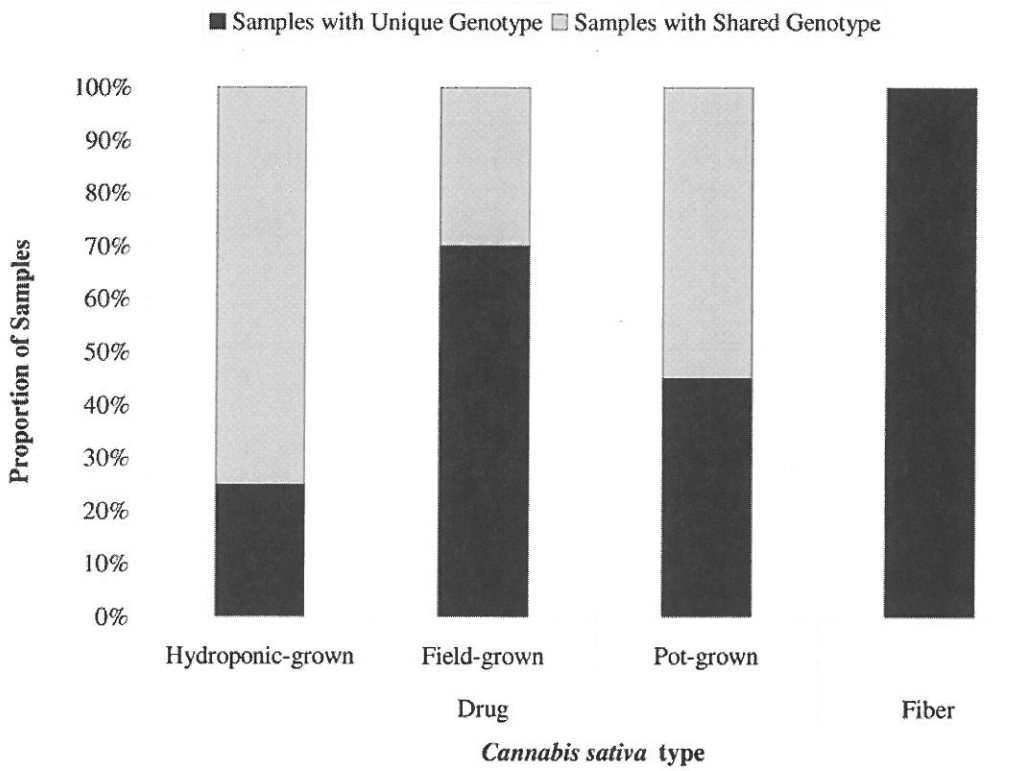


Figure 3.2. Multilocus genotype resolution over 10 short tandem repeat loci showing the proportion of fibre and drug samples resolved to a unique genotype for increasing combinations of loci.

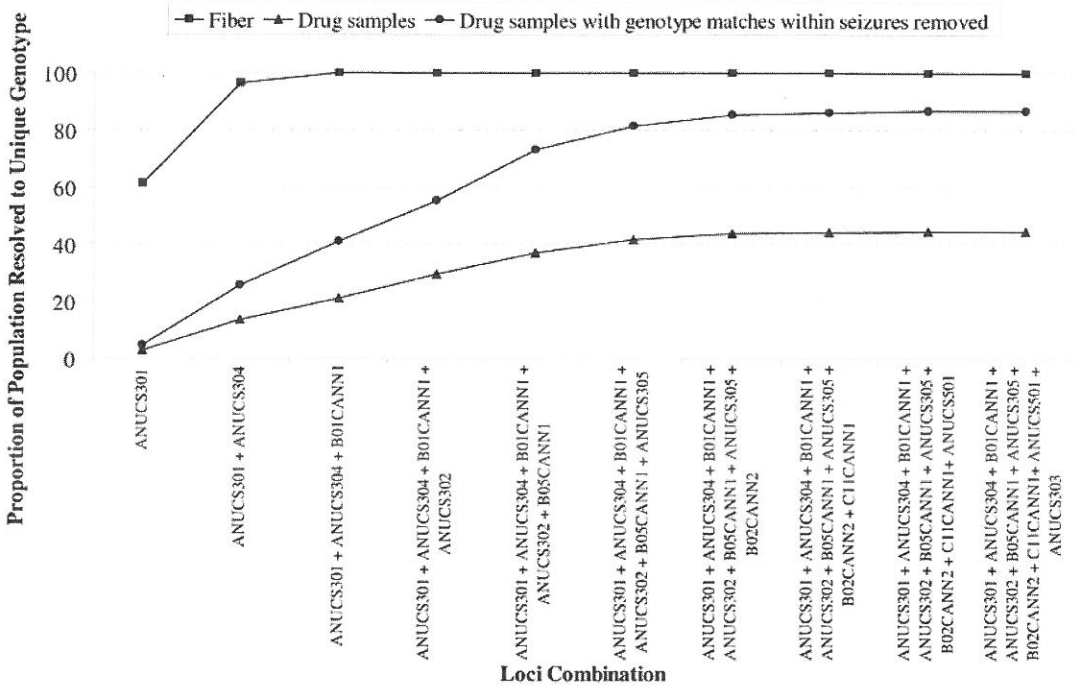


Table 3.2. Summary of *Cannabis sativa* STR loci allelic characteristics with respect to various population groupings of samples used in this study. Loci are listed in the order that provided maximum multilocus genotype resolution.

Grouping		ANUCS301	ANUCS304	B01-CANN1	ANUCS302	B05-CANN1	ANUCS305	B02-CANN2	C11-CANN1	ANUCS501	ANUCS303
Cannabis	*Na*	24	21	14	9	6	9	4	7	4	9
N 341	*Ne*	5.663	5.662	3.279	3.666	3.019	3.093	2.650	2.735	2.313	2.167
	Ho	0.478	0.246	0.516	0.299	0.572	0.548	0.537	0.422	0.437	0.299
	He	0.823	0.823	0.695	0.727	0.669	0.677	0.623	0.634	0.568	0.539
	FI	0.419	0.701	0.257	0.589	0.145	0.190	0.138	0.334	0.230	0.445
Fibre	*Na*	19	18	11	8	5	9	4	5	4	9
N 57	*Ne*	12.078	5.530	3.840	4.149	2.888	5.348	2.748	3.391	1.672	3.173
	Ho	0.667	0.509	0.439	0.474	0.614	0.719	0.649	0.193	0.351	0.491
	He	0.917	0.819	0.740	0.759	0.654	0.813	0.636	0.705	0.402	0.685
	FI	0.273	0.379	0.407	0.376	0.061	0.115	-0.021	0.726	0.127	0.283
Drug	*Na*	18	14	11	6	4	4	4	7	4	4
N 271	*Ne*	4.194	4.546	3.082	2.933	2.946	2.713	2.497	2.463	2.430	1.896
	Ho	0.439	0.188	0.520	0.269	0.557	0.498	0.509	0.480	0.469	0.247
	He	0.763	0.780	0.675	0.659	0.661	0.631	0.600	0.594	0.588	0.472
	FI	0.423	0.759	0.230	0.591	0.157	0.211	0.151	0.192	0.204	0.477

Na = No. of Different Alleles
Ne = No. of Effective Alleles = 1 / (Sum p_i^2)
Ho = Observed Heterozygosity = No. of Hets / N
He = Expected Heterozygosity = 1 - Sum p_i^2
FI = Fixation Index = (He - Ho) / He = 1 - (Ho / He)
Where p_i is the frequency of the i-th allele for the population & Sum p_i^2 is the sum of the squared population allele frequencies.

Table 3.2 -continued

Grouping		ANUCS301	ANUCS304	B01-CANN1	ANUCS302	ANUCS305	B02-CANN2	C11-CANN1	B05-CANN1	ANUCS501	ANUCS303
Field-grown	*Na*	13	11	8	6	3	3	5	3	3	3
N 103	*Ne*	3.377	4.326	3.165	3.151	2.502	2.443	2.386	2.991	2.385	1.599
	Ho	0.408	0.223	0.515	0.311	0.524	0.485	0.524	0.524	0.466	0.155
	He	0.704	0.769	0.684	0.683	0.600	0.591	0.581	0.666	0.581	0.375
	FI	0.421	0.710	0.248	0.545	0.127	0.178	0.098	0.212	0.197	0.585
Hydroponic-grown	*Na*	14	11	7	5	3	4	5	4	3	3
N 82	*Ne*	4.110	4.665	2.733	2.231	2.057	2.643	2.080	2.666	2.564	1.841
	Ho	0.537	0.146	0.561	0.305	0.585	0.622	0.390	0.707	0.598	0.354
	He	0.757	0.786	0.634	0.552	0.514	0.622	0.519	0.625	0.610	0.457
	FI	0.291	0.814	0.115	0.447	-0.139	-0.001	0.248	-0.132	0.020	0.226
Pot-grown	*Na*	10	7	7	4	4	3	7	4	3	4
N 71	*Ne*	4.338	2.416	3.268	3.156	3.179	2.191	2.971	2.429	2.153	2.302
	Ho	0.338	0.183	0.465	0.183	0.324	0.408	0.451	0.394	0.296	0.211
	He	0.769	0.586	0.694	0.683	0.685	0.544	0.663	0.588	0.536	0.566
	FI	0.561	0.688	0.330	0.732	0.527	0.249	0.321	0.330	0.448	0.627

Table 3.2 -continued

Grouping		ANUCS301	ANUCS304	B01-CANN1	ANUCS302	ANUCS305	B02-CANN2	C11-CANN1	B05-CANN1	ANUCS501	ANUCS303
ACT	*Na*	15	11	7	5	4	4	5	4	4	3
N 109	*Ne*	4.546	4.562	2.620	3.239	3.469	2.542	2.179	2.736	2.417	2.238
	Ho	0.477	0.239	0.486	0.294	0.440	0.459	0.404	0.404	0.468	0.266
	He	0.780	0.781	0.618	0.691	0.712	0.607	0.541	0.634	0.586	0.553
	FI	0.388	0.694	0.214	0.575	0.381	0.244	0.254	0.364	0.202	0.519
South Australia	*Na*	6	7	4	4	3	3	4	3	3	2
N 44	*Ne*	2.174	3.376	2.900	2.480	2.029	1.948	1.964	2.927	2.985	1.198
	Ho	0.364	0.136	0.591	0.409	0.636	0.591	0.591	0.727	0.705	0.136
	He	0.540	0.704	0.655	0.597	0.507	0.487	0.491	0.658	0.665	0.165
	FI	0.327	0.806	0.098	0.315	-0.255	-0.214	-0.204	-0.105	-0.059	0.175
Victoria	*Na*	5	7	6	5	3	3	3	3	3	3
N 39	*Ne*	2.263	2.110	2.317	1.831	2.091	2.504	2.208	2.184	2.048	1.139
	Ho	0.462	0.077	0.692	0.051	0.615	0.487	0.615	0.538	0.462	0.077
	He	0.558	0.526	0.568	0.454	0.522	0.601	0.547	0.542	0.512	0.122
	FI	0.173	0.854	-0.218	0.887	-0.180	0.189	-0.125	0.007	0.098	0.369
Western Australia	*Na*	13	10	8	6	3	3	7	4	3	4
N 71	*Ne*	4.523	3.893	3.596	2.796	2.191	2.381	3.374	2.715	1.797	2.288
	Ho	0.423	0.225	0.408	0.296	0.479	0.549	0.437	0.662	0.324	0.408
	He	0.779	0.743	0.722	0.642	0.544	0.580	0.704	0.632	0.443	0.563
	FI	0.458	0.697	0.434	0.540	0.119	0.053	0.379	-0.048	0.270	0.274
Tasmania	*Na*	2	3	3	2	2	3	2	3	2	1
N 8	*Ne*	1.438	2.133	2.909	2.000	1.133	1.855	1.969	2.246	1.600	1.000
	Ho	0.375	0.000	0.750	0.000	0.125	0.500	0.625	0.875	0.500	0.000
	He	0.305	0.531	0.656	0.500	0.117	0.461	0.492	0.555	0.375	0.000
	FI	-0.231	1.000	-0.143	1.000	-0.067	-0.085	-0.270	-0.577	-0.333	#N/A

Table 3.3. Summary of the number of private alleles found within groups. a) Private alleles within drug versus fibre samples. b) Private alleles within states and their exclusive state of origin when only drug growth types were compared.

a.

Locus	Drug/Fibre	Allele
ANUCS301	Fibre	208
	Fibre	211
	Drug	214
	Drug	217
	Fibre	250
	Fibre	256
	Drug	262
	Drug	265
	Fibre	276
ANUCS304	Fibre	141
	Drug	147
	Fibre	165
	Drug	171
	Fibre	177
	Fibre	183
	Fibre	186
	Drug	210
	Fibre	216
	Fibre	222
B01CANN1	Fibre	311
	Fibre	314
	Fibre	320
	Drug	344
	Drug	362
	Drug	371
ANUCS302	Fibre	142
	Drug	148
	Fibre	163
	Fibre	166
ANUCS303	Fibre	139
	Fibre	154
	Fibre	157
	Fibre	160
	Fibre	163
ANUCS305	Fibre	151
	Fibre	157
	Fibre	160
	Fibre	163
	Fibre	167
C11CANN1	Drug	158
	Drug	176
B05CANN1	Fibre	227
	Fibre	230
	Drug	245

b.

Locus	State	Drug Growth Type	Allele
ANUCS301	WA	Field	205
	ACT	Pot	217
	WA	Hydroponic	220
	WA	Field	223
	VIC	Hydroponic	259
	ACT	Field	262
	ACT	Field	265
	ACT	Hydroponic	268
ANUCS304	VIC	Field	147
	ACT	Hydroponic	168
	WA	Pot	180
	ACT	Field	198
B01CANN1	VIC	Hydroponic	323
	WA	Pot	335
	SA	Field	338
	WA	Hydroponic	341
	ACT	Field	362
ANUCS302	-	Field	148*
ANUCS305	ACT	Pot	148
C11CANN1	WA	Pot	161
B02CANN2	ACT	Hydroponic	170

*Although private to field grown, upon subdivision allele was shared between field-grown samples among states

3.3.3 Genotypic Patterns

Multiple occurrences of the same genotype were common within seizures consisting of multiple plants and were more frequent within rather than among seizures. In total, 38 of the 47 shared genotypes were only found within a single seizure. Shared drug genotypes were most frequently found within hydroponically-grown samples (57% of the total) while unique drug genotypes were mostly found in field-grown samples (49% of the total) (Fig. 3.1). Despite the removal of shared genotypes from the analysis, as expected, for most loci there was significant deviation from Hardy-Weinberg Equilibrium, and some Linkage Disequilibrium was evident (full data not shown).

Nine of the 47 shared genotypes were found among seizures, with three of these being present in seizures from two or more states, denoted genotypes *F*, *M* and *N* (Figs 3.3a & 3.3b). Seizures of hydroponically-grown samples from SA had a high degree of genotype sharing, with seven of the 13 seizures of hydroponically-grown samples from SA sharing the same genotype, denoted *P*. Five of these seven seizures were exclusively genotype *P*. Victorian hydroponic seizures also showed similar levels of genotype sharing within and among independent seizures, with six of the 15 independent hydroponic seizures consisting exclusively of the genotype *F*. Genotype *F* was also found in several independent hydroponic seizures from SA and in one unknown growth type seizure from the ACT. The remaining genotypes shared within states, including the two genotypes shared between states (*M* shared between WA and the ACT; *N* shared between VIC, WA and the ACT), were not found in as high abundance between independent seizures as that of genotypes *F* and *P*.

The average *RMP* estimate for all recovered drug genotypes was 5.4×10^{-8} with a range of 9.6×10^{-7} to 9.5×10^{-20}. The *RMP* estimate for all *C. sativa* genotypes recovered was 5.0×10^{-9} with a range of 9.6×10^{-8} to 3.1×10^{-25}. The *RMP* estimates for the shared genotypes: *BB*; *EE*; *K*; *N*; and *P*, were notably smaller than the average *RMP* for the drug samples (Fig. 3.4), which suggests that rare alleles were present in these genotypes. The *RMP* estimates for the remaining shared genotypes: *B*; *F*; *M*; and *Z*; were larger than the average *RMP* for the drug samples, which suggests that these genotypes were composed of more common alleles. The *PI* and *PIsibs* for all drug genotypes recovered were estimated to be 2.4×10^{-8} and 5.5×10^{-4} respectively, and 2.3×10^{-9} and 3.1×10^{-4} respectively for all *C. sativa* genotypes recovered.

3.3.4 Allelic Diversity in *Cannabis sativa*

A total of 106 alleles were detected over all 10 STR loci for the 510 *C. sativa* samples. Within the drug samples, 76 alleles were detected of which 14 were unique to the drug type of *C. sativa*. Within the fibre samples, 92 alleles were detected with 30 being unique to only the fibre type of *C. sativa*. Overall, the number of alleles per locus ranged from 23 (ANUCS301) to 4 (ANUCS501 and B02-CANN1) (Table 3.2).

On average over the 10 STR loci, the fibre group revealed considerably more alleles than the drug sample group (Fig. 3.5a). Consequently, private alleles were more common in fibre samples (Table 3.3a). The average *Na*, average *Ne* and the average number of unique alleles were similar for the Field, Hydroponic, and Pot grown drug growth type groups (Fig. 3.5b). However, the average *He* was considerably lower for the overall hydroponic drug group. Allelic diversity was also variable among the state drug growth groups (Figs 3.5 & 3.6). At a locus by locus level there was variation in the *Na* and the frequency of alleles among the drug growth groups (Fig. 3.5), with the average *Na* for the ACT and WA drug groups being similar and higher than the average number of alleles for VIC and SA drug populations (Fig. 3.5c). The average *He* was highest for the ACT and WA drug groups, with considerable decrease in this measure within the SA, VIC and TAS groups. For most loci, allelic distribution and frequency was uneven among the drug and fibre groups and also within drug growth type groups as well as among states. An example for two loci can be seen in Figures 3.6 and 3.7, with uneven frequency of some alleles among different groups. The overall allele frequency data is reported in Tables 6.2 and 6.3.

Figure 3.3. The distribution of shared multilocus genotypes among seizures. a) All except three of the genotypes shared among seizures were found within one state. b) Genotypes *F*, *N*, and *M* were shared between states.

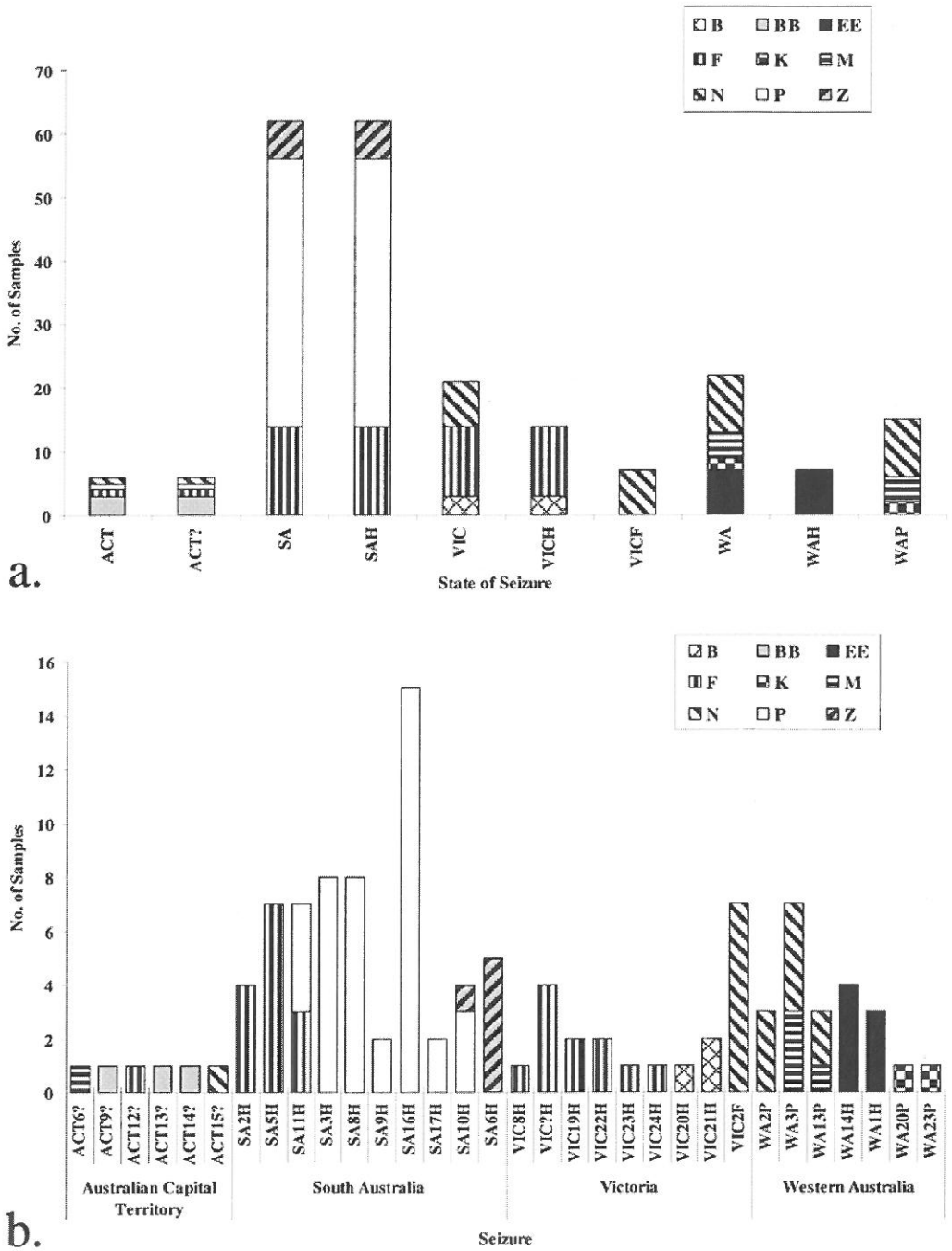


Figure 3.4. Random Match Probability (*RMP*) estimates for the shared genotypes in comparison with the mean *RMP* calculated from all genotypes calculated from drug seizures only.

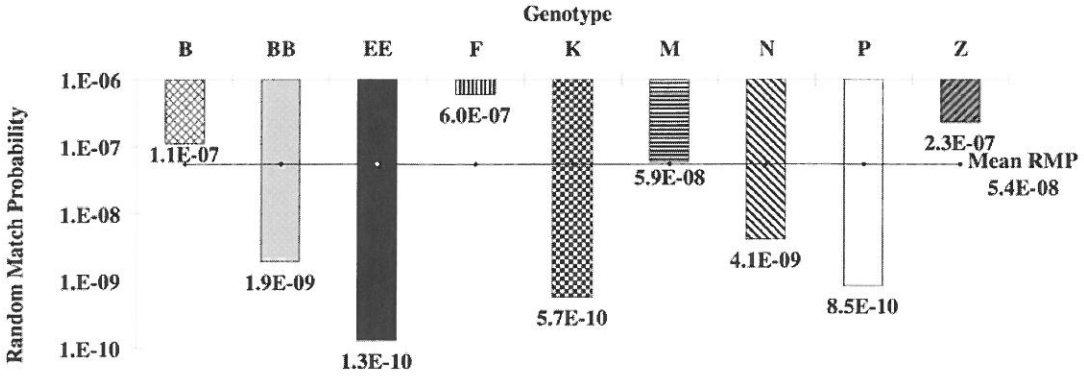


Figure 3.5. The average Number of Alleles (*Na*), the Average Number of Effective alleles (*Ne*), the average number of private alleles, and the average Expected Heterozygosity (*He*) observed over various *Cannabis sativa* sample groups. a) overall *C. sativa*, fibre and drug varieties, b) *C. sativa* drug growth-type, c) *C. sativa* drug samples divided into the Australian state of origin.

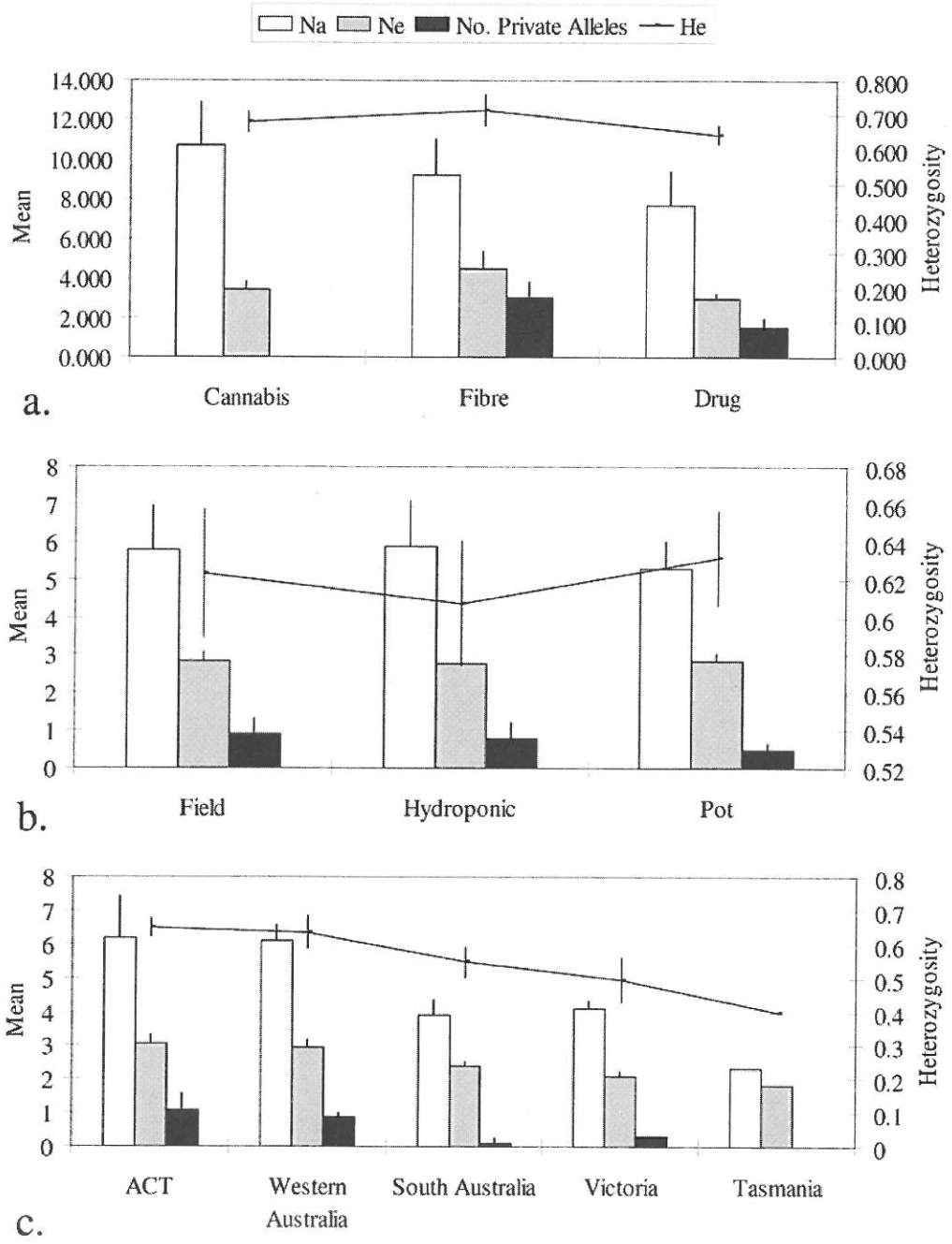


Figure 3.6. Locus ANUCS301 allele frequencies for a) both fibre and drug, b) field-, hydroponic- and pot-grown, and c) drugs from each Australian state represented.

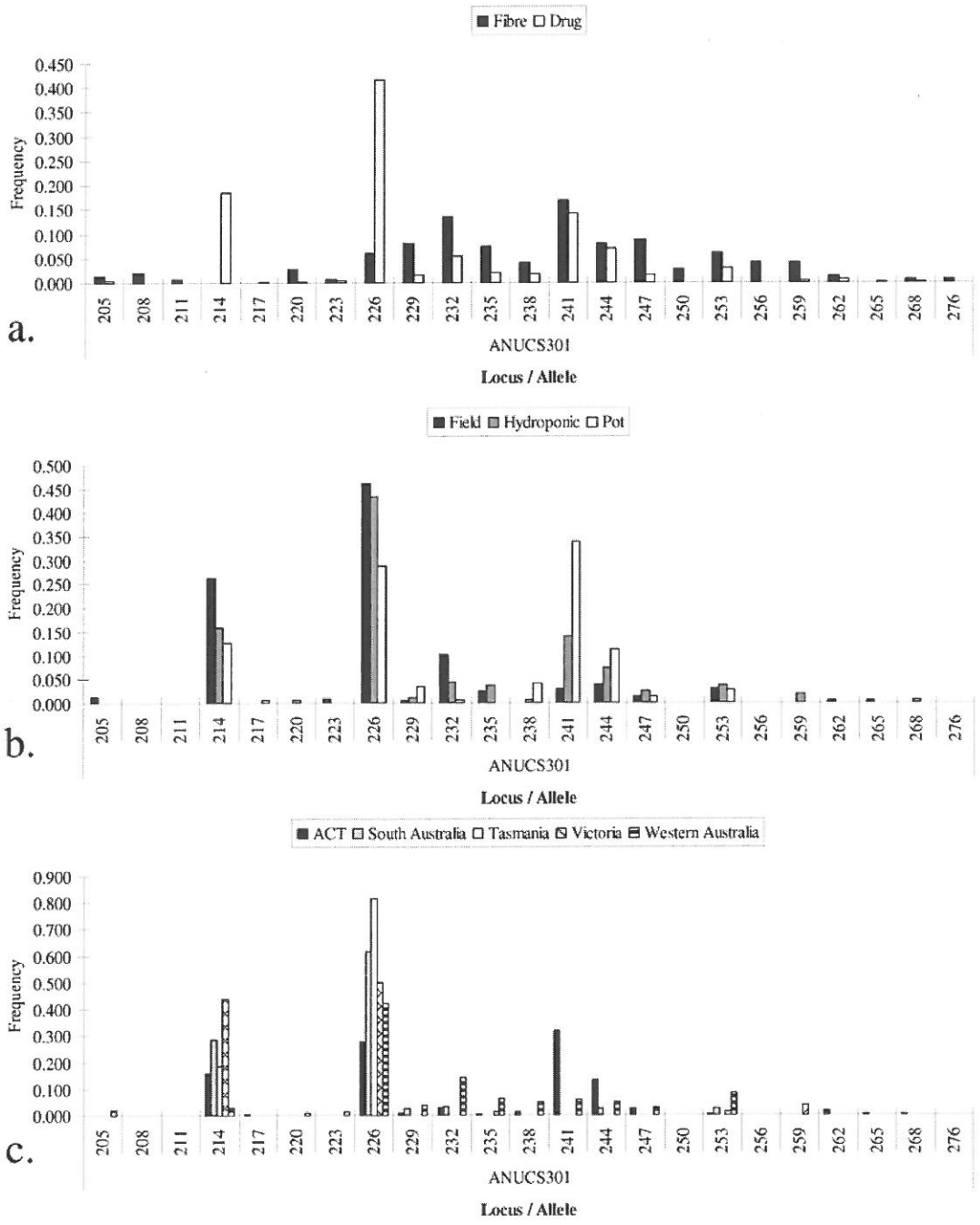
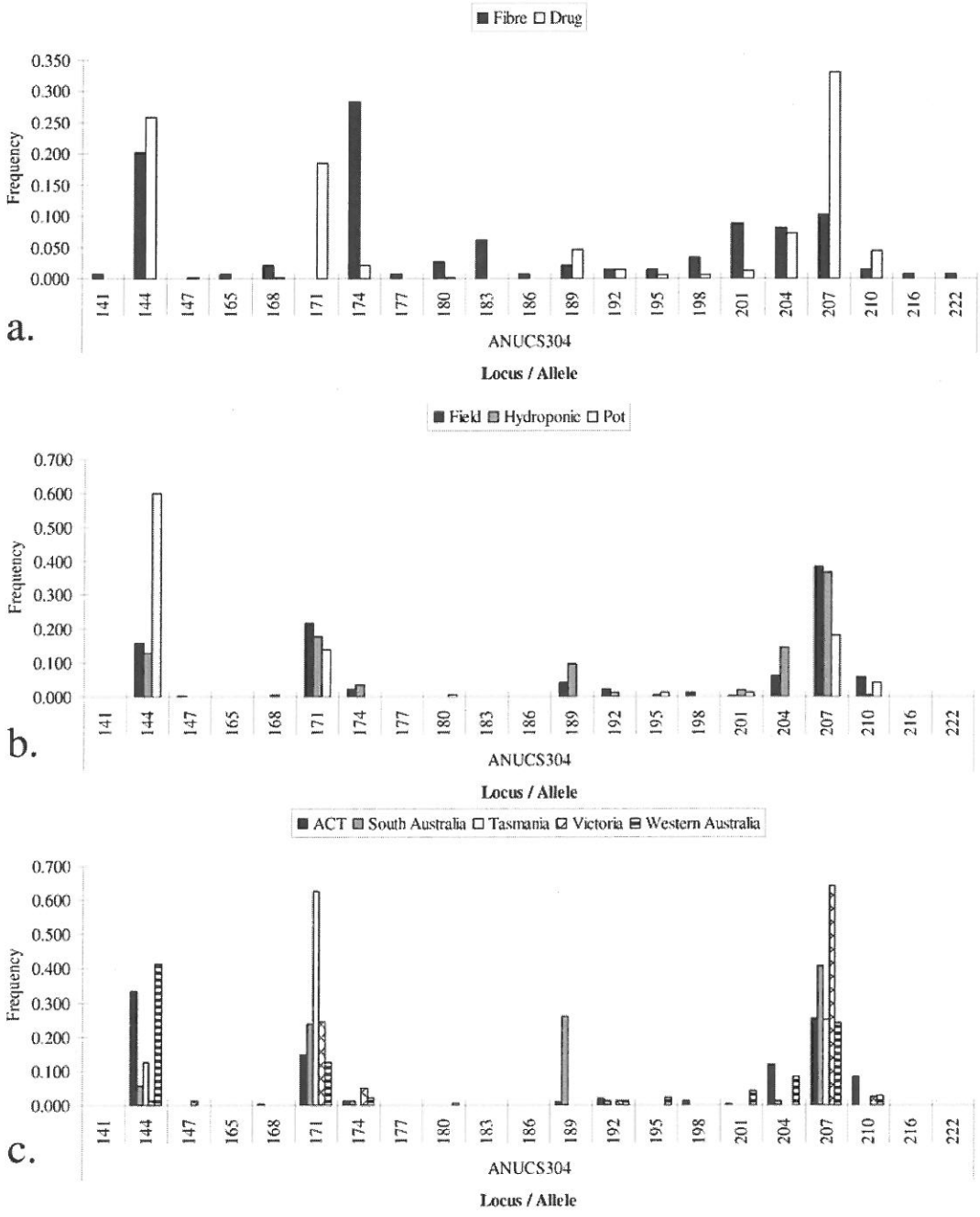


Figure 3.7. Locus ANUCS304 allele frequencies for a) both fibre and drug, b) field-, hydroponic- and pot-grown, and c) drugs from each Australian state represented.



3.3.5 Ability to Distinguish Between Fibre and Drug Sample Populations

The Analysis of Molecular Variance measures revealed that there was significant genetic differentiation (F_{ST} = 0.094 $P > 0.001$) among the fibre and drug samples, with this difference accounting for 9% of the total genetic variance. This was notably higher than the level of differentiation detected between drug and fibre samples reported in Gilmore et al. (2003), where a different subset of *C. sativa* STRs were used. Within the drug samples, the degree of genetic differentiation among the state of origin groups was similar to that among the fibre and drug groups (F_{ST} = 0.077, $P > 0.001$), however, the degree of genetic differentiation among the drug growth-type groups was lower (F_{ST} = 0.041, $P > 0.001$).

Despite the modest differentiation of only 9% of the total genetic variation among drug and fibre samples, the genotype likelihood biplot shown in Figure 3.8 shows minimal overlap between the two types of *C. sativa*. As a consequence, we would predict that assignment tests will, more frequently than not, correctly identify an unknown *C. sativa* sample as being either a drug or fibre variety. Population assignment based on *Log (L)* values for the 13 ambiguous *C. sativa* samples belonging to a single group of germinated seedlings obtained from the AFP, suggested that 9 of the samples had a genotype that most likely belonged in the drug population, with the remaining 4 having a genotype most likely belonging to the fibre population. However, given some overlap of the drug and fibre groups (Fig. 3.8) due to the genetic similarity of the populations, we predict that assignment tests may not be definitive for all samples.

Table 3.4 summarises the outcomes of assignment tests for a subset of samples that were randomly extracted from our database and excluded from the frequency calculation underpinning the subsequent assignment tests. Based on *Log(L)* values for a total of 120 samples, on average 92% of the drug subset samples were correctly identified as drug, while 100% of the fibre subset were correctly identified as fibre.

Furthermore, the simulation options provided by GENECLASS (Piry et al., 2004) allowed us to assess probable population inclusion. When we set $P > 0.01$ for inclusion, 89% of drug samples and 92% of fibre samples were assigned correctly to their respective group. However, for the same set of samples, 65% of the drug samples could not be ruled out as possibly belonging to the fibre group. Similarly, 8% of the fibre samples could not be ruled out as belonging to the drug group. As would be expected, at the $P > 0.001$ level, both correct and incorrect assignments increased slightly (Table 3.4). This suggests that the genetic similarity of some drug and fibre genotypes in this study across these 10 STR loci precluded categorical separation of all drug and fibre samples and that there is a need for some caution in the interpretation of assignment tests based only on *Log (L)* values.

Despite the 8% genetic differentiation among the drug samples when they were grouped into their Australian state of origin, discrete clustering was not apparent in genotype likelihood biplots between these groups (data not shown). Therefore it appears that it may not be possible to assign a state of origin to an Australian seizure. This is not surprising, given some sharing of genotypes among the states as outlined above. Additionally, given the low level of genetic differentiation (4%) separating the drug growth-type groups, genotype likelihood biplots between these groups did not show discrete non-overlapping clusters (data not shown). Consequently, it appears that it may not be possible to assign a drug growth-type to an Australian seizure.

Figure 3.8. Genotype likelihood biplot showing the discrimination between drug and fibre samples.

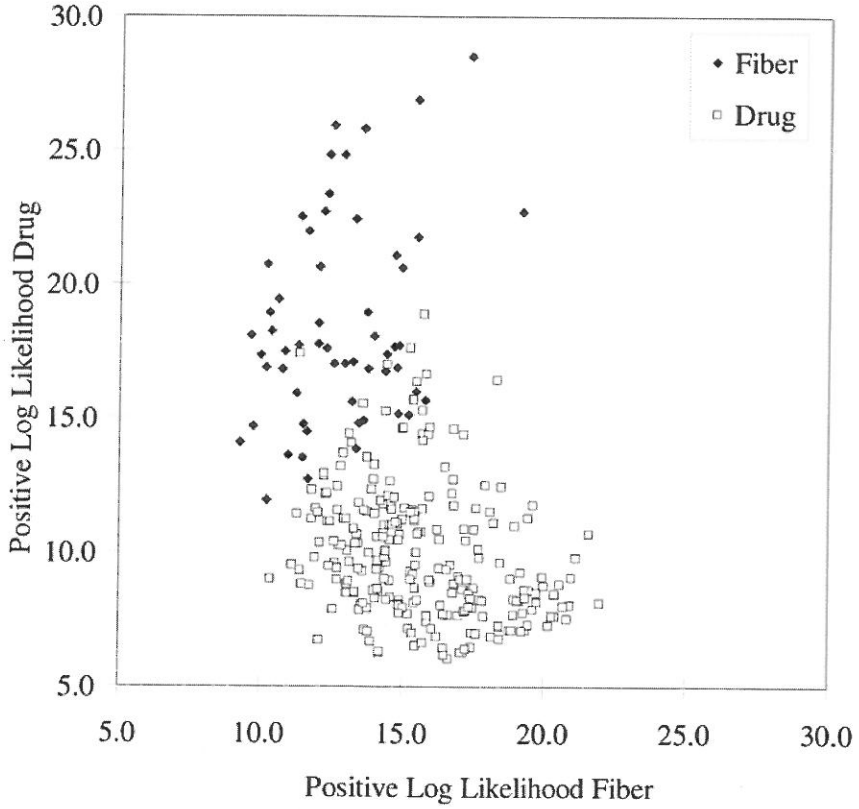


Table 3.4. Results of population assignment tests for drug and fibre samples of *Cannabis sativa*. The proportion of samples placed in their correct population are indicated from Log likelihood [*Log (L)*] values and simulated probability of inclusion

			Simulated Probability of Inclusion			
***C. sativa* type population**	**Random *C. sativa* sample subset**	***Log (L)* - Placement in Actual Group**	***P* > 0.01 - Drug**	***P* > 0.01 - Fibre**	***P* > 0.001 - Drug**	***P* > 0.001 - Fibre**
Drug	1	92%	79%	71%	92%	75%
	2	92%	92%	58%	96%	83%
	3	100%	96%	58%	100%	63%
	4	88%	88%	67%	92%	75%
	5	88%	92%	71%	92%	79%
	Average	92%	89%	65%	94%	75%
Fibre	1	100%	0%	80%	20%	80%
	2	100%	20%	100%	20%	100%
	3	100%	20%	80%	60%	80%
	4	100%	0%	100%	0%	100%
	5	100%	0%	100%	40%	100%
	Average	100%	8%	92%	28%	92%

3.4 Discussion

3.4.1 Genetic Diversity of Australian *Cannabis sativa*

To our knowledge, we have built the world's first *Cannabis sativa* genetic database. Based on the genetic analysis of STR loci, the current standard in human forensic analysis (Butler, 2006), the database contains multilocus genotype data across 10 STR loci for approximately 500 *C. sativa* plants representing drug seizures from five Australian states and territories and a selection of fibre samples. While additional STR loci are available for *C. sativa*, and have been used successfully for population studies of the plant (Gilmore et al., 2003), our selection of the 10 loci used in this study was based on the need to use developmentally validated STR loci that most closely matched the standards in human forensic analysis and avoided many of the interpretive challenges common with STRs (Hauge & Litt, 1993; Hoffman & Amos, 2005).

Concurring with the study of Gilmore et al. (2003), the analysis of our database revealed that fibre varieties were genetically more diverse than drug varieties of *C. sativa*. For example, while fibre samples represented only 11% of the total number of samples tested, these samples contained 86% of the total allelic diversity. Furthermore, 28% of the total of 106 alleles were only found in fibre samples. Moreover, all of the fibre samples tested had a unique multilocus genotype across the 10 STR loci. This finding of high genetic diversity within the fibre samples is consistent with obligate outcrossing and long distance wind-dispersed pollen that likely characterises this dioecious plant (Ranalli, 2004). It is also apparent that a wide genetic base has been sourced by the hemp industry.

Despite the lower genetic diversity of drug versus fibre samples, a high proportion of drug samples in our database did exhibit a unique multilocus genotype across the 10 STR loci. These genetically distinct samples were found among field-, hydroponic- and pot-grown drug samples, but were most frequent in field-grown samples. Of the total of 106 alleles, 13% of the alleles detected were unique to the drug samples.

3.4.2 Genotypic Patterns among Australian *Cannabis sativa*

Unique multilocus genotypes were common amongst the Australian *C. sativa* samples that we analysed, with multilocus genotype sharing occurring only amongst the drug samples. Our finding of multilocus genotype sharing among some drug samples, and the lack of any genotype sharing among the fibre samples is of interest. The challenge in the case of *C. sativa* (and many other plants) is that unlike humans (except identical twins), some genotype sharing due to clonal propagation can be expected. However, this genotype sharing may also be due to lack of sufficient resolution at the set of 10 STR loci used in the study. Here we evaluate the evidence concerning these two alternatives that could explain the sharing of genotypes.

In human forensic analysis, multiple STR loci are required to 'individualise' each human (except identical twins). For example, in the United States of America, the use of the 13 STR loci of the Combined DNA Index System (CODIS) enables sufficient discrimination within the human population. Calculation of *PI* can provide an indication of whether we have sufficient genetic resolution with the 10 STR loci, or not. For the CODIS loci set, the *PI* between profiles of two unrelated persons in a randomly mating population of Caucasian Americans is estimated to be 1.74×10^{-15} or one in 575 trillion (Samuels & Asplen, 2000). Similarly, in Australia a set of 9 STR loci have been certified as being sufficiently discriminatory for use in human forensic analysis (Walsh & Buckleton, 2007). If fewer loci are used in these two jurisdictions, unrelated individuals may share the same genotype due to chance alone. Similarly, in the case of *C. sativa*, if insufficient loci are used, unrelated samples may share the same genotype.

Our database analysis based on *PI* estimates indicated that, on average, the chance of obtaining identical genotypes across the 10 STR loci by sexual reproduction in a randomly mating population of *C. sativa* is approximately one in 400 million. Therefore, we would not expect to encounter identical genotypes by chance alone in our database of approximately 500 *C. sativa* samples. However, given that the random mating assumption underlying the calculation of *PI* is violated due to significant deviation from Hardy-Weinberg Equilibrium, we recommend the use of a more conservative estimate of probability. The statistic *PIsibs* provides an estimate of the probability of two samples, including genetically related samples, having the same identical genotype across the loci in question. From our database, the estimates of *PIsibs* across the 10 STR loci are in the order of one in three thousand. Therefore, in our database of some 500 samples we still would not expect to encounter shared genotypes as a consequence of chance, even allowing for closely related individuals within the sample set. Consequently, shared genotypes between two separate plants are likely to be due to them both having the same genetic origin.

An alternative way to assess whether our 10 STR markers provide sufficient resolution is to empirically determine the rate at which unique genotypes are recovered with increasing combinations of loci within the database itself. Our analysis revealed that for the genetically more diverse fibre samples the combination of three or four loci was more than sufficient to 'individualise' all of the 57 genotypes (see Fig. 3.2). For the less diverse drug samples, most unique genotypes were recovered with 7 or 8 loci, with subsequent additional loci failing to find substantial numbers of extra genotypes.

Additionally, all of the 13 samples of unknown *C. sativa* type (Set 2) included in this study had unique genotypes. As these samples originated from individual germinated seeds, this outcome was not entirely unexpected and concurs with the *PIsibs* estimates from our database that indicates that shared genotypes among related *C. sativa* plants will be unlikely. With this in mind, both the empirical assessment of genotype discrimination and probability estimates strongly suggest the 10 STR loci used in this study provide adequate resolution to distinguish between unique genotypes in our database of 500 samples.

Further support for clonal propagation as the basis for genotype sharing can be provided by an evaluation of the distribution of genotype sharing. If genotype sharing was merely a consequence of insufficient genetic resolution we would expect the degree of sharing to be spread across the samples, irrespective of their growth type. In the case of *C. sativa*, we predict that drug seizures of hydroponically-grown material will have a high likelihood of containing plants derived by clonal propagation (ACC, 2007), while drug seizures from field-grown crops are expected to contain fewer clonally propagated plants. Our findings concur with these predictions. The majority of samples with shared genotypes (57%) occurred within hydroponic seizures (Fig. 3.1), while far fewer shared genotypes found within field-grown seizures (17%). Further support for clonal propagation as the basis for genotype sharing is provided by the patterns of sharing within versus among seizures. The overwhelming majority of shared genotypes, 38 out of 47 (81%), were detected within seizures. Of the remaining nine genotypes shared among seizures, all but three were exclusive to a single Australian state. On the weight of evidence we conclude that the genotype sharing we have detected in our database is predominantly, if not exclusively, a consequence of clonal propagation. Below we explore the forensic implications of this finding.

3.4.3 Forensic Applications and Limitations

Our genetic database and associated analysis has been completed 'blind' with the only information provided with the samples being the varietal type of *C. sativa*, the state of origin and (where known) the growth type of the drug samples (hydroponic-, pot- or field-grown). We were not provided with any other information such as known or suspected linkages among seizures.

Such additional knowledge would allow us to better assess the forensic value of the database. In the absence of this information, our comments on the forensic applications remain somewhat speculative.

The patterns of genotype sharing that we have uncovered in our database suggest some variation in the form of drug production within Australia. We infer that the production consists of two types of perpetrator: many small independent growers using all types of growing methods leading to the proliferation of unique multilocus genotypes; and organised crime syndicates of a variety of operational size leading to the proliferation of shared multilocus genotypes.

We have already argued that the sharing of genotypes most likely reflects a common origin via clonal propagation. Consequently, the finding of shared genotypes among seizures is most likely due to either a common supplier, or direct links among seizures. One example of shared genotype was genotype *P* (Fig. 3.3b) which was exclusive to South Australian hydroponic samples and found amongst several seizures. The *RMP* value for this genotype was approximately 2 orders of magnitude lower than the average *RMP*, indicating that multiple occurrences of this genotype should be unlikely in the drug population in our database. Given that this genotype is quite distinctive and was recovered from multiple seizures, connectivity through clonal propagation between the seizures can be implied. Similarly, other cases of potential linkage are implied by genotype sharing among the states as indicated in Figure 3.3. If this genetic knowledge reinforces suspected linkages from other evidence, this combined knowledge may aid in prosecution.

It is of interest to note that despite the inability to categorically assign a drug growth-type by population assignment methods, the unknown growth-type of some of the drug seizures from the ACT (Set 1) may be inferred by their genotype sharing. No genotypes of hydroponically-grown samples were shared with pot- or field-grown growth type samples, leading us to predict that the sample of seizure 'ACT12?' with genotype *F*, was most likely hydroponically-grown. Conversely, the samples of seizure 'ACT6?' with genotype *M*, and of seizure 'ACT15?' with genotype *N*, are unlikely to be hydroponically-grown, being genotypes shared with field- and pot-growth types (Fig. 3b). With this type of linkage in mind, it would be of value to combine genetic and non-genetic evidence to assess the possible basis of genotype sharing among the states for genotypes such as those of *F, M and N* found in this study. For example, are these potent drug varieties shared among interstate consortia? Or merely sourced independently from a single supplier?

Notwithstanding the potential intelligence information provided by genetic analysis of *C. sativa* drug seizures, it is presently not possible to categorically assign a state of origin to an Australian seizure. As already noted, there is some sharing of genotypes among states, and this likely underestimates the degree of human assisted gene flow that occurs between the states. Nonetheless, there were state-by-state differences in alleles and allele frequency that may become even more pronounced as the database expands. It is possible that *C. sativa* drug seizures from other countries may exhibit more informative differences than among states within Australia (Gilmore et al., 2003) but this analysis was beyond the scope of the present study.

The genetic similarity that we identified among fibre and drug varieties reflects their common evolutionary origin and is likely a consequence of historical or contemporary gene flow between fibre and field-grown drug crops and poses several challenges for the law enforcement community. Nonetheless, the combination of low genetic diversity within drug samples and the presence of unique fibre and drug specific alleles has the potential to provide strong indication as to the likelihood of a sample being of drug versus fibre origin. Furthermore, notwithstanding the moderate genetic differentiation between the drug and fibre samples, our assignment test results indicated that, more often than not, drug and fibre samples could be readily distinguished.

The population assignment results for the 13 samples of unknown *C. sativa* type (Set 2) are of interest. Given that these samples were from a single group of seeds held by the AFP, the samples were most likely of the drug variety. However, despite this, population assignment testing indicated that the genotypes of some of the samples were more likely to be of fibre rather than drug origin. Given this result and some equivocal outcomes for the simulated population assignment tests, population assignment test outcomes need to be considered cautiously. It is well known that large population sizes are needed for robust estimates of allele frequencies. Therefore, the addition of samples of both drug and fibre type to our database will likely improve the reliability of assignment tests in the future.

Ideally a DNA test for drug versus fibre varieties of *C. sativa* would be based on the direct analysis of the gene/s responsible for THC regulation. However, until such a test is available it may be possible to enhance the results of nuclear STRs with organelle DNA haplotype data that also provides some discrimination among fibre and drug varieties of *C. sativa* (Gilmore et al., 2007). The study of Gilmore et al. (2007) showed that some organelle DNA haplotype groupings in *C. sativa* largely were associated with either drug or fibre type plants. Whilst Gilmore et al. (2007) noted there was still some overlap between drug and fibre types based on organelle haplotypes, these markers coupled with the set of nuclear STRs used here may achieve the necessary resolution between drug and fibre plants. A further solution to aid the identification of drug versus fibre plants may be a DNA profile register of fibre varieties, analogous to the DNA registers proposed to assist with the legal trafficking of wildlife (Palsboll et al., 2006).

Given the limitations we have identified, what practical recommendations can we make? The detection of genotype sharing among multiple drug seizures may provide objective and independent corroboration of suspected linkages. Equally, this genetic evidence may refute evidence of linkages. We suggest that with appropriate consideration there will be a range of circumstances where genetic analysis of *C. sativa* seizures will be of forensic value, be it for prosecutor or defence assistance in drug related crime or for intelligence gathering for other investigations. It is apparent that genetic knowledge, including the finding of shared genotypes within and among seizures, has potential intelligence value. However, as noted in human forensics, genetic analysis must complement, rather than replace, other forms of evidence (Lynch & McNally, 2003).

Chapter four: General Conclusion

The overall objective of this project was to develop and implement a DNA typing technology for *Cannabis sativa* and to enable its subsequent transfer from the research laboratory to the forensic community. We have achieved our objectives by establishing the accuracy and reliability of this technology through developmental validation, and by the subsequent compilation of a genetic database for some 500 *C. sativa* samples representing drug seizures from multiple states of Australia. While it was disappointing that we were unable to source samples from all states and territories of Australia (as originally planned), we have worked successfully with multiple jurisdictions. The role played by these jurisdictions in providing DNA samples was critical to the success of this project. With the establishment of this first *C. sativa* genetic database, the next step in the implementation of *C. sativa* DNA typing can now be handed to established forensic laboratories, with discussion on the transfer of this technology having already begun. The final step will be realised when this technology is evaluated in the courtroom.

Chapter five: References

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Chapter six: Appendix

Table 6.1. Multilocus genotypes of drug and fibre varieties of *Cannabis sativa* obtained from this investigation. The Random Match Probability (*RMP*) of a given DNA profile and genotype designation is indicated.

Genotype Designation	*RMP* – Drug Genotypes Only	*RMP* – All C. sativa genotypes	*C. sativa* variety	ANUCS301		ANUCS304		B01-CANN1		ANUCS302		ANUCS305		B02-CANN2		C11-CANN1		B05-CANN1		ANUCS501		ANUCS303	
A	$6.4x10^{-10}$	$2.1x10^{-10}$	Drug	214	214	171	192	317	326	139	154	145	154	167	173	155	155	236	242	88	93	145	145
AA	$1.0x10^{-08}$	$4.8x10^{-09}$	Drug	226	232	207	207	329	329	139	154	154	154	167	173	155	158	239	242	88	88	145	145
B	$1.1x10^{-07}$	$1.6x10^{-08}$	Drug	214	214	207	207	326	326	154	154	145	154	164	173	155	155	236	239	88	88	145	145
BB	$1.9x10^{-09}$	$1.5x10^{-09}$	Drug	226	238	207	207	326	329	139	139	142	154	167	173	155	158	239	242	88	88	145	151
C	$2.6x10^{-07}$	$3.6x10^{-08}$	Drug	214	214	207	207	326	326	154	154	154	154	164	164	155	158	236	239	88	88	145	145
CC	$4.8x10^{-10}$	$2.6x10^{-10}$	Drug	226	244	189	189	326	326	139	151	142	154	164	167	155	158	239	242	88	93	145	145
D	$1.4x10^{-07}$	$1.5x10^{-08}$	Drug	214	226	144	207	326	326	154	154	145	154	164	173	158	158	239	242	88	98	145	151
DD	$1.3x10^{-09}$	$2.2x10^{-09}$	Drug	226	247	144	144	326	329	139	139	154	154	164	167	155	158	242	242	88	88	145	145
E	$1.7x10^{-08}$	$1.9x10^{-09}$	Drug	214	226	171	171	317	326	151	151	145	154	164	164	155	158	236	239	88	98	145	145
EE	$1.3x10^{-10}$	$7.4x10^{-11}$	Drug	235	253	144	144	329	329	154	154	142	154	164	164	158	158	239	242	88	88	145	151
F	$5.9x10^{-07}$	$5.5x10^{-08}$	Drug	214	226	207	207	317	326	154	154	145	154	164	173	155	158	236	239	88	98	145	145
FF	$1.7x10^{-11}$	$1.7x10^{-11}$	Drug	241	241	144	144	317	317	145	154	148	148	164	164	155	155	236	236	88	88	151	151
G	$1.1x10^{-07}$	$1.3x10^{-08}$	Drug	214	226	207	207	326	326	154	154	145	154	167	173	155	158	236	236	88	98	145	145
GG	$7.8x10^{-12}$	$9.4x10^{-12}$	Drug	241	241	144	144	317	326	145	145	148	148	164	173	155	155	236	236	88	88	151	151
H	$8.8x10^{-08}$	$6.5x10^{-09}$	Drug	214	226	207	207	326	329	154	154	145	154	164	173	155	158	239	242	98	98	145	145
HH	$7.1x10^{-11}$	$6.9x10^{-11}$	Drug	241	241	144	144	317	326	145	154	148	148	164	164	155	155	236	236	88	88	151	151
I	$1.5x10^{-09}$	$3.1x10^{-10}$	Drug	226	226	144	144	326	329	154	154	154	154	164	167	155	155	242	245	93	98	145	151
II	$5.9x10^{-11}$	$5.0x10^{-11}$	Drug	241	241	144	144	317	326	145	154	148	148	164	173	155	155	236	236	88	88	151	151
J	$6.6x10^{-10}$	$8.7x10^{-11}$	Drug	226	226	144	144	329	329	139	154	154	154	164	164	155	158	239	245	93	98	145	145
JJ	$1.2x10^{-11}$	$9.1x10^{-12}$	Drug	241	241	144	144	317	326	145	154	148	148	173	173	155	155	236	236	88	88	151	151
K	$5.7x10^{-10}$	$3.8x10^{-10}$	Drug	226	226	144	144	329	332	139	139	154	154	164	167	155	158	242	242	88	98	145	145

Table 6.1 continued

Genotype Designation	*RMP* – Drug Genotypes Only	*RMP* – All C. sativa genotypes	*C. sativa* variety	ANUCS301		ANUCS304		B01-CANN1		ANUCS302		ANUCS305		B02-CANN2		C11-CANN1		B05-CANN1		ANUCS501		ANUCS303	
KK	$1.3x10^{-10}$	$9.2x10^{-11}$	Drug	241	241	144	144	317	326	154	154	148	148	164	164	155	155	236	236	88	88	151	151
L	$3.3x10^{-13}$	$1.7x10^{-12}$	Drug	226	226	168	171	326	344	154	154	154	154	167	170	155	158	239	242	93	98	145	145
LL	$9.5x10^{-12}$	$1.2x10^{-11}$	Drug	241	241	144	144	326	326	145	145	148	148	164	164	155	155	236	236	88	88	151	151
M	$5.8x10^{-08}$	$6.6x10^{-09}$	Drug	226	226	171	171	326	326	154	154	154	154	164	167	155	158	239	242	98	98	145	145
MM	$7.8x10^{-12}$	$9.1x10^{-12}$	Drug	241	241	144	144	326	326	145	145	148	148	164	173	155	155	236	236	88	88	151	151
N	$4.1x10^{-09}$	$3.7x10^{-10}$	Drug	226	226	171	171	326	344	154	154	154	154	164	167	155	158	239	242	98	98	145	145
NN	$7.2x10^{-11}$	$6.7x10^{-11}$	Drug	241	241	144	144	326	326	145	154	148	148	164	164	155	155	236	236	88	88	151	151
O	$6.0x10^{-11}$	$9.7x10^{-12}$	Drug	226	226	171	192	326	329	151	151	154	154	164	167	155	158	239	245	88	98	145	151
OO	$5.9x10^{-11}$	$4.9x10^{-11}$	Drug	241	241	144	144	326	326	145	154	148	148	164	173	155	155	236	236	88	88	151	151
P	$8.4x10^{-10}$	$1.9x10^{-10}$	Drug	226	226	189	189	317	329	139	154	142	154	164	173	155	155	236	242	93	98	145	145
PP	$1.2x10^{-11}$	$8.9x10^{-12}$	Drug	241	241	144	144	326	326	145	154	148	148	173	173	155	155	236	236	88	88	151	151
Q	$2.1x10^{-09}$	$1.6x10^{-10}$	Drug	226	226	207	207	317	326	151	151	145	154	164	164	158	158	236	236	98	98	145	145
QQ	$8.9x10^{-12}$	$8.1x10^{-12}$	Drug	241	241	171	171	329	329	151	151	154	154	164	167	155	155	239	242	88	93	142	151
R	$1.7x10^{-09}$	$1.2x10^{-10}$	Drug	226	226	207	207	317	326	151	151	145	154	164	173	158	158	236	236	98	98	145	145
RR	$1.1x10^{-11}$	$1.8x10^{-11}$	Drug	241	241	204	204	329	329	154	154	142	154	164	167	155	155	242	242	88	93	142	145
S	$3.4x10^{-08}$	$1.8x10^{-09}$	Drug	226	226	207	207	317	329	154	154	154	154	164	173	158	158	236	239	98	98	145	145
SS	$6.6x10^{-13}$	$1.2x10^{-12}$	Drug	241	247	144	210	326	362	139	154	142	154	164	164	152	155	242	242	88	93	145	145
T	$5.3x10^{-07}$	$9.6x10^{-08}$	Drug	226	226	207	207	326	329	154	154	142	154	164	164	155	155	239	242	88	98	145	145
TT	$1.0x10^{-12}$	$1.2x10^{-12}$	Drug	241	247	207	207	329	332	139	139	142	154	164	173	158	176	239	242	88	93	145	151
U	$2.5x10^{-08}$	4.7x10-$^{-09}$	Drug	226	226	207	207	329	329	154	154	154	154	164	164	155	158	242	242	88	93	145	151
UU	$1.9x10^{-09}$	$2.3x10^{-09}$	Drug	247	253	207	207	326	329	139	154	154	154	164	164	155	155	236	242	88	88	145	151
V	$1.7x10^{-09}$	$4.5x10^{-10}$	Drug	226	226	210	210	317	326	145	154	142	154	164	167	155	158	239	242	93	98	145	145
W	$4.3x10^{-09}$	$3.1x10^{-09}$	Drug	226	229	144	144	329	329	154	154	142	154	164	167	155	158	239	242	88	88	145	151
X	$6.2x10^{-09}$	$3.7x10^{-09}$	Drug	226	229	144	171	329	329	154	154	142	154	164	167	155	158	239	242	88	88	145	151
Y	$5.4x10^{-09}$	$2.5x10^{-09}$	Drug	226	232	144	207	329	329	154	154	142	154	164	167	155	155	236	242	88	98	151	151
Z	$2.3x10^{-07}$	$5.7x10^{-08}$	Drug	226	232	207	207	326	329	154	154	154	154	164	164	155	158	236	239	88	88	145	151

Table 6.1 continued

Genotype Designation	*RMP* – Drug Genotypes Only	*RMP* – All C. sativa genotypes	*C. sativa* variety	ANUCS301		ANUCS304		B01-CANN1		ANUCS302		ANUCS305		B02-CANN2		C11-CANN1		B05-CANN1		ANUCS501		ANUCS303	
2	$6.8x10^{-13}$	$1.0x10^{-12}$	Drug	205	205	207	207	326	329	139	154	154	154	164	167	158	158	239	242	88	88	148	151
3	$9.8x10^{-13}$	$1.5x10^{-11}$	Drug	205	232	174	207	326	329	139	154	142	154	164	167	152	158	239	242	88	88	145	148
7	$2.6x10^{-11}$	$1.1x10^{-11}$	Drug	214	214	144	144	317	317	145	145	142	145	164	164	158	164	236	239	88	93	145	145
8	$1.1x10^{-09}$	$5.4x10^{-10}$	Drug	214	214	144	207	326	326	145	145	142	154	164	164	158	164	236	239	88	88	145	151
9	$8.3x10^{-12}$	$4.1x10^{-12}$	Drug	214	214	144	207	326	326	151	151	142	142	164	164	164	164	236	236	88	88	145	145
10	$2.6x10^{-11}$	$2.1x10^{-12}$	Drug	214	214	171	171	317	317	151	151	154	154	173	173	155	155	236	236	98	98	145	145
11	$2.7x10^{-09}$	$4.0x10^{-10}$	Drug	214	214	171	171	317	326	139	151	145	154	164	173	155	158	239	239	88	93	145	145
12	$1.0x10^{-09}$	$7.9x10^{-11}$	Drug	214	214	171	171	317	326	154	154	145	145	164	173	158	158	239	239	88	88	145	145
13	$1.3x10^{-09}$	$2.4x10^{-10}$	Drug	214	214	171	171	326	326	151	151	145	154	164	164	155	155	236	242	88	93	145	145
14	$1.1x10^{-09}$	$1.2x10^{-10}$	Drug	214	214	171	171	326	326	154	154	145	145	173	173	155	155	236	239	88	88	145	145
15	$2.7x10^{-08}$	$3.1x10^{-09}$	Drug	214	214	171	171	326	326	154	154	154	154	164	173	155	158	239	242	88	93	145	145
16	$1.2x10^{-09}$	$1.8x10^{-10}$	Drug	214	214	207	207	317	317	151	151	145	154	164	173	155	158	236	236	88	88	145	145
17	$2.4x10^{-08}$	$3.9x10^{-09}$	Drug	214	214	207	207	317	317	154	154	154	154	164	164	155	158	236	242	88	93	145	145
18	$2.7x10^{-09}$	$5.2x10^{-10}$	Drug	214	214	207	207	317	326	145	145	145	154	164	173	158	158	236	239	88	88	145	145
19	$9.1x10^{-10}$	$1.4x10^{-10}$	Drug	214	214	207	207	317	326	151	151	145	145	164	164	155	158	236	236	88	88	145	145
20	$1.1x10^{-09}$	$1.5x10^{-10}$	Drug	214	214	207	207	317	326	151	151	145	154	173	173	155	158	239	239	88	88	145	145
21	$1.6x10^{-08}$	$1.9x10^{-09}$	Drug	214	214	207	207	317	326	151	151	154	154	164	173	155	158	236	239	88	98	145	145
22	$2.0x10^{-08}$	$1.7x10^{-09}$	Drug	214	214	207	207	317	326	154	154	145	145	164	173	155	158	236	239	88	98	145	145
23	$8.0x10^{-09}$	$6.8x10^{-10}$	Drug	214	214	207	207	317	326	154	154	145	154	173	173	158	158	236	239	88	88	145	145
24	$1.5x10^{-07}$	$2.0x10^{-08}$	Drug	214	214	207	207	317	326	154	154	154	154	164	164	155	158	236	239	88	93	145	145
25	$7.3x10^{-09}$	$7.4x10^{-10}$	Drug	214	214	207	207	326	326	154	154	145	145	164	164	158	158	236	239	88	88	145	145
26	$7.5x10^{-08}$	$1.2x10^{-08}$	Drug	214	214	207	207	326	326	154	154	154	154	164	173	155	155	239	242	88	93	145	145
27	$8.6x10^{-09}$	$1.8x10^{-09}$	Drug	214	214	207	207	326	329	145	151	145	154	164	164	155	158	239	242	88	93	145	145
28	$2.5x10^{-09}$	$3.0x10^{-10}$	Drug	214	214	207	207	326	329	151	151	145	154	164	173	155	158	236	236	88	93	145	145
29	$4.9x10^{-10}$	$2.5x10^{-10}$	Drug	214	226	144	144	317	317	145	145	142	154	164	164	155	164	236	236	88	98	145	145
30	$5.1x10^{-11}$	$7.0x10^{-11}$	Drug	214	226	144	144	317	326	145	145	142	154	164	164	155	164	236	236	88	93	142	151

Table 6.1 continued

Genotype Designation	*RMP* – Drug Genotypes Only	*RMP* – All C. sativa genotypes	*C. sativa* variety	ANUCS301		ANUCS304		B01-CANN1		ANUCS302		ANUCS305		B02-CANN2		C11-CANN1		B05-CANN1		ANUCS501		ANUCS303	
31	$1.6x10^{-08}$	$5.5x10^{-09}$	Drug	214	226	144	144	326	329	139	139	154	154	164	164	158	158	239	242	88	88	145	145
32	$1.7x10^{-09}$	$2.4x10^{-10}$	Drug	214	226	171	171	317	317	151	151	145	154	164	164	155	155	236	236	88	98	145	145
33	$2.5x10^{-08}$	$2.0x10^{-09}$	Drug	214	226	171	171	317	317	154	154	145	154	164	173	155	158	239	239	88	98	145	145
34	$1.5x10^{-08}$	$1.1x10^{-09}$	Drug	214	226	171	171	317	317	154	154	154	154	173	173	155	158	236	239	88	98	145	145
35	$6.7x10^{-09}$	$7.9x10^{-10}$	Drug	214	226	171	171	317	326	139	151	154	154	173	173	155	158	239	239	88	98	145	145
36	$1.2x10^{-08}$	$1.3x10^{-09}$	Drug	214	226	171	171	317	329	151	151	154	154	164	164	155	158	236	239	88	98	145	145
37	$1.8x10^{-07}$	$1.4x10^{-08}$	Drug	214	226	171	171	326	326	154	154	145	154	164	173	155	158	236	239	88	98	145	145
38	$3.6x10^{-07}$	$3.3x10^{-08}$	Drug	214	226	171	171	326	326	154	154	154	154	164	164	155	158	236	239	88	98	145	145
39	$4.5x10^{-08}$	$2.1x10^{-08}$	Drug	214	226	171	174	326	326	154	154	154	154	164	173	155	158	239	242	88	98	145	145
40	$3.2x10^{-10}$	$4.2x10^{-11}$	Drug	214	226	189	189	317	329	139	151	142	154	164	173	155	158	236	239	98	98	145	145
41	$1.4x10^{-08}$	$1.4x10^{-09}$	Drug	214	226	207	207	317	317	154	154	145	154	173	173	155	155	239	239	88	98	145	145
42	$9.9x10^{-10}$	$8.4x10^{-11}$	Drug	214	226	207	207	317	326	151	151	145	145	164	173	158	158	236	236	88	98	145	145
43	$3.8x10^{-09}$	$4.8x10^{-10}$	Drug	214	226	207	207	317	326	151	151	145	154	173	173	155	155	236	236	88	98	145	145
44	$2.1x10^{-08}$	$3.1x10^{-09}$	Drug	214	226	207	207	317	326	151	151	145	154	164	173	155	155	239	239	88	98	145	145
45	$6.4x10^{-09}$	$5.7x10^{-10}$	Drug	214	226	207	207	317	326	151	151	145	154	164	173	158	158	236	236	88	98	145	145
46	$7.6x10^{-08}$	$9.0x10^{-09}$	Drug	214	226	207	207	317	326	151	151	154	154	164	173	155	158	236	239	88	98	145	145
47	$7.2x10^{-07}$	$7.5x10^{-08}$	Drug	214	226	207	207	317	326	154	154	145	154	164	164	155	158	236	239	88	98	145	145
48	$9.6x10^{-07}$	$9.3x10^{-08}$	Drug	214	226	207	207	317	326	154	154	154	154	164	173	155	158	236	239	88	98	145	145
49	$2.6x10^{-08}$	$1.8x10^{-09}$	Drug	214	226	207	207	317	326	154	154	154	154	173	173	158	158	236	236	88	98	145	145
50	$9.5x10^{-09}$	$1.9x10^{-09}$	Drug	214	226	207	207	317	329	151	151	145	154	164	167	155	155	239	239	88	98	145	145
51	$4.1x10^{-07}$	$3.7x10^{-08}$	Drug	214	226	207	207	317	329	154	154	154	154	164	173	155	158	236	239	88	98	145	145
52	$3.3x10^{-07}$	$4.7x10^{-08}$	Drug	214	226	207	207	326	326	154	154	145	154	164	164	155	158	236	236	88	88	145	145
53	$5.9x10^{-07}$	$5.3x10^{-08}$	Drug	214	226	207	207	326	326	154	154	145	154	164	173	155	158	236	239	88	98	145	145
54	$6.6x10^{-08}$	$5.2x10^{-09}$	Drug	214	226	207	207	326	326	154	154	145	154	173	173	155	158	239	239	88	98	145	145
55	$9.5x10^{-08}$	$6.7x10^{-09}$	Drug	214	226	207	207	326	326	154	154	145	154	164	173	158	158	239	239	88	98	145	145
56	$2.7x10^{-08}$	$1.7x10^{-09}$	Drug	214	226	207	207	326	326	154	154	154	154	173	173	158	158	236	236	88	98	145	145
57	$1.0x10^{-08}$	$6.9x10^{-10}$	Drug	214	226	207	207	326	329	154	154	145	145	164	173	158	158	236	236	88	98	145	145
58	$2.0x10^{-08}$	$4.2x10^{-09}$	Drug	214	244	144	144	317	326	154	154	145	154	164	164	155	158	236	236	88	93	145	145

Table 6.1 continued

Genotype Designation	*RMP* – Drug Genotypes Only	*RMP* – All C. sativa genotypes	*C. sativa* variety	ANUCS301		ANUCS304		B01-CANN1		ANUCS302		ANUCS305		B02-CANN2		C11-CANN1		B05-CANN1		ANUCS501		ANUCS303	
59	$1.2x10^{-12}$	$1.0x10^{-12}$	Drug	214	244	144	207	317	317	145	145	142	145	164	164	164	164	236	236	88	93	145	145
60	$4.0x10^{-12}$	$4.4x10^{-12}$	Drug	214	244	144	207	317	317	145	145	154	154	164	164	158	164	236	236	88	93	142	151
61	$3.9x10^{-12}$	$3.7x10^{-12}$	Drug	214	244	144	207	317	326	145	145	142	142	164	164	164	164	239	239	88	93	145	145
62	$4.0x10^{-11}$	$1.4x10^{-11}$	Drug	214	244	207	207	317	326	151	151	142	142	164	164	158	164	239	239	88	93	145	151
63	$3.8x10^{-13}$	$2.5x10^{-13}$	Drug	217	241	144	144	317	317	145	154	148	148	164	173	155	155	236	236	88	88	151	151
67	$1.7x10^{-16}$	$7.5x10^{-15}$	Drug	220	241	144	201	326	341	139	154	154	154	167	167	167	167	236	239	88	88	151	151
69	$4.0x10^{-12}$	$3.1x10^{-12}$	Drug	223	223	144	144	326	329	154	154	142	142	164	164	155	155	239	242	88	88	145	151
71	$6.5x10^{-09}$	$1.5x10^{-09}$	Drug	226	226	144	144	317	329	151	151	154	154	167	173	155	158	239	242	88	98	145	145
72	$1.7x10^{-08}$	$5.8x10^{-09}$	Drug	226	226	144	144	326	326	148	154	142	154	164	167	155	158	236	242	88	88	145	145
73	$3.9x10^{-09}$	$6.8x10^{-10}$	Drug	226	226	144	144	326	329	154	154	154	154	164	173	155	155	242	245	88	93	145	145
74	$8.8x10^{-08}$	$1.6x10^{-08}$	Drug	226	226	144	144	326	371	154	154	154	154	164	164	155	158	239	242	88	88	145	145
75	$3.5x10^{-09}$	$1.7x10^{-09}$	Drug	226	226	144	144	329	329	139	139	154	154	164	167	155	158	242	242	88	98	145	145
76	$2.9x10^{-10}$	$1.3x10^{-10}$	Drug	226	226	144	144	329	329	148	154	142	154	164	164	155	158	239	242	88	88	145	148
77	$4.1x10^{-09}$	$9.2x10^{-10}$	Drug	226	226	144	144	329	329	154	154	154	154	167	167	158	158	239	242	88	98	145	151
78	$2.2x10^{-11}$	$5.6x10^{-12}$	Drug	226	226	144	144	371	371	148	154	142	142	164	167	155	158	239	242	88	88	145	145
79	$3.1x10^{-10}$	$1.3x10^{-10}$	Drug	226	226	144	144	371	371	154	154	142	154	167	167	155	155	239	242	88	88	145	151
80	$1.0x10^{-08}$	$2.5x10^{-09}$	Drug	226	226	144	171	329	329	154	154	142	142	164	164	155	155	236	236	88	88	145	151
81	$1.5x10^{-12}$	$4.0x10^{-12}$	Drug	226	226	144	189	338	338	154	154	142	154	164	164	155	158	236	236	98	98	145	145
82	$2.0x10^{-10}$	$4.7x10^{-11}$	Drug	226	226	144	207	326	326	145	154	142	142	164	164	155	176	239	239	98	98	151	151
83	$3.7x10^{-10}$	$9.3x10^{-11}$	Drug	226	226	144	210	326	371	154	154	154	154	167	167	155	158	239	239	88	93	151	151
84	$1.3x10^{-12}$	$2.0x10^{-13}$	Drug	226	226	147	192	326	344	154	154	154	154	167	167	155	158	239	242	98	98	145	145
86	$3.7x10^{-10}$	$1.1x10^{-10}$	Drug	226	226	171	171	317	317	139	151	142	154	164	164	155	164	239	242	93	98	145	145
87	$1.3x10^{-08}$	$3.3x10^{-09}$	Drug	226	226	171	171	317	326	139	151	145	154	164	173	155	155	236	242	88	93	145	145
88	$3.4x10^{-09}$	$7.3x10^{-10}$	Drug	226	226	171	171	317	326	139	151	154	154	164	164	155	155	239	239	93	93	145	145
89	$3.0x10^{-09}$	$2.3x10^{-10}$	Drug	226	226	171	171	317	326	151	151	154	154	164	173	155	158	236	236	98	98	145	145
90	$1.2x10^{-09}$	$3.8x10^{-10}$	Drug	226	226	171	171	323	326	139	154	154	154	164	164	155	158	239	239	88	88	145	145
91	$4.7x10^{-10}$	$1.9x10^{-10}$	Drug	226	226	171	171	326	326	139	151	154	154	164	164	155	167	239	242	93	93	145	145

Table 6.1 continued

Genotype Designation	*RMP* – Drug Genotypes Only	*RMP* – All C. sativa genotypes	*C. sativa* variety	ANUCS301		ANUCS304		B01-CANN1		ANUCS302		ANUCS305		B02-CANN2		C11-CANN1		B05-CANN1		ANUCS501		ANUCS303	
92	$4.2x10^{-09}$	$2.7x10^{-10}$	Drug	226	226	171	171	326	326	154	154	145	154	173	173	155	155	236	236	98	98	145	145
93	$8.5x10^{-09}$	$4.5x10^{-10}$	Drug	226	226	171	171	326	326	154	154	145	154	164	164	158	158	236	236	98	98	145	145
94	$5.9x10^{-09}$	$6.0x10^{-10}$	Drug	226	226	171	171	326	329	151	151	154	154	164	164	155	155	236	239	98	98	145	145
95	$5.8x10^{-09}$	$4.0x10^{-10}$	Drug	226	226	171	171	326	329	151	151	154	154	164	173	155	158	236	239	98	98	145	145
96	$1.1x10^{-08}$	$1.1x10^{-09}$	Drug	226	226	171	171	326	329	154	154	142	154	164	167	158	158	239	242	98	98	145	145
97	$1.8x10^{-11}$	$1.2x10^{-11}$	Drug	226	226	171	174	317	329	151	151	154	154	167	173	155	155	239	239	98	98	151	151
98	$7.4x10^{-09}$	$1.5x10^{-09}$	Drug	226	226	171	192	326	326	139	154	145	154	164	164	155	158	239	239	88	93	145	151
99	$1.4x10^{-08}$	$3.1x10^{-09}$	Drug	226	226	171	192	326	326	154	154	142	154	164	167	155	158	239	239	88	93	145	145
100	$6.5x10^{-10}$	$1.0x10^{-09}$	Drug	226	226	174	174	317	326	154	154	154	154	164	173	158	158	239	239	88	98	145	145
102	$1.3x10^{-14}$	$3.3x10^{-13}$	Drug	226	226	174	174	326	332	145	145	142	154	167	167	155	155	236	236	98	98	142	151
103	$7.3x10^{-10}$	$6.7x10^{-11}$	Drug	226	226	189	189	317	317	154	154	154	154	164	164	155	158	236	236	98	98	145	145
104	$2.1x10^{-10}$	$1.5x10^{-11}$	Drug	226	226	189	189	317	317	154	154	154	154	164	164	158	158	236	236	98	98	145	145
105	$4.3x10^{-12}$	$3.8x10^{-12}$	Drug	226	226	201	210	326	326	139	154	142	148	167	167	155	158	239	239	98	98	151	151
106	$1.0x10^{-14}$	$1.0x10^{-13}$	Drug	226	226	204	210	332	332	133	154	142	142	164	164	155	164	239	239	88	88	145	151
107	$7.6x10^{-09}$	$6.2x10^{-10}$	Drug	226	226	207	207	317	326	151	151	154	154	164	164	158	158	236	239	98	98	145	145
108	$1.0x10^{-07}$	$9.3x10^{-09}$	Drug	226	226	207	207	317	326	154	154	145	154	164	173	155	158	239	239	93	98	145	145
109	$9.0x10^{-08}$	$6.3x10^{-09}$	Drug	226	226	207	207	317	326	154	154	145	154	164	173	155	158	239	239	98	98	145	145
110	$6.0x10^{-07}$	$8.7x10^{-08}$	Drug	226	226	207	207	317	326	154	154	154	154	164	164	155	155	239	239	88	98	145	145
111	$5.5x10^{-08}$	$3.6x10^{-09}$	Drug	226	226	207	207	317	326	154	154	154	154	173	173	155	158	236	239	98	98	145	145
112	$2.0x10^{-07}$	$5.9x10^{-08}$	Drug	226	226	207	207	326	326	139	154	145	154	164	164	155	155	239	242	88	93	145	145
113	$1.0x10^{-08}$	$1.2x10^{-09}$	Drug	226	226	207	207	326	326	151	151	145	154	164	164	155	158	239	239	93	98	145	145
114	$9.0x10^{-08}$	$6.2x10^{-09}$	Drug	226	226	207	207	326	326	154	154	145	154	164	173	155	158	239	239	98	98	145	145
115	$5.9x10^{-08}$	$3.6x10^{-09}$	Drug	226	226	207	207	326	326	154	154	145	154	164	164	158	158	236	239	98	98	145	145
116	$3.9x10^{-07}$	$3.9x10^{-08}$	Drug	226	226	207	207	326	326	154	154	154	154	164	164	155	158	236	239	93	98	145	145
117	$2.3x10^{-08}$	$4.0x10^{-09}$	Drug	226	226	207	207	329	329	139	154	154	154	164	173	158	158	239	242	88	88	145	151
118	$7.5x10^{-09}$	$1.0x10^{-09}$	Drug	226	226	207	207	329	329	154	154	154	154	164	164	158	158	242	242	88	93	145	151
119	$3.2x10^{-09}$	$2.8x10^{-10}$	Drug	226	226	210	210	326	326	154	154	154	154	164	164	158	158	236	236	88	98	145	145

Table 6.1 continued

Genotype Designation	*RMP* – Drug Genotypes Only	*RMP* – All C. sativa genotypes	*C. sativa* variety	ANUCS301		ANUCS304		B01-CANN1		ANUCS302		ANUCS305		B02-CANN2		C11-CANN1		B05-CANN1		ANUCS501		ANUCS303	
120	$2.5x10^{-09}$	$1.1x10^{-09}$	Drug	226	229	144	171	317	329	154	154	145	154	167	173	155	158	236	242	88	93	145	151
121	$6.6x10^{-15}$	$4.3x10^{-14}$	Drug	226	229	198	198	329	344	139	154	142	154	164	173	152	155	239	242	88	88	151	151
122	$3.7x10^{-10}$	$9.9x10^{-10}$	Drug	226	232	144	144	326	329	139	154	142	154	164	167	152	158	239	242	88	88	145	148
123	$4.3x10^{-12}$	$1.3x10^{-12}$	Drug	226	232	171	192	326	326	148	148	154	154	164	167	155	158	236	242	88	98	145	151
124	$1.0x10^{-07}$	$3.0x10^{-08}$	Drug	226	232	204	207	326	329	154	154	154	154	164	164	155	158	236	239	88	88	145	151
125	$3.9x10^{-12}$	$2.5x10^{-12}$	Drug	226	241	171	192	317	344	139	139	142	145	167	167	155	155	239	239	88	98	145	145
126	$6.8x10^{-13}$	$4.1x10^{-13}$	Drug	226	241	207	207	317	371	139	154	142	154	164	164	176	176	236	242	88	98	148	151
127	$1.0x10^{-10}$	$2.6x10^{-11}$	Drug	226	241	210	210	317	317	139	139	142	154	164	164	155	158	236	239	98	98	145	145
128	$8.3x10^{-12}$	$2.0x10^{-11}$	Drug	226	244	144	144	317	326	148	154	142	154	164	167	152	155	242	242	88	88	148	151
129	$4.2x10^{-11}$	$1.5x10^{-11}$	Drug	226	244	144	144	326	326	151	151	142	154	164	164	158	164	239	239	93	93	145	151
130	$3.3x10^{-12}$	$3.5x10^{-12}$	Drug	226	244	144	207	317	317	145	145	142	154	164	164	158	164	239	239	93	93	142	145
131	$4.3x10^{-10}$	$2.8x10^{-10}$	Drug	226	244	171	171	317	326	139	139	142	154	164	164	152	155	239	242	93	98	145	145
132	$3.9x10^{-09}$	$1.0x10^{-09}$	Drug	226	244	171	171	317	326	151	151	154	154	164	164	155	155	239	242	88	93	145	145
134	$3.0x10^{-10}$	$2.8x10^{-10}$	Drug	226	244	207	207	326	329	139	139	142	142	167	173	155	155	242	242	88	93	145	145
135	$4.5x10^{-13}$	$2.7x10^{-13}$	Drug	226	244	210	210	326	326	151	151	142	142	167	167	152	158	236	239	93	98	145	145
136	$2.3x10^{-12}$	$5.6x10^{-13}$	Drug	226	244	210	210	326	326	151	151	142	145	167	167	158	158	236	239	93	98	145	145
138	$1.8x10^{-11}$	$2.1x10^{-11}$	Drug	226	247	171	171	326	326	139	139	142	154	164	164	158	158	239	242	88	98	148	151
139	$3.0x10^{-10}$	$2.6x10^{-10}$	Drug	226	247	171	171	329	329	139	139	142	154	164	173	155	155	239	242	88	88	145	145
142	$7.0x10^{-13}$	$1.2x10^{-12}$	Drug	226	253	144	207	371	371	139	145	142	142	164	167	152	155	239	242	88	88	151	151
143	$2.7x10^{-08}$	$3.6x10^{-09}$	Drug	226	253	171	171	326	329	154	154	154	154	164	164	155	158	239	239	88	98	145	145
145	$4.2x10^{-12}$	$3.7x10^{-12}$	Drug	226	253	195	204	326	326	139	139	142	154	167	173	155	155	239	239	98	98	145	151
146	$5.0x10^{-12}$	$1.3x10^{-11}$	Drug	226	253	207	207	326	329	154	154	142	142	167	167	152	152	242	242	88	88	145	151
147	$4.7x10^{-13}$	$4.2x10^{-13}$	Drug	226	262	198	210	326	329	139	148	142	154	167	173	155	158	239	242	88	98	145	151
148	$1.3x10^{-14}$	$1.1x10^{-13}$	Drug	226	262	204	204	317	329	139	139	142	154	167	173	155	164	239	242	78	98	145	151
154	$3.6x10^{-14}$	$3.3x10^{-13}$	Drug	229	238	144	144	329	329	154	154	142	154	164	167	152	152	242	242	88	88	145	148
155	$6.1x10^{-17}$	$7.4x10^{-16}$	Drug	229	238	144	144	371	371	154	154	142	154	164	167	152	152	236	242	88	88	148	148
156	$4.5x10^{-16}$	$2.8x10^{-15}$	Drug	229	238	144	144	371	371	154	154	142	154	164	167	167	167	242	242	88	88	145	148

Table 6.1 continued

Genotype Designation	*RMP* – Drug Genotypes Only	*RMP* – All C. sativa genotypes	*C. sativa* variety	ANUCS301		ANUCS304		B01-CANN1		ANUCS302		ANUCS305		B02-CANN2		C11-CANN1		B05-CANN1		ANUCS501		ANUCS303	
157	$4.3x10^{-15}$	$1.6x10^{-14}$	Drug	229	238	144	144	371	371	154	154	154	154	167	167	152	152	242	242	88	88	145	145
159	$9.4x10^{-20}$	$4.7x10^{-17}$	Drug	229	244	207	210	326	335	139	145	142	142	167	167	161	176	236	239	88	88	148	148
163	$4.5x10^{-15}$	$1.1x10^{-14}$	Drug	232	232	144	201	371	371	145	145	145	145	164	164	155	155	236	242	88	88	151	151
164	$2.1x10^{-09}$	$2.2x10^{-09}$	Drug	232	232	144	207	329	329	139	154	142	154	164	167	155	158	239	242	88	88	145	145
169	$3.3x10^{-12}$	$1.0x10^{-11}$	Drug	232	232	204	204	326	326	139	145	142	145	164	167	155	155	236	242	88	88	151	151
170	$5.1x10^{-13}$	$1.3x10^{-12}$	Drug	232	232	204	204	326	326	145	145	142	145	164	164	155	155	242	242	88	88	151	151
171	$4.2x10^{-10}$	$7.5x10^{-10}$	Drug	232	232	207	207	326	329	139	154	142	154	164	167	152	158	239	242	88	88	145	151
172	$1.1x10^{-10}$	$1.8x10^{-10}$	Drug	232	232	207	207	329	329	139	154	142	154	164	167	152	158	239	242	88	88	145	145
174	$1.4x10^{-14}$	$7.6x10^{-14}$	Drug	232	235	144	204	326	326	145	145	142	145	164	164	167	167	236	242	88	93	151	151
177	$9.4x10^{-13}$	$3.2x10^{-12}$	Drug	232	235	204	204	326	326	145	145	142	145	164	167	155	155	236	242	88	88	151	151
178	$6.8x10^{-15}$	$1.1x10^{-14}$	Drug	232	235	204	204	371	371	145	145	142	145	164	164	155	155	236	242	88	88	151	151
179	$6.7x10^{-15}$	$5.2x10^{-14}$	Drug	232	235	204	207	326	326	145	145	142	142	164	164	167	167	242	242	88	88	151	151
180	$1.2x10^{-09}$	$1.2x10^{-09}$	Drug	232	241	171	171	326	326	139	154	154	154	167	167	155	155	239	242	88	93	145	151
181	$9.2x10^{-09}$	$4.1x10^{-09}$	Drug	232	241	171	171	326	329	154	154	154	154	164	167	155	155	239	242	88	93	145	145
182	$1.2x10^{-11}$	$2.3x10^{-11}$	Drug	232	241	171	171	329	329	139	154	154	154	167	167	155	155	239	242	88	93	142	151
188	$1.7x10^{-12}$	$2.1x10^{-12}$	Drug	232	247	207	207	326	326	151	151	154	154	164	173	155	176	242	242	88	88	151	151
189	$6.6x10^{-10}$	$5.1x10^{-10}$	Drug	232	247	207	207	329	329	139	154	142	154	164	164	155	155	239	239	88	98	145	145
193	$6.6x10^{-12}$	$7.3x10^{-11}$	Drug	235	238	174	174	326	329	151	154	142	154	164	164	155	158	236	239	88	88	145	151
194	$1.9x10^{-13}$	$9.3x10^{-13}$	Drug	235	241	144	144	326	326	139	139	142	154	167	173	167	167	239	239	88	88	151	151
195	$2.4x10^{-13}$	$1.0x10^{-12}$	Drug	235	241	144	144	326	326	139	139	154	154	167	173	167	167	239	239	88	88	151	151
197	$2.5x10^{-11}$	$2.1x10^{-11}$	Drug	235	241	204	204	326	329	145	154	145	154	164	164	155	155	239	239	88	93	151	151
199	$1.8x10^{-17}$	$2.2x10^{-14}$	Drug	235	259	144	174	326	326	133	154	142	142	167	167	152	152	236	239	88	88	142	151
201	$5.9x10^{-14}$	$2.3x10^{-13}$	Drug	238	238	144	144	329	329	154	154	142	154	167	167	152	152	242	242	88	88	145	145
205	$1.9x10^{-12}$	$2.4x10^{-12}$	Drug	241	241	144	144	317	317	145	145	148	148	164	173	155	155	236	236	88	88	151	151
206	$3.0x10^{-12}$	$2.3x10^{-12}$	Drug	241	241	144	144	317	317	145	154	148	148	173	173	155	155	236	236	88	88	151	151
207	$3.3x10^{-11}$	$2.3x10^{-11}$	Drug	241	241	144	144	317	317	154	154	148	148	164	164	155	155	236	236	88	88	151	151
208	$2.7x10^{-11}$	$1.7x10^{-11}$	Drug	241	241	144	144	317	317	154	154	148	148	164	173	155	155	236	236	88	88	151	151

Table 6.1 continued

Genotype Designation	*RMP* – Drug Genotypes Only	*RMP* – All C. sativa genotypes	*C. sativa* variety	ANUCS301		ANUCS304		B01-CANN1		ANUCS302		ANUCS305		B02-CANN2		C11-CANN1		B05-CANN1		ANUCS501		ANUCS303	
210	$9.4x10^{-12}$	$1.2x10^{-11}$	Drug	241	241	144	144	317	326	145	145	148	148	164	164	155	155	236	236	88	88	151	151
211	$1.1x10^{-10}$	$6.7x10^{-11}$	Drug	241	241	144	144	317	326	154	154	148	148	164	173	155	155	236	236	88	88	151	151
212	$2.3x10^{-11}$	$1.2x10^{-11}$	Drug	241	241	144	144	317	326	154	154	148	148	173	173	155	155	236	236	88	88	151	151
213	$1.6x10^{-12}$	$1.6x10^{-12}$	Drug	241	241	144	144	326	326	145	145	148	148	173	173	155	155	236	236	88	88	151	151
214	$2.3x10^{-11}$	$1.2x10^{-11}$	Drug	241	241	144	144	326	326	154	154	148	148	173	173	155	155	236	236	88	88	151	151
216	$1.3x10^{-09}$	$1.0x10^{-09}$	Drug	241	241	144	210	317	326	145	154	142	148	164	167	155	158	236	239	88	88	145	151
217	$2.5x10^{-09}$	$1.4x10^{-09}$	Drug	241	241	144	210	317	326	154	154	142	148	164	167	155	158	236	239	88	88	145	151
218	$1.3x10^{-09}$	$1.0x10^{-09}$	Drug	241	241	171	171	326	329	154	154	154	154	164	167	155	155	239	242	88	93	142	145
221	$2.4x10^{-16}$	$4.3x10^{-15}$	Drug	241	241	201	201	326	341	154	154	154	154	167	167	167	167	236	239	88	88	151	151
222	$2.8x10^{-11}$	$3.4x10^{-11}$	Drug	241	241	204	204	326	329	139	139	154	154	164	167	155	155	239	239	88	93	151	151
227	$5.8x10^{-11}$	$1.2x10^{-10}$	Drug	241	241	207	210	317	329	139	139	142	154	164	167	155	155	239	242	88	93	142	145
228	$3.1x10^{-12}$	$1.2x10^{-12}$	Drug	241	241	210	210	329	329	154	154	154	154	167	167	155	155	236	236	93	93	145	145
231	$1.0x10^{-12}$	$1.4x10^{-12}$	Drug	241	244	189	210	326	329	145	154	142	142	164	167	152	155	242	242	93	93	145	145
232	$1.9x10^{-10}$	$2.0x10^{-10}$	Drug	241	244	204	204	326	326	139	145	145	154	164	167	155	155	239	239	88	93	145	145
233	$5.8x10^{-10}$	$6.4x10^{-10}$	Drug	241	244	204	204	326	326	139	145	154	154	164	167	155	155	236	239	88	93	145	145
234	$9.3x10^{-13}$	$4.5x10^{-12}$	Drug	241	244	204	204	326	326	139	145	154	154	164	167	155	155	239	239	88	93	142	142
235	$3.3x10^{-13}$	$1.0x10^{-12}$	Drug	241	244	204	204	326	326	139	154	145	145	164	167	155	155	239	239	88	93	142	142
241	$4.8x10^{-12}$	$2.3x10^{-12}$	Drug	241	244	204	204	326	329	154	154	145	154	167	167	155	155	239	239	88	93	145	145
242	$8.0x10^{-14}$	$1.0x10^{-13}$	Drug	244	244	144	144	317	326	145	145	142	142	164	164	164	164	236	239	93	93	145	145
245	$9.7x10^{-12}$	$9.2x10^{-12}$	Drug	244	244	144	207	317	326	145	145	145	154	164	164	155	158	236	239	93	93	142	151
246	$4.1x10^{-15}$	$7.2x10^{-15}$	Drug	244	244	144	210	326	326	139	139	142	142	167	167	176	176	236	239	88	88	151	151
247	$1.5x10^{-14}$	$1.7x10^{-13}$	Drug	244	244	180	201	329	329	139	139	154	154	167	167	155	155	236	236	88	93	145	145
248	$1.0x10^{-12}$	$1.5x10^{-12}$	Drug	244	244	189	207	317	317	154	154	142	154	167	167	152	158	242	242	88	88	151	151
249	$2.7x10^{-12}$	$2.9x10^{-12}$	Drug	244	244	204	204	326	326	145	154	145	145	164	164	155	155	239	239	88	93	142	145
250	$4.4x10^{-11}$	$2.1x10^{-11}$	Drug	244	244	204	204	326	326	145	154	145	154	164	164	155	155	236	239	93	93	145	145
251	$4.1x10^{-12}$	$3.9x10^{-12}$	Drug	244	244	204	204	326	326	154	154	145	154	164	167	155	155	239	239	93	93	142	145
253	$1.3x10^{-12}$	$1.1x10^{-12}$	Drug	244	265	207	210	326	326	145	154	142	154	167	167	155	158	242	242	88	88	151	151
254	$4.5x10^{-14}$	$1.3x10^{-13}$	Drug	244	268	204	204	326	326	145	145	154	154	164	167	155	155	236	239	93	93	142	145

Table 6.1 continued

Genotype Designation	*RMP* – Drug Genotypes Only	*RMP* – All C. sativa genotypes	*C. sativa* variety	ANUCS301		ANUCS304		B01-CANN1		ANUCS302		ANUCS305		B02-CANN2		C11-CANN1		B05-CANN1		ANUCS501		ANUCS303	
256	$9.1x10^{-15}$	$1.0x10^{-14}$	Drug	247	253	144	144	317	332	139	154	145	154	173	173	176	176	236	239	88	88	145	151
261	$5.6x10^{-12}$	$1.2x10^{-11}$	Drug	253	253	144	144	329	329	139	139	142	154	164	167	155	155	242	242	88	93	145	151
262	$4.0x10^{-17}$	$1.8x10^{-15}$	Drug	253	253	144	201	332	335	139	139	142	145	167	167	155	167	236	236	88	88	145	151
263	$5.0x10^{-12}$	$5.5x10^{-12}$	Drug	253	253	171	192	326	329	139	151	142	154	164	167	155	158	242	242	88	93	145	145
265	$5.0x10^{-16}$	$7.1x10^{-16}$	Drug	253	253	195	195	326	371	139	139	142	142	164	173	155	155	236	242	98	98	145	151
269	$3.4x10^{17}$	$3.1x10^{-15}$	Drug	259	259	171	174	326	332	154	154	142	145	167	167	152	152	236	239	88	88	142	145
271	$4.8x10^{-15}$	$5.1x10^{-14}$	Drug	262	262	207	207	317	329	139	154	142	142	164	164	155	155	236	242	78	93	142	145
1		$1.7x10^{-22}$	Fibre	205	205	144	144	323	329	163	166	148	154	167	170	155	167	239	239	88	98	148	148
4		$8.5x10^{-18}$	Fibre	208	208	144	144	317	326	139	139	154	160	170	170	152	152	239	242	88	88	145	145
5		$3.5x10^{-18}$	Fibre	208	238	144	144	326	329	139	154	151	151	164	167	152	167	230	239	88	88	148	151
6		$2.4x10^{-16}$	Fibre	211	247	144	195	329	329	154	154	151	151	164	167	155	164	239	239	88	88	145	145
64		$7.4x10^{-17}$	Fibre	220	229	144	189	329	329	139	145	148	151	164	167	152	155	236	239	88	93	151	154
65		$1.0x10^{-14}$	Fibre	220	235	204	204	326	329	151	151	148	151	164	164	152	155	239	242	88	93	145	145
66		$2.4x10^{-14}$	Fibre	220	238	144	189	326	326	139	145	148	154	164	167	152	152	236	242	93	93	145	151
68		$9.2x10^{-17}$	Fibre	220	247	144	144	326	326	151	151	151	154	170	173	152	155	236	242	88	93	145	157
70		$7.4x10^{-19}$	Fibre	223	235	183	183	326	329	139	139	145	154	170	170	167	167	239	242	88	93	145	145
85		$9.8x10^{-18}$	Fibre	226	226	165	183	326	326	151	151	148	151	167	167	155	155	236	239	88	93	148	163
101		$1.5x10^{-14}$	Fibre	226	226	174	174	320	320	139	139	154	154	164	167	152	152	239	242	88	88	142	145
133		$5.4x10^{-15}$	Fibre	226	244	201	201	317	326	133	145	142	151	164	167	155	155	236	236	88	93	148	148
137		$4.7x10^{-14}$	Fibre	226	247	168	201	326	326	133	145	154	154	167	167	155	155	236	242	78	88	145	148
140		$2.5x10^{-13}$	Fibre	226	247	198	198	326	326	145	145	154	154	164	167	155	155	242	242	78	88	145	151
141		$6.8x10^{-13}$	Fibre	226	247	198	201	326	326	145	145	142	142	164	167	155	155	236	242	88	93	145	148
144		$1.1x10^{-14}$	Fibre	226	253	174	180	326	326	133	133	154	154	164	167	161	161	239	242	88	88	145	145
149		$1.0x10^{-14}$	Fibre	229	229	174	174	317	317	133	139	154	154	164	167	167	167	242	242	88	88	145	145
150		$2.2x10^{-17}$	Fibre	229	229	174	207	317	317	133	133	145	145	167	167	161	161	236	242	88	88	142	145
151		$4.2x10^{-15}$	Fibre	229	232	174	174	317	338	139	139	148	148	164	167	155	155	239	242	78	88	142	145
152		$2.5x10^{-18}$	Fibre	229	232	174	180	317	338	145	145	148	154	164	167	161	161	239	239	88	88	142	142
153		$4.3x10^{-18}$	Fibre	229	235	174	174	320	338	133	133	154	160	164	164	155	155	236	239	88	88	142	142

Table 6.1 continued

Genotype Designation	*RMP* – Drug Genotypes Only	*RMP* – All C. sativa genotypes	*C. sativa* variety	ANUCS301		ANUCS304		B01-CANN1		ANUCS302		ANUCS305		B02-CANN2		C11-CANN1		B05-CANN1		ANUCS501		ANUCS303	
158		$3.4x10^{-16}$	Fibre	229	244	174	180	317	317	139	139	142	154	164	167	164	164	242	242	78	88	142	145
160		$6.9x10^{-17}$	Fibre	229	247	144	183	314	326	145	154	148	154	164	167	152	152	236	239	88	88	148	148
161		$8.7x10^{-15}$	Fibre	229	253	174	207	320	320	133	133	154	154	164	167	155	155	242	242	88	88	145	145
162		$1.6x10^{-16}$	Fibre	229	259	174	174	317	326	133	133	142	151	164	167	167	167	242	242	88	88	145	145
165		$1.1x10^{-20}$	Fibre	232	232	174	174	320	332	139	139	154	160	164	167	161	161	239	242	78	78	142	145
166		$1.4x10^{-19}$	Fibre	232	232	174	174	320	338	139	145	142	154	167	167	161	161	239	239	78	88	142	142
167		$2.0x10^{-14}$	Fibre	232	232	192	204	326	326	139	154	142	151	164	173	152	152	239	242	88	88	145	148
168		$4.2x10^{-16}$	Fibre	232	232	198	201	326	326	133	145	142	157	164	164	155	155	236	239	88	88	148	148
173		$1.3x10^{-13}$	Fibre	232	235	144	183	326	326	145	151	142	163	167	173	155	155	239	242	88	88	145	148
175		$3.1x10^{-25}$	Fibre	232	235	174	174	341	341	139	142	148	151	164	164	164	164	242	242	78	78	142	142
176		$1.3x10^{-15}$	Fibre	232	235	192	195	329	329	139	139	145	157	164	167	155	155	236	239	88	88	151	151
184		$2.9x10^{-14}$	Fibre	232	244	174	180	317	338	139	145	154	154	164	167	155	155	242	242	78	88	142	145
185		$4.2x10^{-17}$	Fibre	232	244	183	189	326	335	145	145	157	163	167	170	155	155	236	239	88	88	145	145
187		$1.7x10^{-16}$	Fibre	232	247	201	201	326	326	145	145	154	163	164	164	167	167	239	242	88	88	148	151
190		$1.9x10^{-12}$	Fibre	232	256	174	174	317	317	133	145	145	154	164	167	155	155	236	242	88	88	145	145
191		$4.5x10^{-17}$	Fibre	232	276	144	183	311	326	139	151	142	151	164	164	152	152	236	239	88	88	145	151
192		$4.2x10^{-20}$	Fibre	235	235	174	174	338	338	139	139	142	148	167	170	161	164	239	242	78	88	142	145
196		$7.1x10^{-15}$	Fibre	235	241	183	216	326	326	133	145	142	163	164	167	155	155	239	239	88	88	145	145
198		$5.3x10^{-20}$	Fibre	235	250	174	174	317	338	139	139	154	160	167	167	152	152	242	242	78	88	142	142
200		$6.3x10^{-12}$	Fibre	235	268	144	144	326	326	139	145	151	154	164	164	155	155	239	242	88	88	145	151
203		$7.7x10^{-17}$	Fibre	238	247	168	201	326	326	139	145	142	167	167	173	155	155	236	236	88	93	148	151
204		$1.1x10^{-15}$	Fibre	238	250	144	198	326	326	133	133	142	163	164	167	155	155	239	242	88	88	145	148
219		$1.6x10^{-12}$	Fibre	241	241	183	207	317	326	133	151	142	142	170	173	155	155	236	239	88	88	145	145
220		$3.6x10^{-17}$	Fibre	241	241	201	201	326	326	139	145	154	160	167	170	155	164	227	239	88	88	151	151
236		$5.1x10^{-17}$	Fibre	241	250	144	186	326	329	139	139	142	160	167	170	152	152	236	236	88	88	145	145
243		$4.5x10^{-12}$	Fibre	244	244	144	144	326	329	133	145	145	148	164	164	152	155	242	242	88	88	145	151
255		$1.4x10^{-15}$	Fibre	247	247	168	201	317	326	133	145	154	157	167	173	155	155	236	236	88	93	145	151

Table 6.1 continued

Genotype Designation	*RMP* – Drug Genotypes Only	*RMP* – All C. sativa genotypes	*C. sativa* variety	ANUCS301		ANUCS304		B01-CANN1		ANUCS302		ANUCS305		B02-CANN2		C11-CANN1		B05-CANN1		ANUCS501		ANUCS303	
257		$2.0x10^{-21}$	Fibre	247	256	177	222	317	338	139	151	151	154	167	170	155	164	236	239	88	88	145	160
258		$7.1x10^{-18}$	Fibre	247	259	174	174	317	317	133	163	145	154	167	167	167	167	242	242	88	88	142	145
259		$1.1x10^{-20}$	Fibre	250	253	174	174	320	338	133	139	148	154	167	167	164	164	239	242	78	88	142	142
260		$3.7x10^{-22}$	Fibre	253	253	141	144	320	326	139	139	160	163	167	170	152	152	239	242	88	88	139	151
264		$2.5x10^{-12}$	Fibre	253	253	174	207	317	326	133	133	148	154	164	167	155	155	242	242	88	88	145	145
266		$1.6x10^{-17}$	Fibre	253	259	174	174	317	317	133	133	142	148	164	164	167	167	242	242	78	88	145	145
267		$3.7x10^{-22}$	Fibre	256	256	174	207	317	317	133	163	148	148	164	167	161	161	242	242	78	78	142	145
268		$3.3x10^{-15}$	Fibre	256	259	174	174	317	317	133	133	148	148	164	164	155	155	239	242	88	88	145	145
270		$3.7x10^{-15}$	Fibre	259	259	174	174	317	317	133	133	154	154	167	167	155	167	239	242	88	88	145	145
202		$1.0x10^{-12}$	Unknown	238	238	144	210	317	326	145	145	142	154	167	167	155	155	236	236	88	88	142	145
209		$1.6x10^{-10}$	Unknown	241	241	144	144	317	326	139	139	142	142	164	167	155	155	239	242	88	88	142	151
223		$2.7x10^{-12}$	Unknown	241	241	204	204	326	332	145	145	142	142	164	164	155	155	236	236	88	88	145	151
224		$2.6x10^{-12}$	Unknown	241	241	204	204	329	335	139	139	142	154	164	167	155	155	236	242	88	88	151	151
225		$2.1x10^{-12}$	Unknown	241	241	207	207	332	335	145	145	142	154	164	167	155	155	236	242	88	88	151	151
226		$1.1x10^{-12}$	Unknown	241	241	207	207	332	335	154	154	142	154	164	167	155	155	236	242	88	88	142	142
229		$1.5x10^{-12}$	Unknown	241	244	174	204	326	326	139	139	142	154	164	167	152	167	236	242	88	93	142	151
230		$4.7x10^{-12}$	Unknown	241	244	174	204	326	329	139	139	145	154	167	167	152	155	236	242	88	93	151	151
237		$4.1x10^{-12}$	Unknown	241	253	207	207	329	329	139	139	142	145	167	167	155	155	242	242	88	88	151	151
238		$5.9x10^{-13}$	Unknown	241	256	201	201	326	332	145	154	142	154	164	167	155	155	236	239	88	88	151	151
239		$9.5x10^{-14}$	Unknown	241	262	144	210	326	326	151	151	142	154	164	167	176	176	236	239	88	88	145	151
240		$3.4x10^{-13}$	Unknown	241	262	207	207	317	326	139	145	142	154	167	167	152	152	242	242	88	88	142	151
252		$8.3x10^{-12}$	Unknown	244	244	207	207	317	329	154	154	142	154	164	167	155	164	236	242	93	93	142	145

Table 6.2. ***Cannabis sativa*** **variety allele frequencies for fibre, drug, and drug growth type varieties. Only one representative sample of each genotype in each seizure was included in the analysis.**

Locus	Allele/n	Cannabis	Fibre	Drug	Field-grown	Hydroponic-grown	Pot-grown
ANUCS301	N	341	57	271	103	82	71
	205	0.007	0.018	0.006	0.015		
	208	0.004	0.026				
	211	0.001	0.009				
	214	0.147		0.185	0.262	0.159	0.127
	217	0.001		0.002			0.007
	220	0.007	0.035	0.002		0.006	
	223	0.004	0.009	0.004	0.010		
	226	0.345	0.079	0.417	0.461	0.433	0.289
	229	0.031	0.105	0.017	0.005	0.012	0.035
	232	0.069	0.158	0.054	0.102	0.043	0.007
	235	0.032	0.096	0.020	0.024	0.037	
	238	0.023	0.035	0.018		0.006	0.042
	241	0.145	0.053	0.142	0.029	0.140	0.338
	244	0.072	0.053	0.072	0.039	0.079	0.113
	247	0.031	0.105	0.017	0.015	0.024	0.014
	250	0.006	0.035				
	253	0.037	0.070	0.030	0.029	0.037	0.028
	256	0.009	0.044				
	259	0.013	0.053	0.006		0.018	
	262	0.009		0.007	0.005		
	265	0.001		0.002	0.005		
	268	0.003	0.009	0.002		0.006	
	276	0.001	0.009				

Locus	Allele/n	Cannabis	Fibre	Drug	Field-grown	Hydroponic-grown	Pot-grown
ANUCS304	N	345	74	271	103	82	71
	141	0.001	0.007				
	144	0.246	0.203	0.258	0.160	0.128	0.599
	147	0.001		0.002	0.005		
	165	0.001	0.007				
	168	0.006	0.020	0.002		0.006	
	171	0.145		0.185	0.218	0.177	0.141
	174	0.077	0.284	0.020	0.024	0.037	
	177	0.001	0.007				
	180	0.007	0.027	0.002			0.007
	183	0.013	0.061				
	186	0.001	0.007				
	189	0.041	0.020	0.046	0.044	0.098	
	192	0.014	0.014	0.015	0.024	0.012	
	195	0.007	0.014	0.006		0.006	0.014
	198	0.012	0.034	0.006	0.015		
	201	0.029	0.088	0.013	0.005	0.018	0.014
	204	0.074	0.081	0.072	0.063	0.146	
	207	0.281	0.101	0.330	0.384	0.366	0.183
	210	0.038	0.014	0.044	0.058	0.006	0.042
	216	0.001	0.007				
	222	0.001	0.007				

Table 6.2 continued

Locus	Allele/n	Cannabis	Fibre	Drug	Field-grown	Hydroponic-grown	Pot-grown
B01CANN1	N	345	74	271	103	82	71
	311	0.001	0.007				
	314	0.001	0.007				
	317	0.235	0.230	0.236	0.243	0.165	0.324
	320	0.013	0.061				
	323	0.003	0.007	0.002		0.006	
	326	0.459	0.405	0.474	0.466	0.506	0.423
	329	0.186	0.115	0.205	0.189	0.287	0.134
	332	0.020	0.034	0.017	0.010	0.018	0.028
	335	0.014	0.054	0.004			0.014
	338	0.017	0.068	0.004	0.010		
	341	0.006	0.014	0.004		0.012	
	344	0.013		0.017	0.019	0.006	0.021
	362	0.001		0.002	0.005		
	371	0.029		0.037	0.058		0.056
Locus	Allele/n	Cannabis	Fibre	Drug	Field-grown	Hydroponic-grown	Pot-grown
ANUCS302	N	345	74	271	103	82	71
	133	0.049	0.216	0.004	0.005	0.006	
	139	0.220	0.351	0.185	0.175	0.195	0.148
	142	0.001	0.007				
	145	0.158	0.236	0.137	0.102	0.061	0.303
	148	0.010		0.013	0.034		
	151	0.132	0.081	0.146	0.199	0.110	0.113
	154	0.423	0.081	0.517	0.485	0.628	0.437
	163	0.004	0.020				
	166	0.001	0.007				

Locus	Allele/n	Cannabis	Fibre	Drug	Field-grown	Hydroponic-grown	Pot-grown
ANUCS305	N	345	74	271	103	82	71
	142	0.223	0.257	0.214	0.228	0.171	0.218
	145	0.145	0.068	0.166	0.228	0.177	0.063
	148	0.093	0.128	0.083			0.310
	151	0.022	0.101				
	154	0.491	0.324	0.537	0.544	0.652	0.408
	157	0.006	0.027				
	160	0.010	0.047				
	163	0.009	0.041				
	167	0.001	0.007				
Locus	Allele/n	Cannabis	Fibre	Drug	Field-grown	Hydroponic-grown	Pot-grown
C11CANN1	N	345	74	271	103	82	71
	152	0.078	0.189	0.048	0.063	0.024	0.056
	155	0.542	0.527	0.546	0.510	0.634	0.507
	158	0.252		0.321	0.393	0.274	0.246
	161	0.020	0.088	0.002			0.007
	164	0.043	0.074	0.035	0.010		0.113
	167	0.046	0.108	0.030	0.024	0.049	0.021
	176	0.017	0.014	0.018		0.018	0.049

Table 6.2 continued

Locus	Allele/n	Cannabis	Fibre	Drug	Field-grown	Hydroponic-grown	Pot-grown
B05CANN1	N	345	74	271	103	82	71
	227	0.001	0.007				
	230	0.001	0.007				
	236	0.341	0.277	0.358	0.316	0.268	0.563
	239	0.368	0.297	0.387	0.359	0.512	0.246
	242	0.283	0.412	0.247	0.325	0.201	0.183
	245	0.006		0.007		0.018	0.007
Locus	**Allele/n**	**Cannabis**	**Fibre**	**Drug**	**Field-grown**	**Hydroponic-grown**	**Pot-grown**
ANUCS501	N	345	74	271	103	82	71
	78	0.026	0.108	0.004			
	88	0.597	0.750	0.555	0.563	0.524	0.620
	93	0.159	0.135	0.166	0.160	0.207	0.127
	98	0.217	0.007	0.275	0.277	0.268	0.254
Locus	**Allele/n**	**Cannabis**	**Fibre**	**Drug**	**Field-grown**	**Hydroponic-grown**	**Pot-grown**
B02CANN2	N	345	74	271	103	82	71
	164	0.510	0.378	0.546	0.558	0.512	0.620
	167	0.284	0.493	0.227	0.214	0.244	0.183
	170	0.020	0.088	0.002		0.006	
	173	0.186	0.041	0.225	0.228	0.238	0.197

Locus	Allele/n	Cannabis	Fibre	Drug	Field-grown	Hydroponic-grown	Pot-grown
ANUCS303	N	345	74	271	103	82	71
	139	0.001	0.007				
	142	0.071	0.196	0.037		0.091	0.028
	145	0.617	0.405	0.675	0.762	0.701	0.514
	148	0.046	0.128	0.024	0.029		0.049
	151	0.258	0.236	0.264	0.209	0.207	0.408
	154	0.001	0.007				
	157	0.001	0.007				
	160	0.001	0.007				
	163	0.001	0.007				

Table 6.3 Allele frequencies in *Cannabis sativa* drug varieties and Australian state of origin. Only one representative sample of each genotype in each independent seizure was included in the analysis.

Locus	Allele/n	ACT	ACTF	ACTH	ACTP	South Australia	SAF	SAH	Tasmania	TASP	Victoria	VICF	VICH	Western Australia	WAF	WAH	WAP
ANUCS301	N	109	31	20	43	44	25	19	8	8	39	19	20	71	28	23	20
	205													0.021	0.054		
	214	0.156	0.274		0.174	0.284	0.380	0.158	0.188	0.188	0.436	0.474	0.400	0.028		0.087	
	217	0.005			0.012												
	220													0.007		0.022	
	223													0.014	0.036		
	226	0.275	0.419	0.150	0.105	0.614	0.560	0.684	0.813	0.813	0.500	0.526	0.475	0.423	0.375	0.435	0.475
	229	0.009	0.016			0.023		0.053						0.035			0.125
	232	0.028	0.032	0.075	0.012	0.034		0.079						0.141	0.339	0.022	
	235	0.005	0.016								0.013		0.025	0.063	0.071	0.109	
	238	0.014												0.049		0.022	0.150
	241	0.317	0.097	0.400	0.547									0.056		0.152	0.025
	244	0.138	0.081	0.300	0.128	0.023	0.020	0.026						0.049	0.036		0.125
	247	0.023	0.032	0.025	0.023									0.028	0.018	0.065	
	253	0.005		0.025		0.023	0.040				0.013		0.025	0.085	0.071	0.087	0.100
	259										0.038		0.075				
	262	0.018	0.016														
	265	0.005	0.016														
	268	0.005		0.025													

ACT–Australian Capital Territory, VIC –Victoria, SA – South Australia, WA – Western Australia, TAS –Tasmania

F – field-grown, P – pot or container-grown, H – hydroponically-grown, ? – unknown growing condition

Table 6.3 continued

Locus	Allele/n	ACT	ACTF	ACTH	ACTP	ACT?	South Australia	SAF	SAH	Tasmania	TASP	Victoria	VICF	VICH	Western Australia	WAF	WAH	WAP
ANUCS304	N	109	31	20	43	15	44	25	19	8	8	39	19	20	71	28	23	20
	144	0.335	0.129	0.025	0.733	0.033	0.057	0.020	0.105	0.125	0.125	0.013		0.025	0.415	0.429	0.326	0.500
	147											0.013	0.026					
	168	0.005		0.025														
	171	0.147	0.226	0.300		0.200	0.239	0.400	0.026	0.625	0.625	0.244	0.211	0.275	0.127	0.054	0.109	0.250
	174	0.014	0.048				0.011	0.020				0.051		0.100	0.021	0.018	0.043	
	180														0.007			0.025
	189	0.009	0.032				0.261	0.140	0.421									
	192	0.018	0.032	0.025		0.033	0.011	0.020				0.013	0.026		0.014	0.018	0.022	
	195														0.021		0.022	0.050
	198	0.014	0.048															
	201	0.005				0.033									0.042	0.018	0.065	0.050
	204	0.119	0.032	0.550		0.067	0.011		0.026						0.085	0.196	0.022	
	207	0.252	0.306	0.075	0.221	0.467	0.409	0.400	0.421	0.250	0.250	0.641	0.684	0.600	0.239	0.250	0.370	0.075
	210	0.083	0.145		0.047	0.167						0.026	0.053		0.028	0.018	0.022	0.050

Table 6.3 continued

Locus	Allele/n	ACT	ACTF	ACTH	ACTP	ACT?	South Australia	SAF	SAH	Tasmania	TASP	Victoria	VICF	VICH	Western Australia	WAF	WAH	WAP
B01-CANN1	N	109	31	20	43	15	44	25	19	8	8	39	19	20	71	28	23	20
	317	0.289	0.274		0.477	0.167	0.318	0.360	0.263	0.250	0.250	0.308	0.368	0.250	0.063	0.018	0.152	0.025
	323											0.013		0.025				
	326	0.523	0.419	0.675	0.500	0.600	0.443	0.500	0.368	0.375	0.375	0.577	0.526	0.625	0.373	0.446	0.370	0.275
	329	0.156	0.242	0.300	0.012	0.200	0.216	0.100	0.368	0.375	0.375	0.051	0.053	0.050	0.338	0.304	0.413	0.300
	332	0.005			0.012							0.026		0.050	0.042	0.036	0.022	0.075
	335														0.014			0.050
	338						0.023	0.040										
	341														0.014		0.043	
	344	0.018	0.032	0.025		0.033						0.026	0.053		0.021			0.075
	362	0.005	0.016															
	371	0.005	0.016												0.134	0.196		0.200

Locus	Allele/n	ACT	ACTF	ACTH	ACTP	ACT?	South Australia	SAF	SAH	Tasmania	TASP	Victoria	VICF	VICH	Western Australia	WAF	WAH	WAP
ANUCS302	N	109	31	20	43	15	44	25	19	8	8	39	19	20	71	28	23	20
	133											0.013		0.025	0.007	0.018		
	139	0.170	0.210	0.225	0.047	0.367	0.193	0.180	0.211			0.013		0.025	0.317	0.250	0.304	0.425
	145	0.248	0.065	0.200	0.488		0.034	0.060				0.026		0.050	0.106	0.250		0.025
	148	0.005	0.016												0.042	0.107		
	151	0.128	0.194	0.100	0.093	0.133	0.205	0.340	0.026	0.500	0.500	0.256	0.316	0.200	0.035		0.109	
	154	0.450	0.516	0.475	0.372	0.500	0.568	0.420	0.763	0.500	0.500	0.692	0.684	0.700	0.493	0.375	0.587	0.550

Table 6.3 continued

Locus	Allele/n	ACT	ACTF	ACTH	ACTP	ACT?	South Australia	SAF	SAH	Tasmania	TASP	Victoria	VICF	VICH	Western Australia	WAF	WAH	WAP
ANUCS305	N	109	31	20	43	15	44	25	19	8	8	39	19	20	71	28	23	20
	142	0.206	0.210	0.075	0.221	0.333	0.159	0.080	0.263			0.064		0.125	0.366	0.536	0.217	0.300
	145	0.170	0.274	0.200	0.081	0.167	0.182	0.200	0.158	0.063	0.063	0.333	0.342	0.325	0.070	0.125	0.043	0.025
	148	0.206			0.512	0.033												
	154	0.417	0.516	0.725	0.186	0.467	0.659	0.720	0.579	0.938	0.938	0.603	0.658	0.550	0.563	0.339	0.739	0.675

Locus	Allele/n	ACT	ACTF	ACTH	ACTP	ACT?	South Australia	SAF	SAH	Tasmania	TASP	Victoria	VICF	VICH	Western Australia	WAF	WAH	WAP
C11-CANN1	N	109	31	20	43	15	44	25	19	8	8	39	19	20	71	28	23	20
	152	0.028	0.081			0.033						0.051		0.100	0.113	0.143		0.200
	155	0.619	0.516	0.925	0.593	0.500	0.614	0.580	0.658	0.438	0.438	0.487	0.447	0.525	0.437	0.482	0.457	0.350
	158	0.261	0.403	0.050	0.198	0.433	0.364	0.380	0.342	0.563	0.563	0.462	0.553	0.375	0.282	0.286	0.326	0.225
	161														0.007			0.025
	164	0.078			0.186	0.033	0.011	0.020							0.007	0.018		
	167						0.011	0.020							0.106	0.071	0.174	0.075
	176	0.014		0.025	0.023										0.049		0.043	0.125

Locus	Allele/n	ACT	ACTF	ACTH	ACTP	ACT?	South Australia	SAF	SAH	Tasmania	TASP	Victoria	VICF	VICH	Western Australia	WAF	WAH	WAP
B05-CANN1	N	109	31	20	43	15	44	25	19	8	8	39	19	20	71	28	23	20
	236	0.417	0.306	0.100	0.733	0.167	0.352	0.300	0.421	0.500	0.500	0.526	0.632	0.425	0.162	0.125	0.152	0.225
	239	0.399	0.387	0.650	0.233	0.567	0.386	0.460	0.289	0.438	0.438	0.423	0.289	0.550	0.345	0.286	0.543	0.200
	242	0.179	0.306	0.225	0.035	0.267	0.261	0.240	0.289	0.063	0.063	0.051	0.079	0.025	0.472	0.589	0.261	0.550
	245	0.005		0.025											0.021		0.043	0.025

Table 6.3 continued

Locus	Allele/n	ACT	ACTF	ACTH	ACTP	ACT?	South Australia	SAF	SAH	Tasmania	TASP	Victoria	VICF	VICH	Western Australia	WAF	WAH	WAP
ANUCS501	N	109	31	20	43	15	44	25	19	8	8	39	19	20	71	28	23	20
	78	0.009				0.067												
	88	0.569	0.548	0.400	0.733	0.367	0.364	0.300	0.447	0.250	0.250	0.513	0.447	0.575	0.711	0.893	0.652	0.525
	93	0.234	0.161	0.500	0.186	0.167	0.307	0.380	0.211			0.013	0.026		0.077	0.054	0.130	0.050
	98	0.188	0.290	0.100	0.081	0.400	0.330	0.320	0.342	0.750	0.750	0.474	0.526	0.425	0.211	0.054	0.217	0.425

Locus	Allele/n	ACT	ACTF	ACTH	ACTP	ACT?	South Australia	SAF	SAH	Tasmania	TASP	Victoria	VICF	VICH	Western Australia	WAF	WAH	WAP
B02-CANN2	N	109	31	20	43	15	44	25	19	8	8	39	19	20	71	28	23	20
	164	0.537	0.435	0.500	0.709	0.300	0.659	0.740	0.553	0.688	0.688	0.474	0.447	0.500	0.514	0.607	0.500	0.400
	167	0.239	0.290	0.475	0.023	0.433	0.068	0.040	0.105	0.063	0.063	0.128	0.079	0.175	0.380	0.375	0.217	0.575
	170	0.005		0.025														
	173	0.220	0.274		0.267	0.267	0.273	0.220	0.342	0.250	0.250	0.397	0.474	0.325	0.106	0.018	0.283	0.025

Locus	Allele/n	ACT	ACTF	ACTH	ACTP	ACT?	South Australia	SAF	SAH	Tasmania	TASP	Victoria	VICF	VICH	Western Australia	WAF	WAH	WAP
ANUCS303	N	109	31	20	43	15	44	25	19	8	8	39	19	20	71	28	23	20
	142	0.073		0.275	0.047	0.033						0.038		0.075	0.007		0.022	
	145	0.541	0.774	0.500	0.337	0.700	0.909	0.960	0.842	1.000	1.000	0.936	1.000	0.875	0.556	0.411	0.609	0.700
	148														0.092	0.107		0.175
	151	0.385	0.226	0.225	0.616	0.267	0.091	0.040	0.158			0.026		0.050	0.345	0.482	0.370	0.125

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