Research Scheme R7238

Molecular markers in Tropical Forestry

Final Report

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Stephen A. Harris

Oxford Forestry Institute,
Department of Plant Sciences,
University of Oxford,
South Parks Road,
Oxford,
OX1 3RB

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Executive Summary

The project had three aims: (i) to identify the types of information that are necessary for the effective dissemination and utilisation of molecular information; (ii) to identify the user groups of such information; (iii) to assess the potential value of a manual on the application of molecular marker technologies in tropical trees.

29 replies were received from the 168 letters sent. Of these 29 replies, 24 organisations had an interest in the use of molecular markers, and of these 13 used them in their research or management. The most popular marker systems were randomly amplified polymorphic DNA (RAPDs) and allozymes. Most laboratories used a single technique, but if two or more techniques were to be combined then allozymes and RAPDs were the preferred choice.

Eleven of the organisations that used molecular markers were interested in the application of markers for the study of genetic diversity. Other uses for the markers were much less frequent and included clone identification, systematic studies and marker-aided selection. The majority of organisations were interested in single questions. The numbers of staff employed by each of the organisations in the field of molecular markers varied from 1 to 16, and ranged from Ph.D. level to technicians. Of the organisations, 46.2% had staff with degrees, 84.6% with staff that had learnt via taught courses and four (30.8%) had staff that had been trained by both degrees and courses. A limited range of, often out-dated, manuals are being used by organisations. Limitations on the use of molecular markers by those organisation that are currently using them are the result of funding, information, facilities and personnel.

Of those organisations that did not use molecular markers, but had an interest in them, 10 considered that they had some knowledge of molecular markers. The knowledge that was available had been obtained almost equally from courses and reading. Eight of the organisations were interested in using markers to look at genetic diversity. Six organisations stated that they are planning to use molecular markers in the next five years. One organisation stated that they were not planning on using molecular markers. Six organisations indicated that they were planning to analyse genetic diversity and in five of these cases it was ranked as the most important application for molecular markers.

Statements of intentions were received from 22 organisations. Ten had no intentions of installing or upgrading laboratory facilities. Two organisations had either recently upgraded or were satisfied with their facilities; in the latter case, any increase in technology needed would be met by using external collaborators. Eleven of the organisations were planning either to install or upgrade laboratories in the next five years. Five organisations considered that Government sources would provide capital funding, and that projects would provide training and running costs, whilst two others considered that Development Agencies would cover costs. One institution considered that projects would provide both capital and training/running costs. From the questionnaire responses it is unclear whether organisations have a clear idea of the full capital costs associated with the establishment of laboratory facilities, including the training of staff, the costs of equipment repair and up-grading and the safe disposal of the many toxic chemicals used in molecular procedures.

Molecular markers were considered to have a useful role in tropical forestry by 27

organisations. Two organisations indicated that molecular markers either had a limited role at the present time or that they were unsure of their role. The reasons for the limited role were associated with the lack of expertise, the difficulty of equipment procurement and the availability of funds.

21 organisations identified molecular markers as having an important role in understanding diversity and differentiation of tropical trees, whilst nine and six organisations identified mating systems and systematics respectively as important roles for molecular markers. 26 organisations considered that a manual was useful as a means of providing standard recipes for non-specialists and in order to be used for teaching and research purposes. Three organisations either questioned the need for a manual or did not have enough experience to provide a considered opinion. Three areas were identified as being particularly important for inclusion within a molecular marker manual; methodology, interpretation and analysis, with a focus on tree-specific problems and case studies.

Techniques that would appear to be of greatest interest for coverage are the DNA-based techniques of RAPDs, amplified fragment length polymorphisms (AFLPs) and microsatellites and allozyme analysis. However, the techniques being used may not be the most appropriate for the questions being addressed. The choice of techniques will be governed by two factors, the facilities available and the questions being addressed. Any manual must contain marker systems that have been 'tried-and-tested', are simple to use and generate high quality data quickly. Given the range of interests that most organisations have, the most useful methods of analysis would be those associated with allozymes, polymerase chain reaction restriction fragment length polymorphisms (PCR-RFLPs), RAPDs, AFLPs and microsatellites. The majority of users preferred paper as the medium for a manual and 17 of the organisations expressed an interest in commenting on an early draft of the manual.

The potential end-user community is primarily composed of researchers; few forest managers/decision makers appear to have an idea of the potential of such marker systems. The manual should: (i) be paper-based and cheap; (ii) self-contained, exploring methodology, interpretation and analysis of data and 'trouble-shooting'; (iii) contain basic, 'tried-and-tested' techniques; (iv) detailed discussions of advantages and disadvantages with appropriate references; (v) include worked examples of interpretation and analysis; (vi) include detailed case studies linked to real development situations; (vii) be written for the non-specialist, under the assumption of no previous knowledge; (viii) be heavily illustrated and referenced.

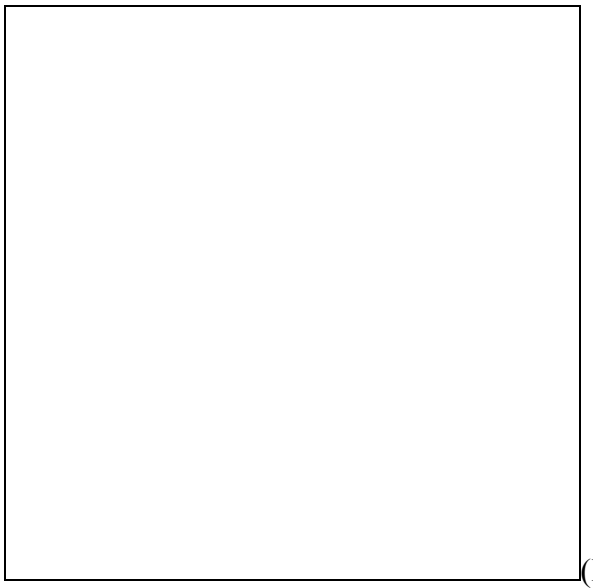
Such a manual could be approached in a number of different ways. The major source of interest is in genetic diversity studies, although other studies are of interest. A manual should have two major user groups; researchers and decision makers/managers. In the case of researchers it is necessary to highlight how studies are undertaken with particular marker systems and provide detailed methods for data generation, interpretation and analysis. Decision makers/managers do not need to have detailed knowledge of methodology, but they do need to know how to interpret molecular data and to have a realistic expectation of the outcome from these data sources. An understanding of the role of molecular data within the context of other data sources, for example, demography, reproductive biology and taxon distribution, is crucial.

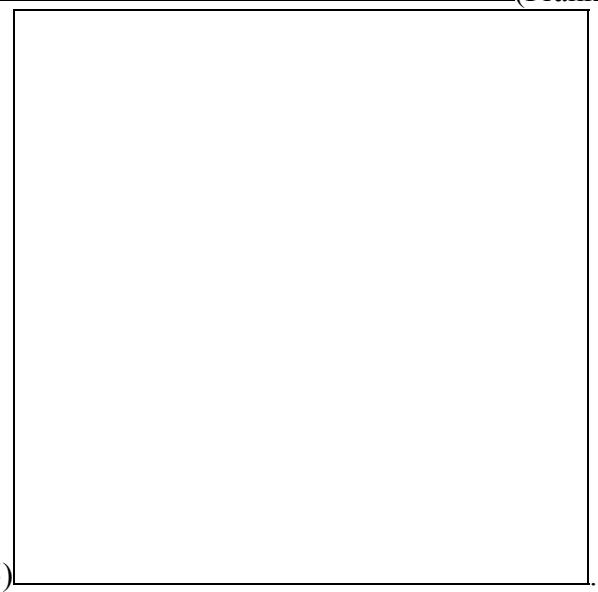
Within any manual there are two problematic situations: (i) initial training; and (ii) up-dating the manual. Training can be approached through the release of the manual in a

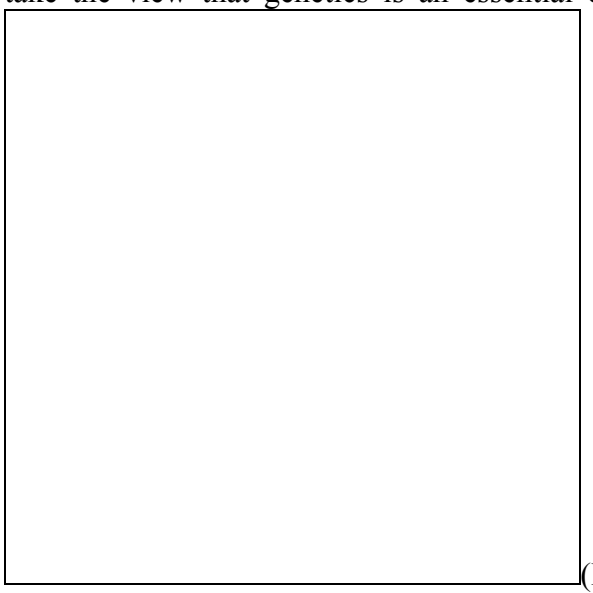
form that would give instructors a source of material, especially since the majority of organisations have well-qualified staff associated with their molecular programmes. In order to up date a manual, particularly in the application and evolution of different techniques (e.g. RAPDs), it would be useful to have either a web-site or list-server for such up-dates. A manual should provide information that will enable users to: (i) ask the correct questions; (ii) determine the genome to analyse; (iii) identify the suitable techniques available; (iv) determine which part of the genome to use.

1.0 Introduction.

Economic, social, ecological, cultural and aesthetic cases have been made for identification, quantification and understanding the distribution and relationships of biological diversity (Kunin and Lawton, 1996). Biological diversity may be assessed at three different levels; the community, the species and the gene

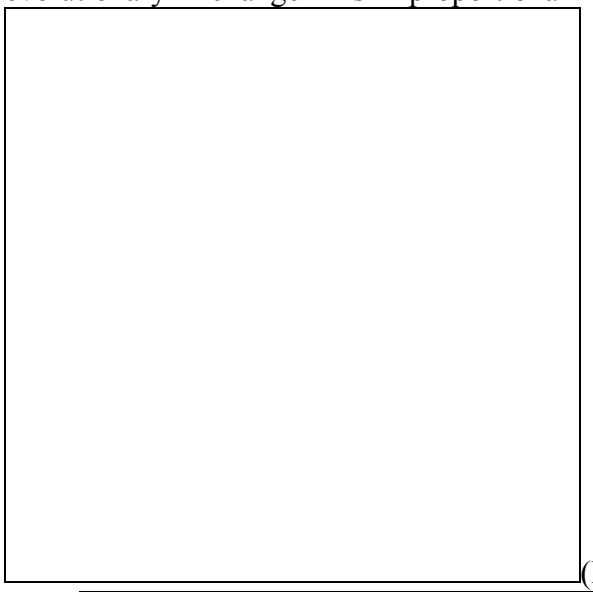
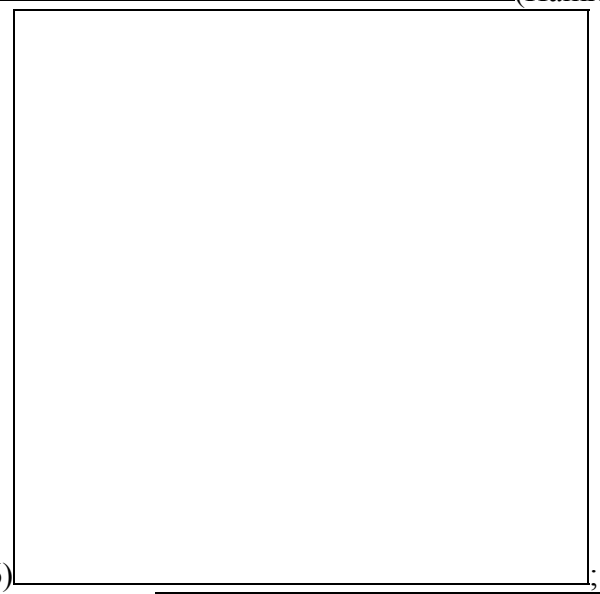
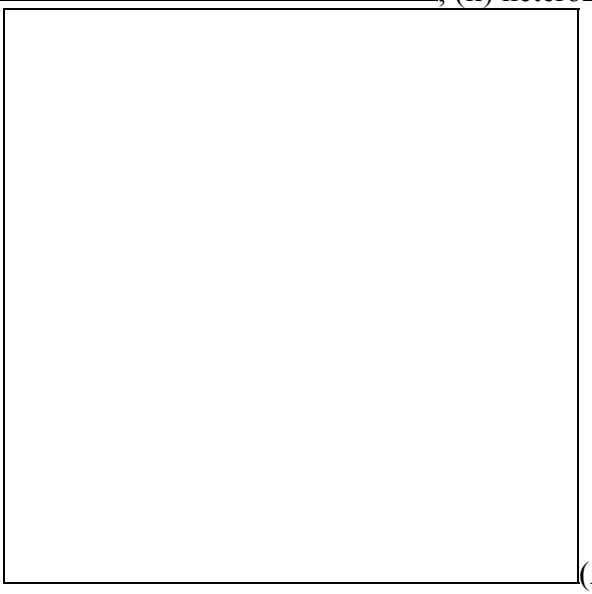
 (Frankel *et al.*,

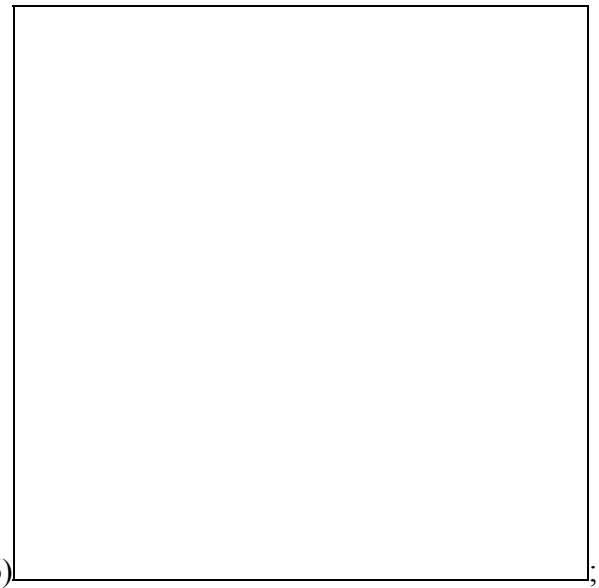
 1995). Whilst the importance of ecological and taxonomic diversity is recognised in conservation programmes, the value of genetic diversity is more controversial; the majority of researchers, either implicitly or explicitly, take the view that genetics is an essential component of any conservation programme

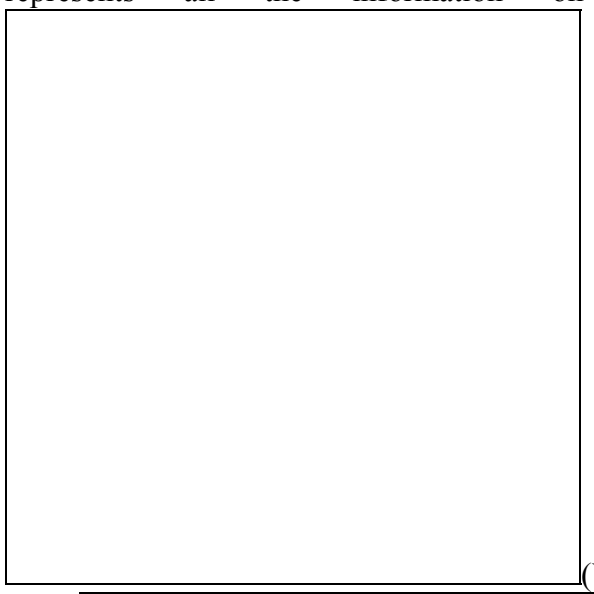
 (Falk and Holsinger, 1991; Hamrick and

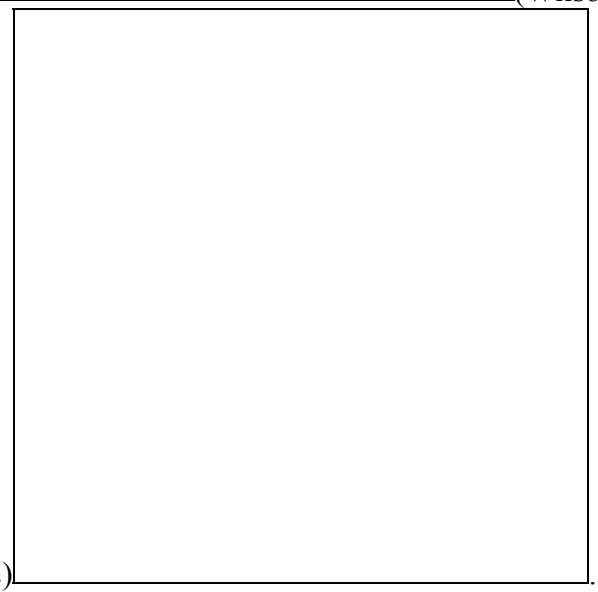
Godt, 1996), although others argue that organisms go extinct for ecological rather than for genetic reasons (Lande, 1988; Schemske *et al.*, 1994).

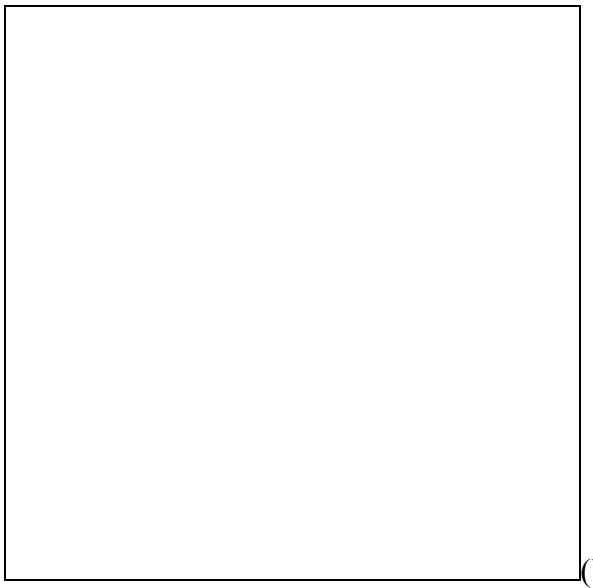
Interest in intraspecific genetic variation is primarily for three reasons: (i) the rate of

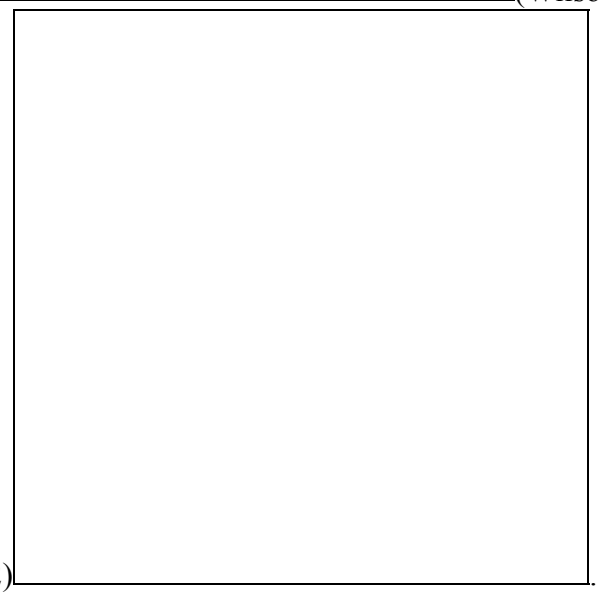
evolutionary change is proportional to the available genetic diversity  (Hamrick and Godt,  1996); (ii) heterozygosity is positively related  to fitness (Allendorf and Leary,

1986)  ; and (iii) the global gene pool represents all the information on the planet's biological processes

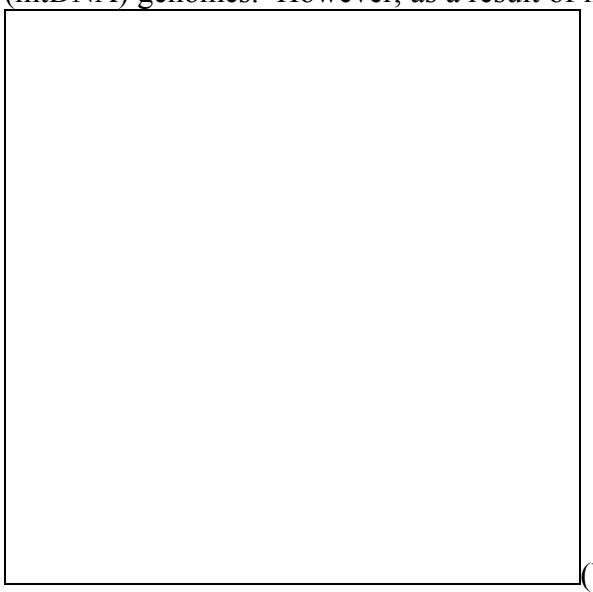
 (Wilson,

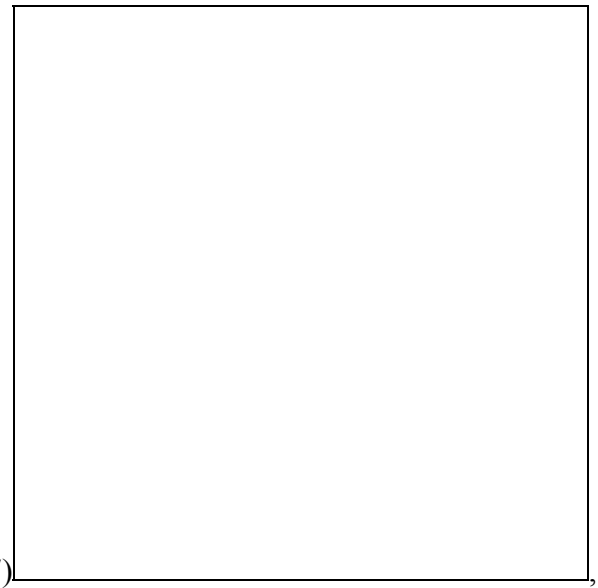
 1992). That is, loss of diversity is likely to decrease the ability of organisms to respond to environmental perturbation and discard anthropocentric biological information

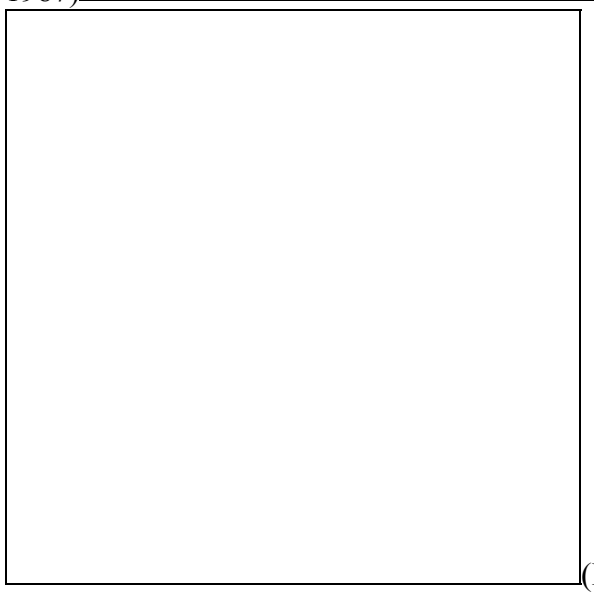
 (Wilson,

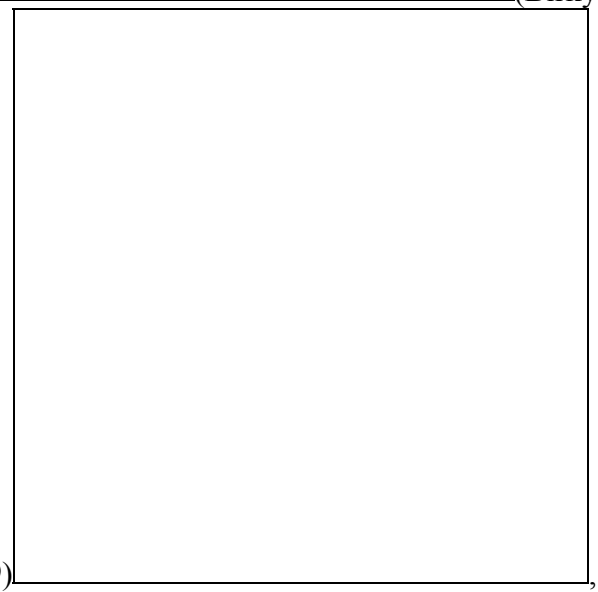
 1992).

Within a plant cell there are nuclear (nDNA), chloroplast (cpDNA) and mitochondrial (mtDNA) genomes. However, as a result of mutation rate variation among these genomes

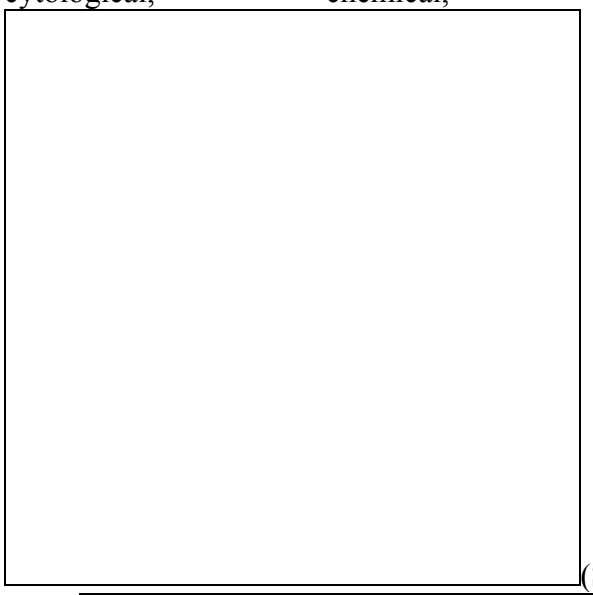
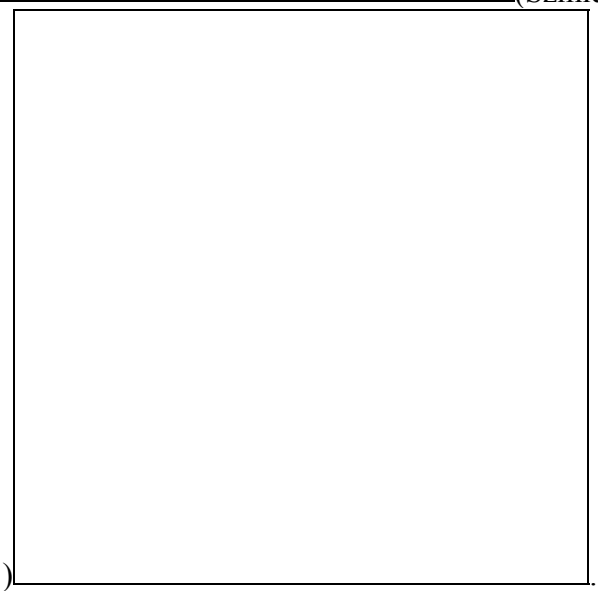
 (Wolfe *et al.*,

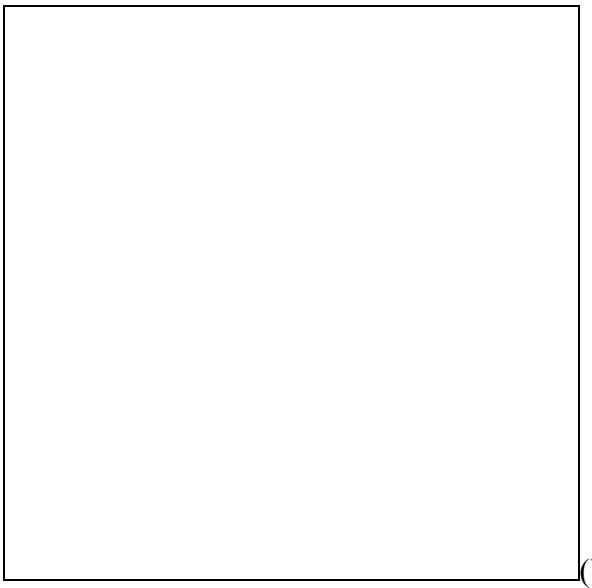
1987)  , and their different inheritance patterns

 (Birky *et al.*,

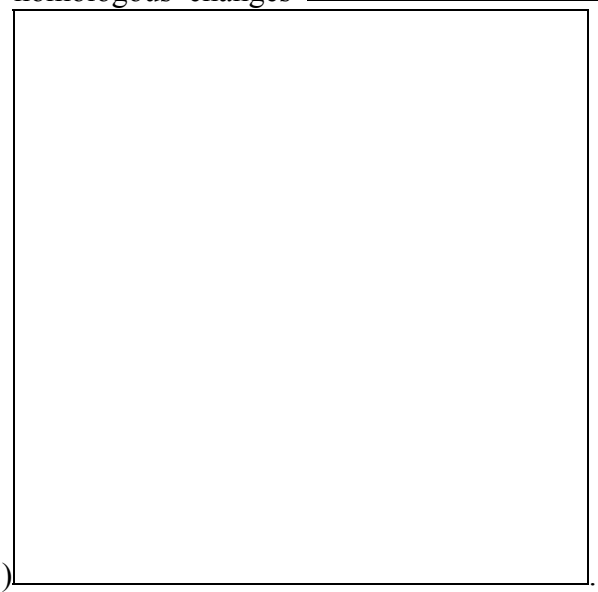
1989)  , markers associated with these genomes are suitable for different types of problem; genome-marker associations place constraints on the questions that may be addressed by a marker system.

1.1 Molecular markers.

Genetic markers are observable traits (the expression of which indicates the presence or absence of certain genes) that are classified into five broad groups: morphological, cytological, chemical, protein and DNA  (Szmidt and Wang,  1991). The characteristics of an ideal genetic marker are: detect qualitative or quantitative variation, show no environmental or developmental influences, show simple codominant inheritance, detect silent nucleotide changes, detect changes in coding and non-coding portions of the genome, detect

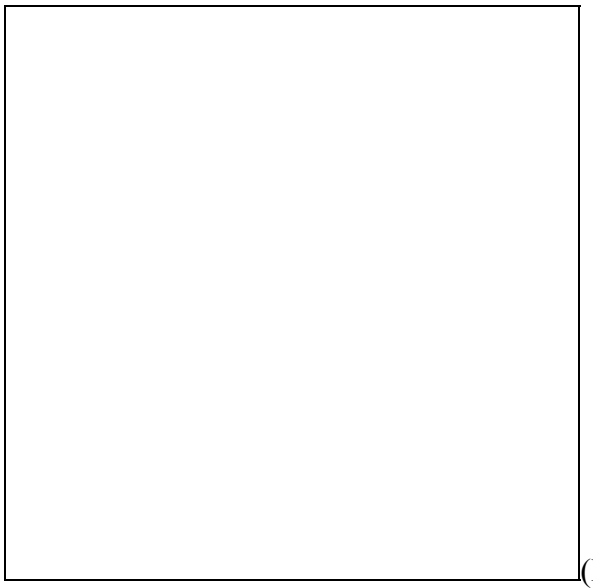
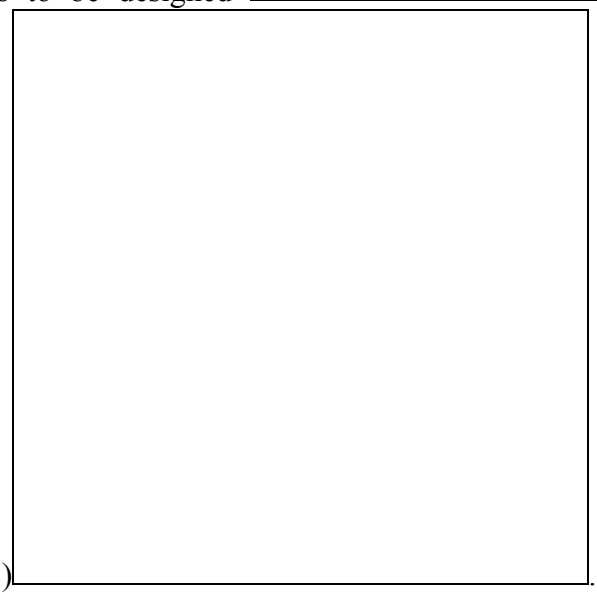
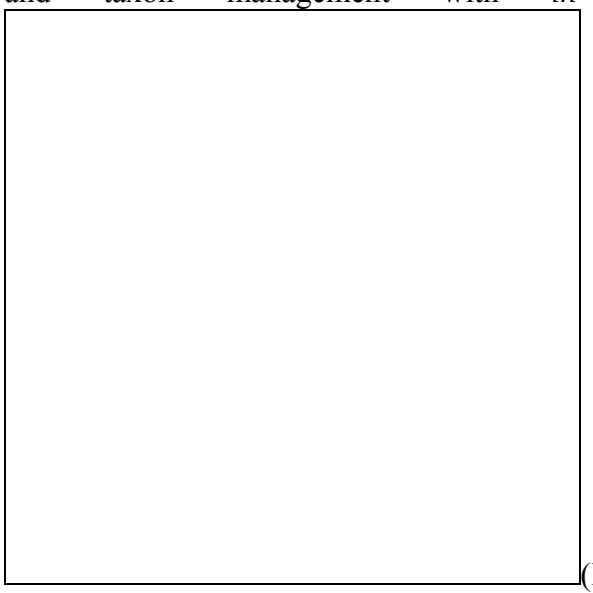


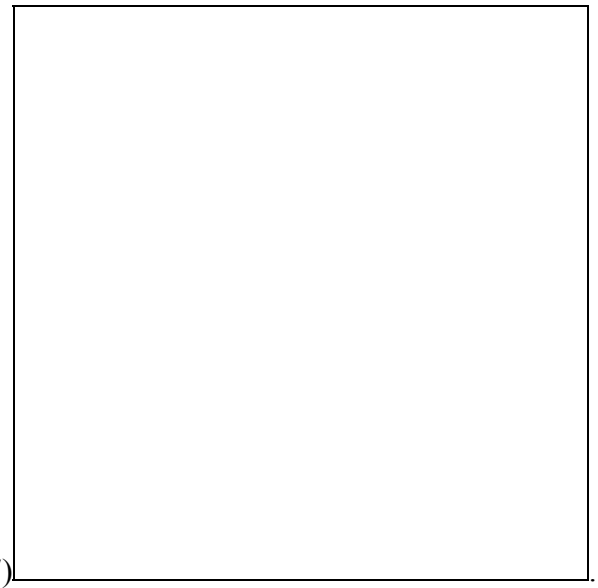
evolutionary homologous changes



(Weising *et al.*, 1995). Such a marker allows the possibility of unambiguously assigning a genotype to a taxon and then using these data either to estimate genetic variation present within and between populations or to compare taxa directly.

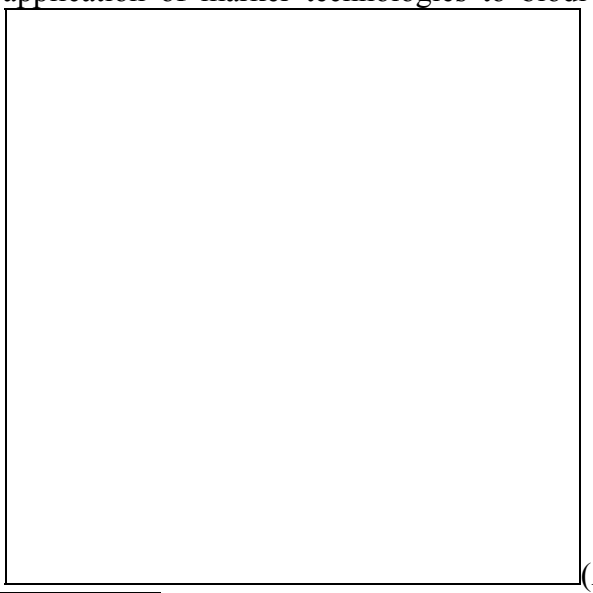
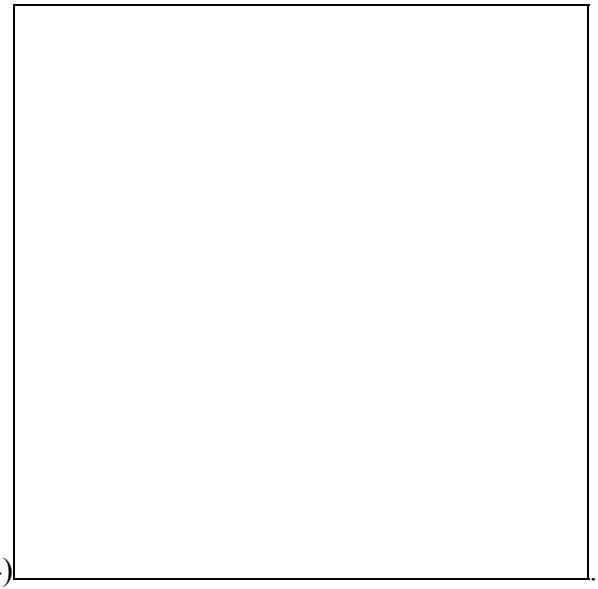
Efficient utilisation, improvement and conservation of taxa must be based on a sound understanding of: phylogeny; the amount and distribution of genetic variation; the design of effective sampling and conservation methods. Crucial to the success of long-term taxon management is an understanding of genetics and demography, enabling biologically

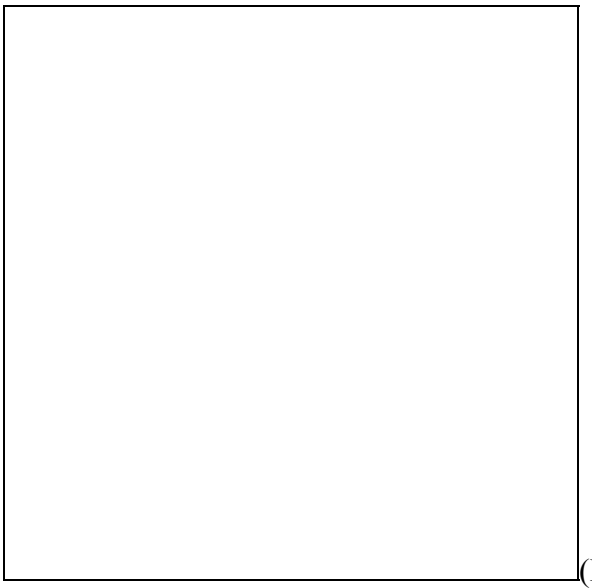
sound strategies to be designed  (Falk and  Holsinger, 1991). Such data are increasingly important in the development of integrated conservation strategies, combining population and taxon management with *in situ* and *ex situ* conservation  (Maxted *et al.*,


1997).

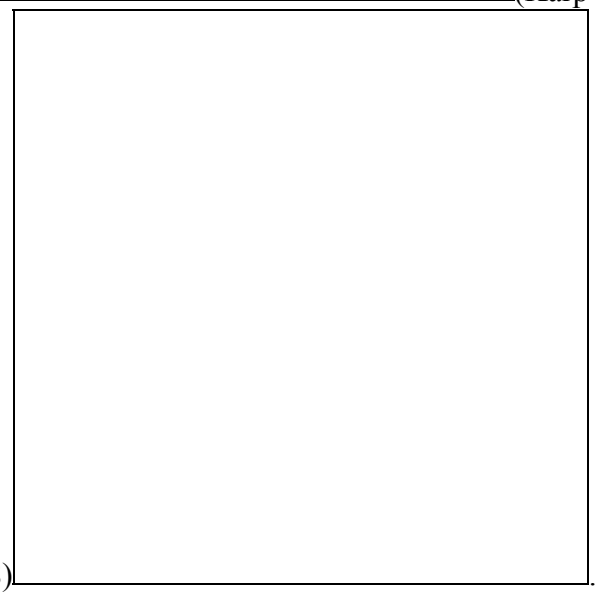
Biodiversity assessment has come to mean different things; the breeder is interested in variation within a particular collection or species' geographic range, whilst the evolutionary biologist is interested in populations and species and understanding the bases of diversity patterns.

1.2 Molecular marker systems.

The perceived importance of genetic variation and the availability of powerful marker systems has lead to the widespread application of marker technologies to biodiversity issues  (Avise, 1994)  . Molecular marker technologies may be broadly grouped into DNA-based and protein-based techniques (Table 1) and numerous publications are available that describe marker techniques in detail (e.g.

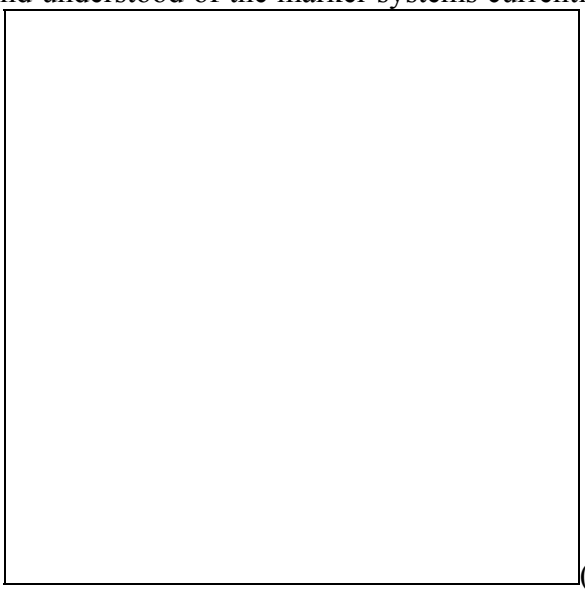


(Karp *et al.*,

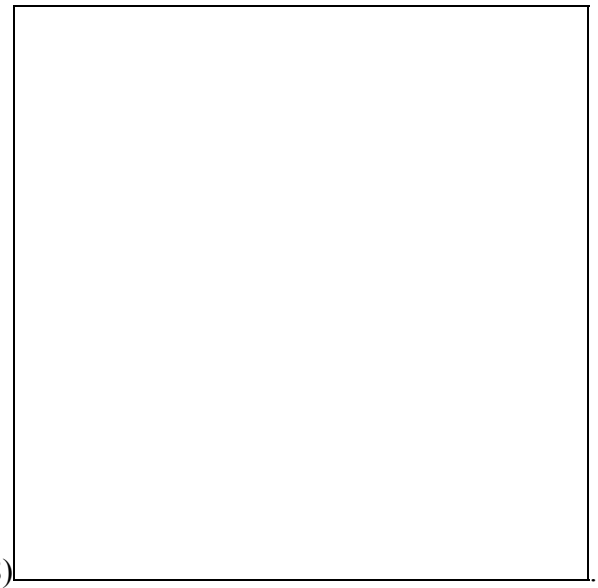


1998).

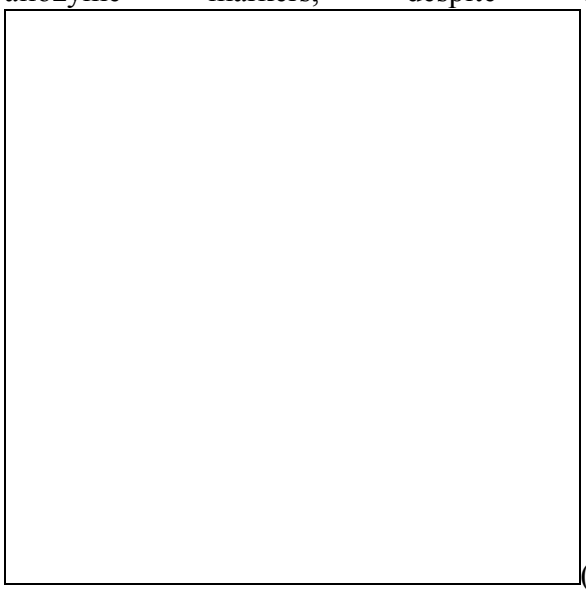
Allozymes are the most widely used and understood of the marker systems currently used



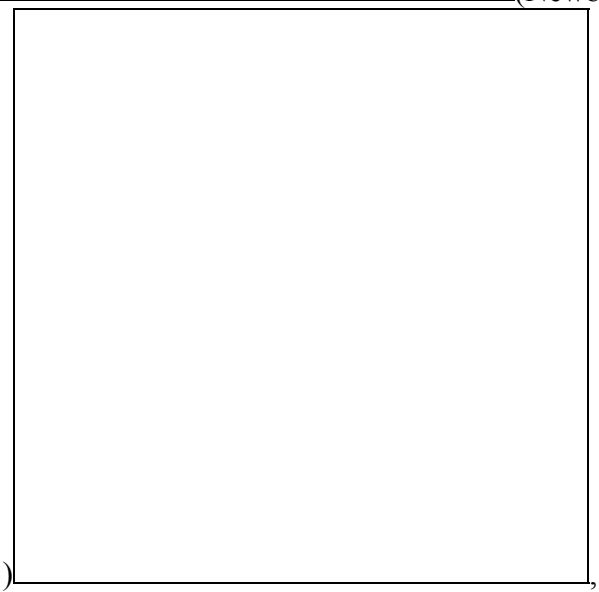
for characterising biological diversity (Butlin



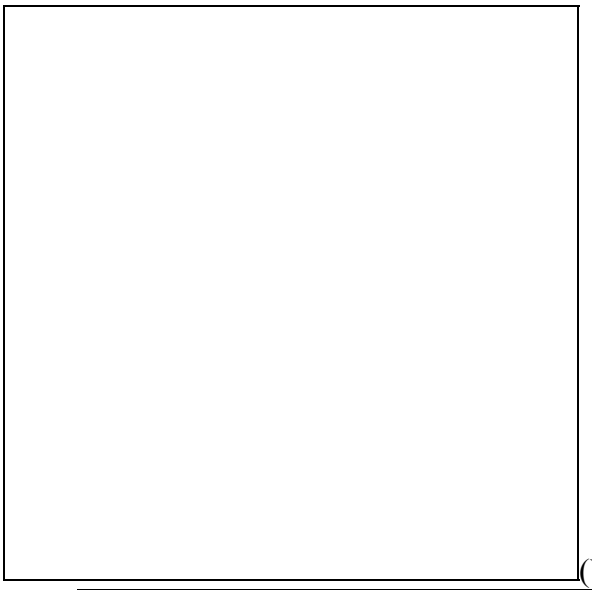
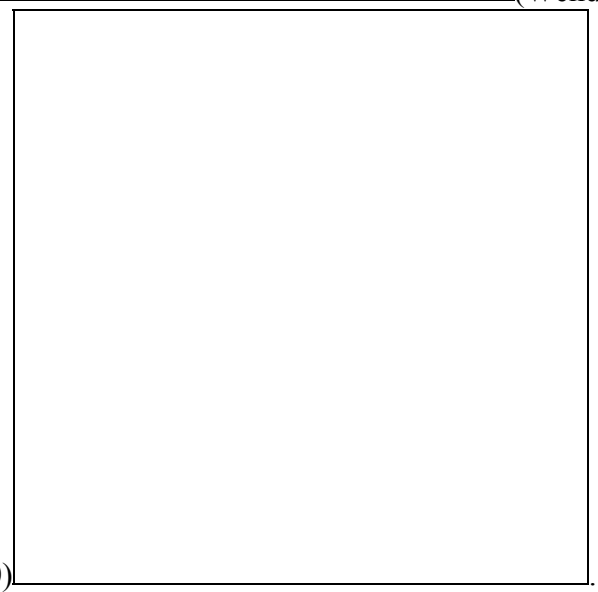
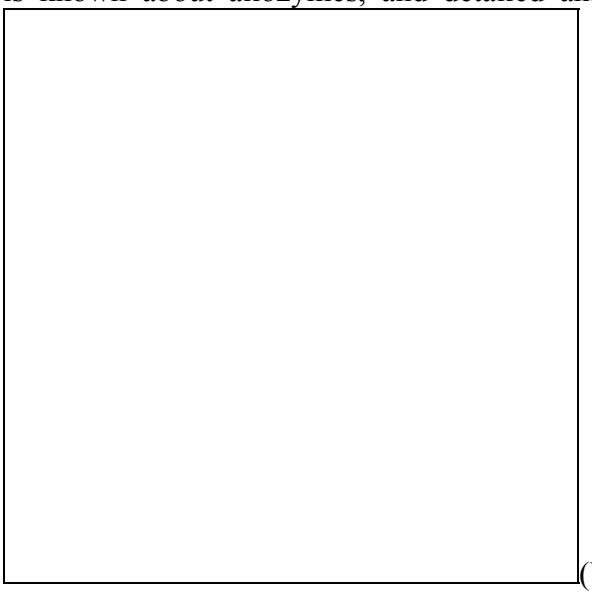
and Tregenza, 1998). Continued interest in allozyme markers, despite arguments against their use

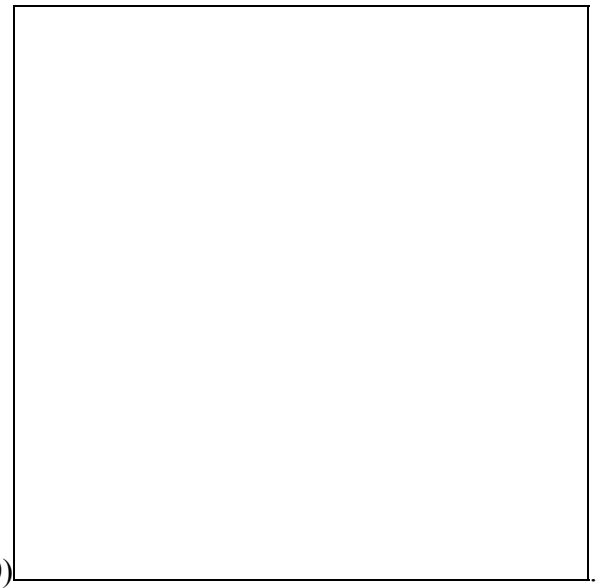


(Newbury and Ford-Lloyd,

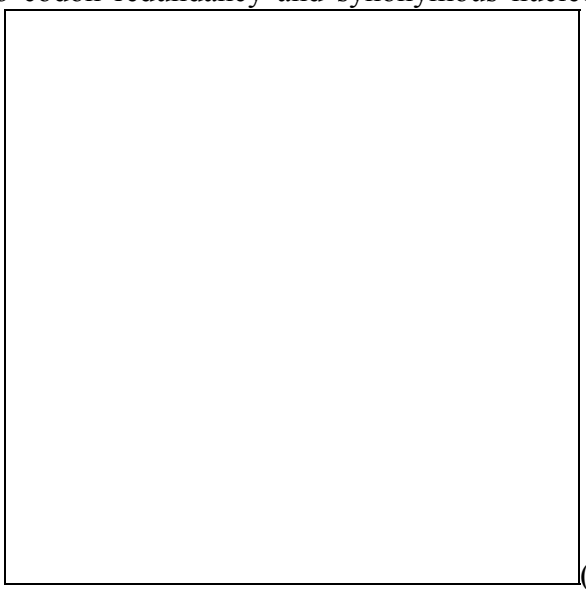


1993), is a result of their codominant expression in most species, cost effectiveness and simplicity

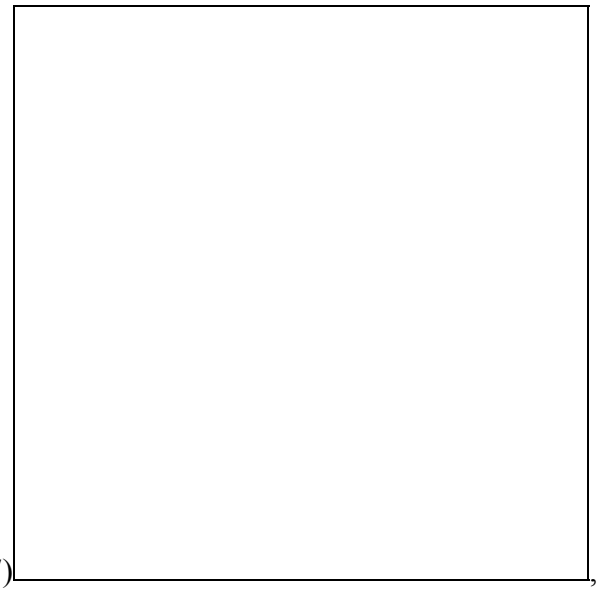
 (Wendel and Weeden,  1990). In addition, considerable information is known about allozymes, and detailed analyses of polyploid speciation are possible  (Weeden and Wendel,



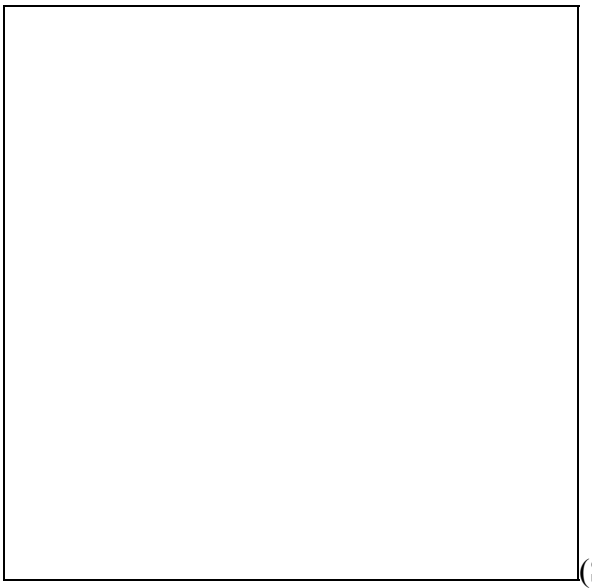
1990). However, allozymes only detect low levels of polymorphism in a limited range of water-soluble, nuclear-encoded enzymes, and gene variation is underestimated due to codon redundancy and synonymous nucleotide



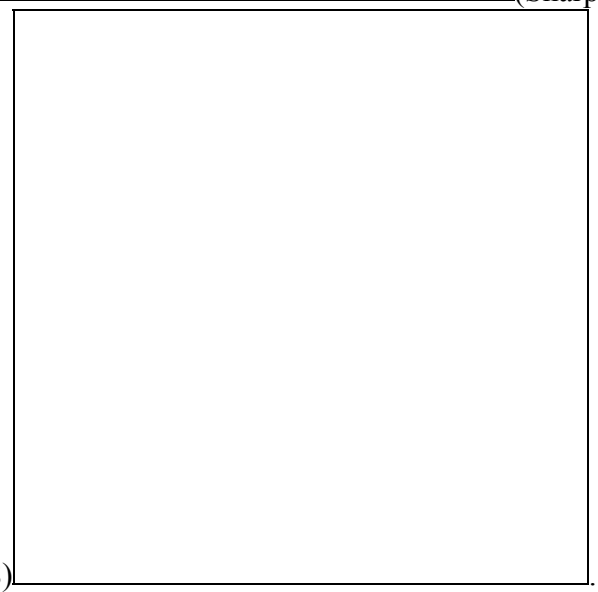
substitutions (Nei,



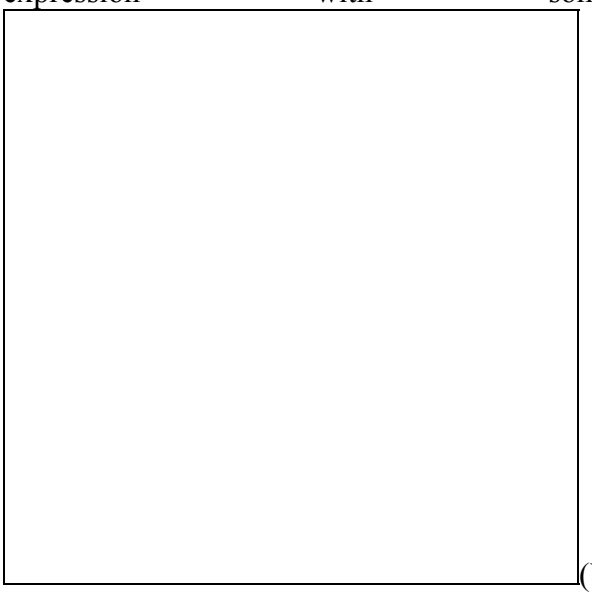
1987), although additional polymorphisms are often identified via isoelectric focusing



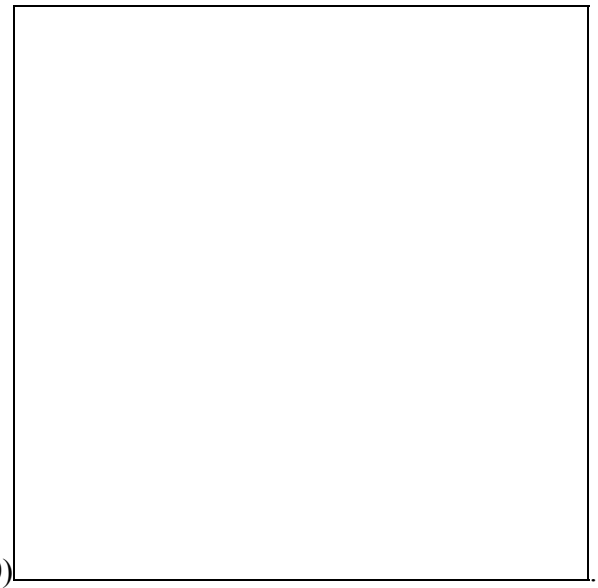
(Sharp *et al.*,



1988). Furthermore, fresh material must be used in allozyme analyses, and there are problems of environmental and ontogenetic expression with some enzyme systems

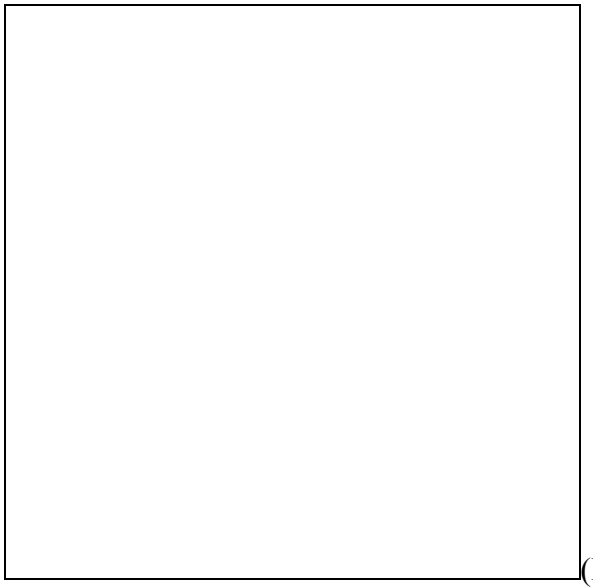


(Wendel and Weeden,

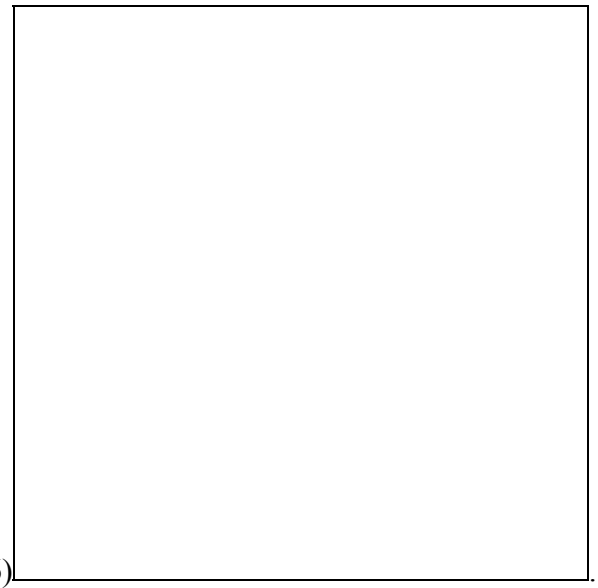


1990).

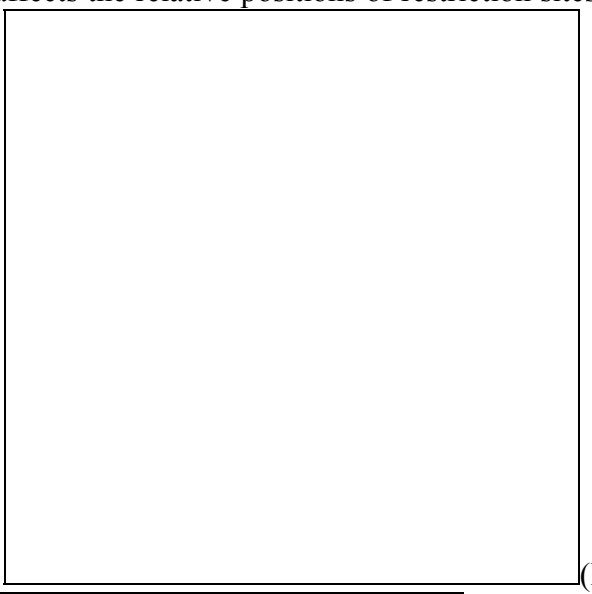
Restriction fragment length polymorphisms (RFLP) analysis uses restriction enzymes (REs) to detect variation in primary DNA structure, followed by Southern blotting and a suitable detection method to reveal the variation in any of the plant genomes



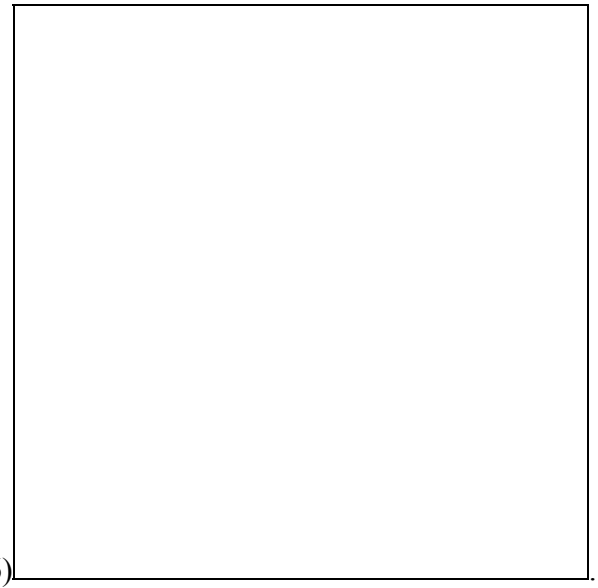
(Dowling *et al.*,



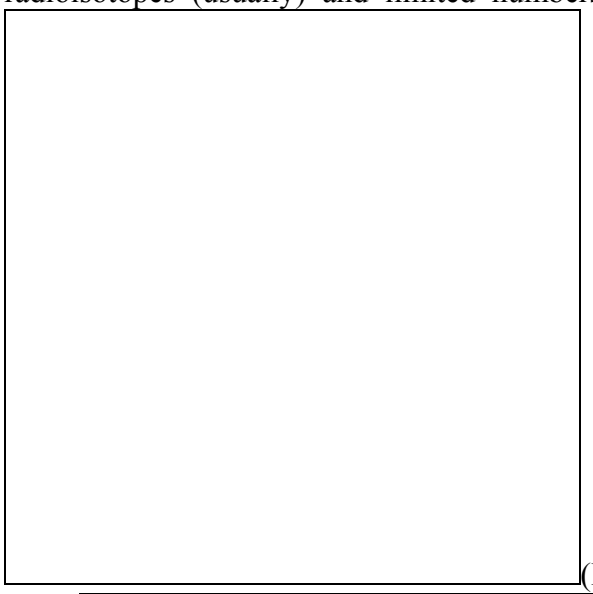
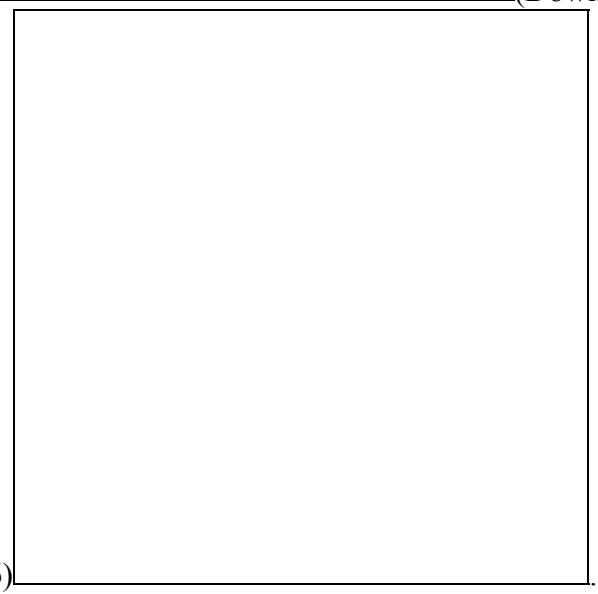
1996). RFLP analysis measures DNA variation that affects the relative positions of restriction sites and is usually codominant in



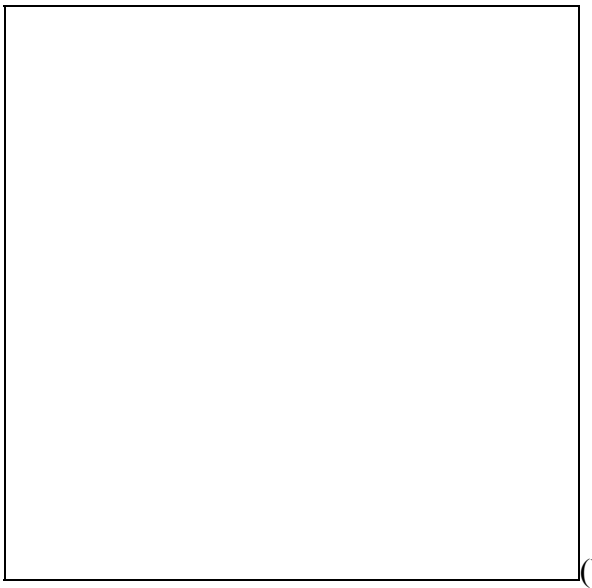
nDNA (Dowling *et al.*,

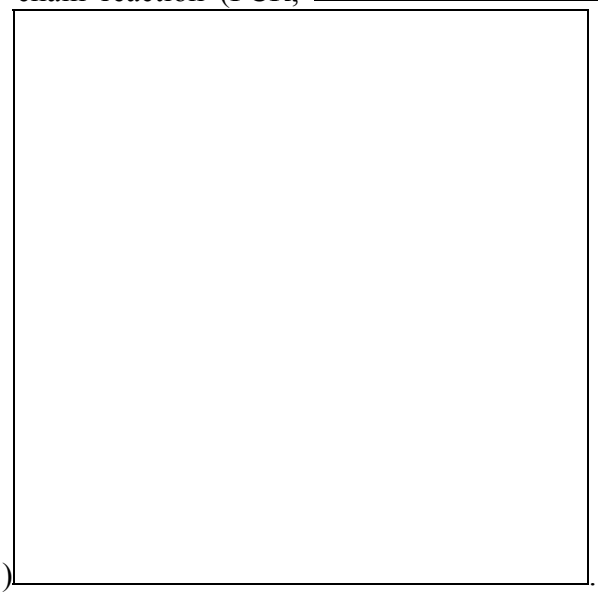


1996). Since DNA fragments migrate logarithmically, changes in large fragments are more difficult to detect than similar size changes in small fragments. RFLP analyses require large amounts of DNA, access to

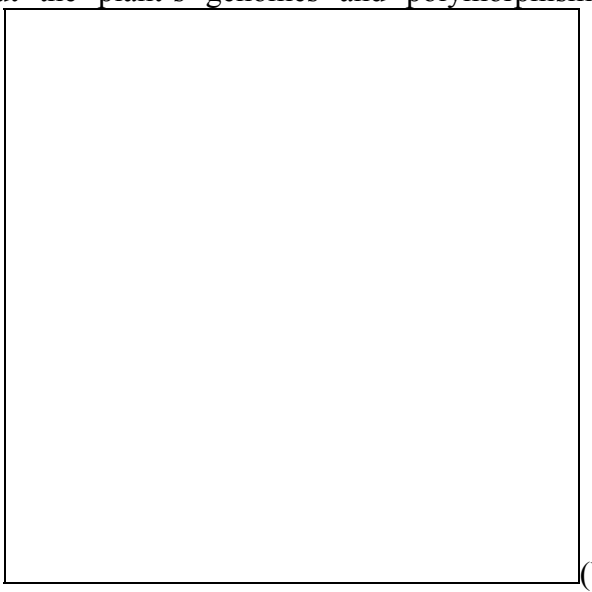
radioisotopes (usually) and limited numbers of suitable nDNA markers are available  (Dowling *et al.*,  1996). However, as with all other DNA-based methods dried leaves may be used as a source of DNA.

Randomly amplified polymorphic DNA (RAPD) analysis utilises single, arbitrary decamer DNA oligonucleotide primers to amplify regions of the genome using the

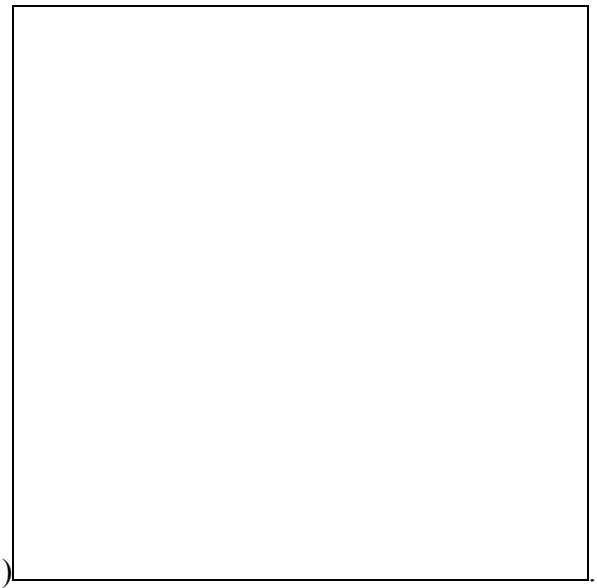
polymerase chain reaction (PCR;  (Williams



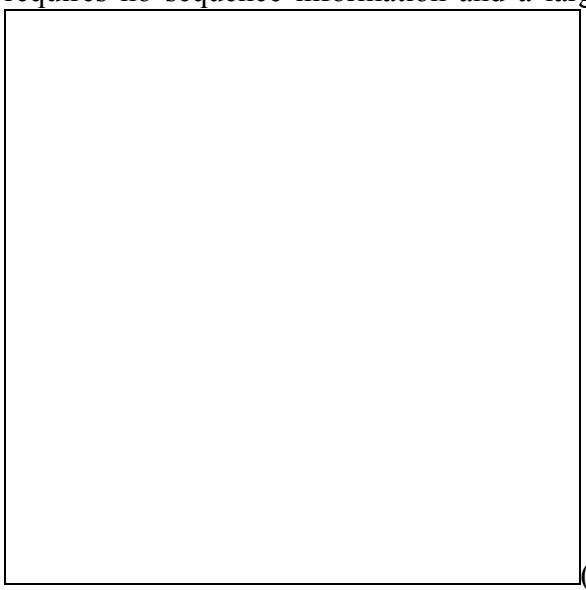
et al., 1993). Priming sites are thought to be randomly distributed throughout the plant's genomes and polymorphism results in



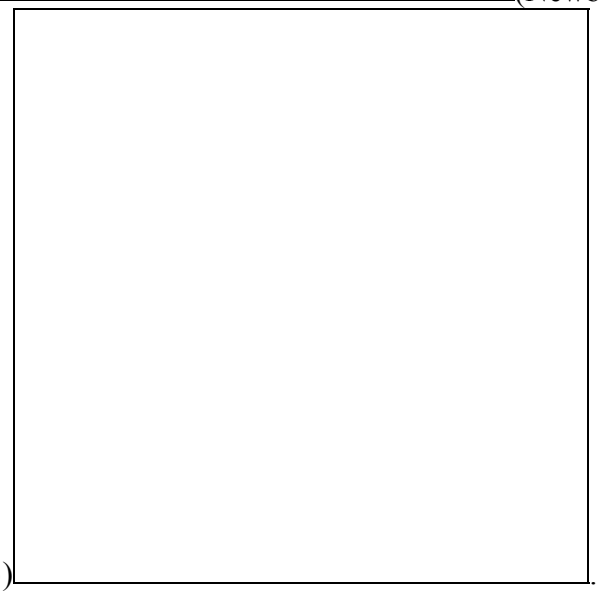
differing amplification products (Williams *et*



al., 1993). The technique is cheap, simple, requires no sequence information and a large number of putative loci may be screened

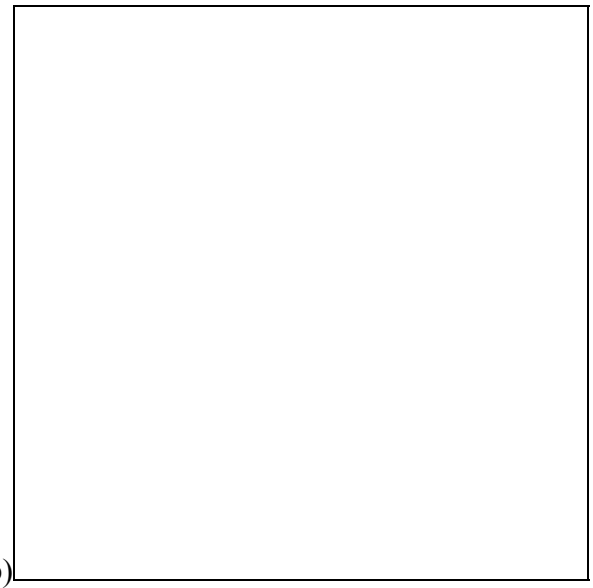


(Newbury and Ford-Lloyd,

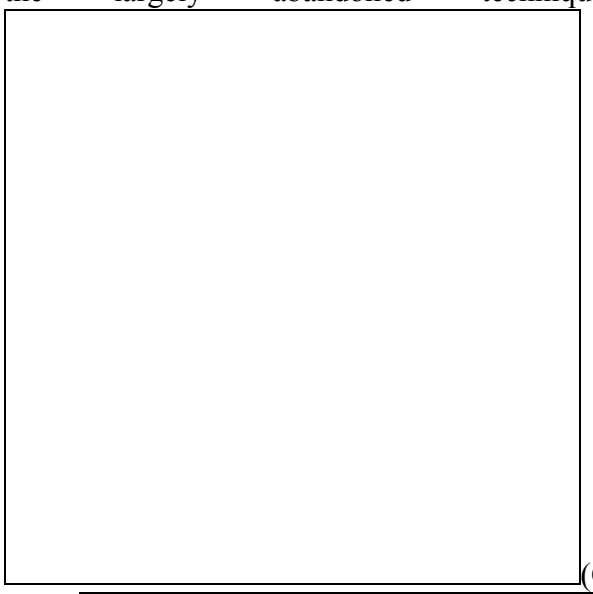


1993). However, the technique has been

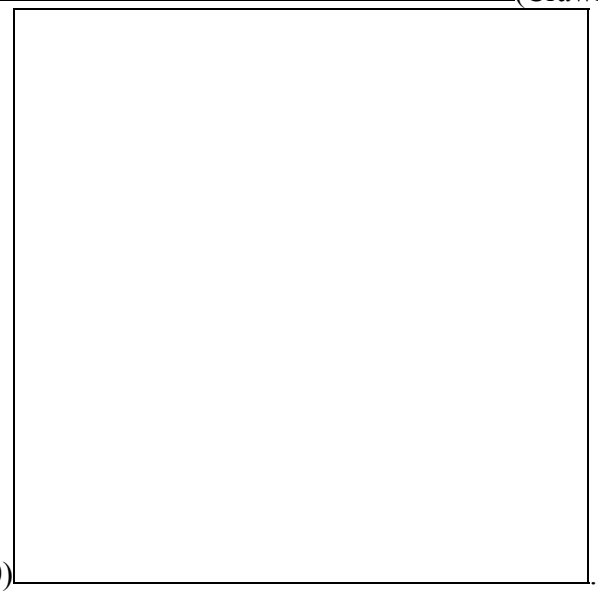
criticised on technical (Jones *et al.*, 1997) and theoretical (Harris, in press-



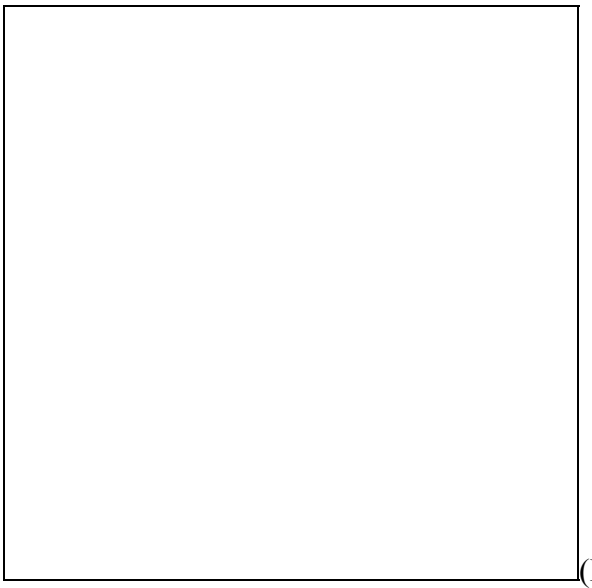
b) grounds, and in these respects is similar to the largely abandoned technique of total protein analyses



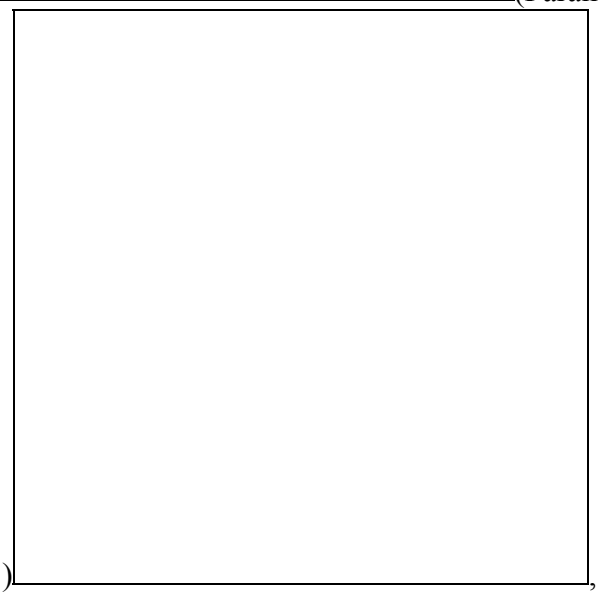
(Crawford,



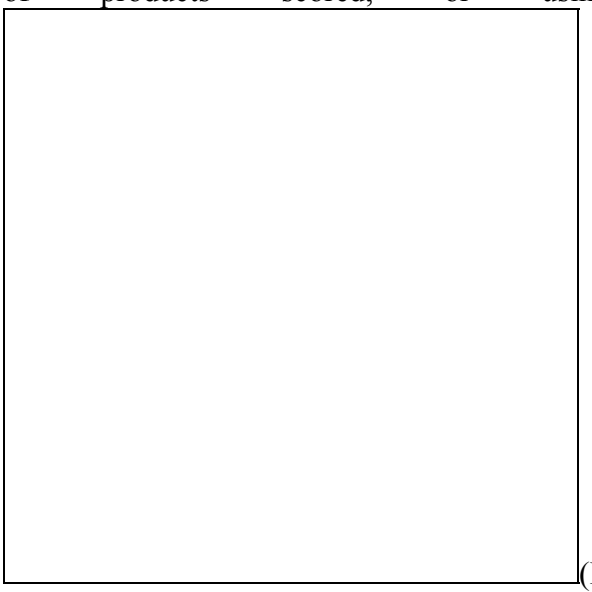
1990). Some of these criticisms may be overcome by the development of sequence characterised amplified regions (SCARs;



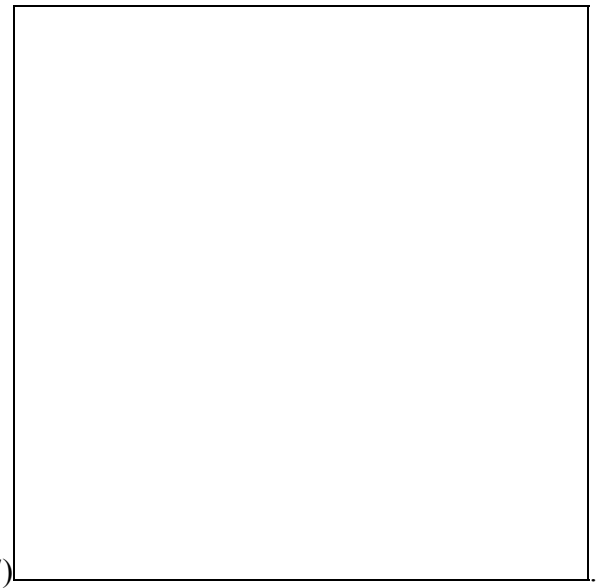
(Paran and Michelmore,

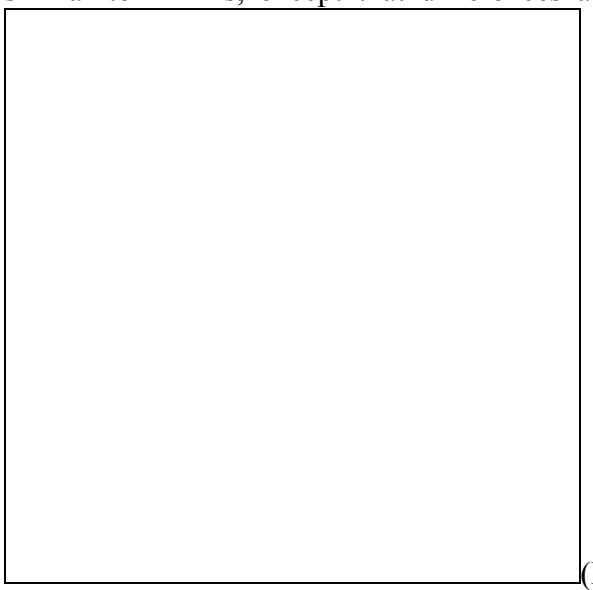
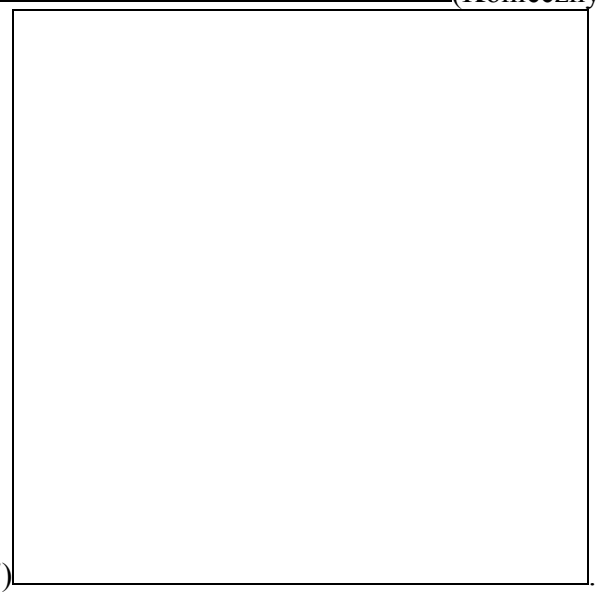


1993), but at the cost of reducing the number of products scored, or using very large sample sizes

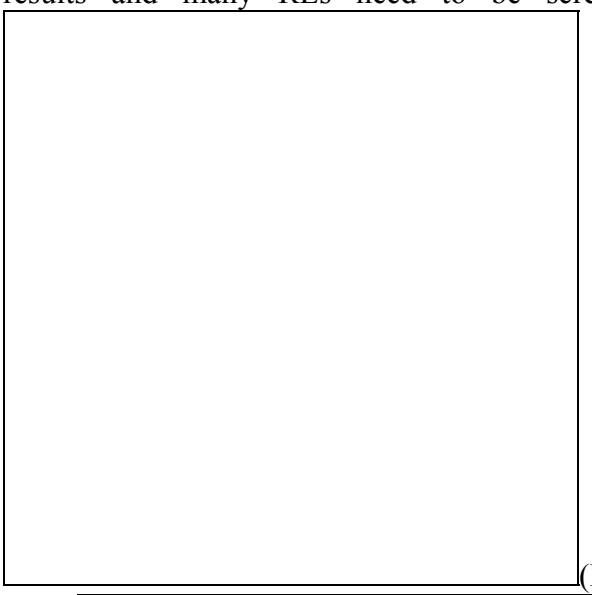


(Furman *et al.*,

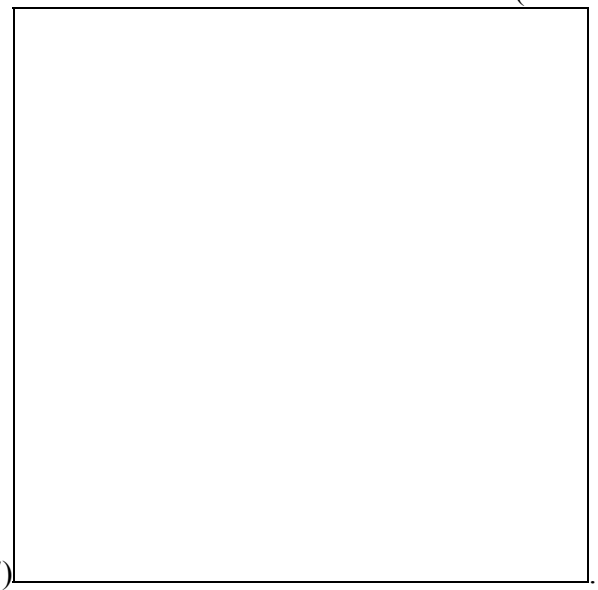
1997)  .

Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLPs) are similar to RFLPs, except that differences are visualised within specific PCR products  (Konieczny and Ausubel, 1993; Rafalski *et*  *al.*, 1997). The technique is cheap and

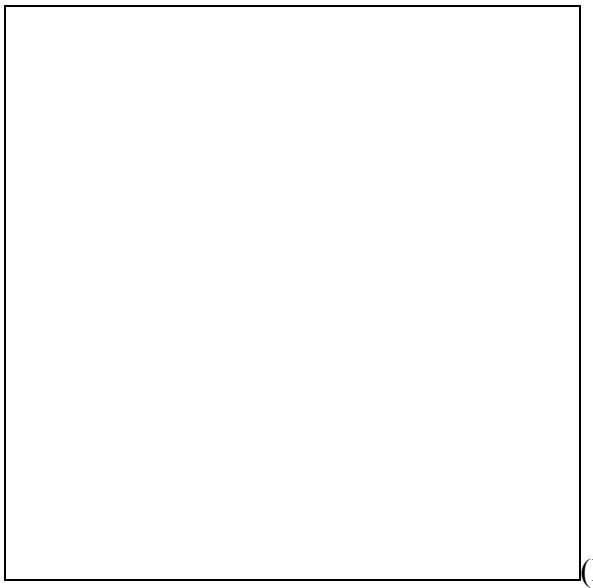
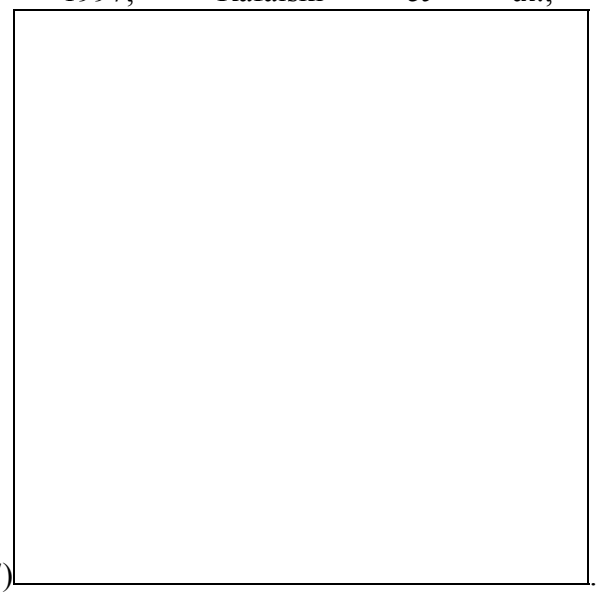
simple once suitable products have been identified, although information content of individual products may be low since short products (≤2kb) give the best amplification results and many REs need to be screened to identify suitable polymorphism

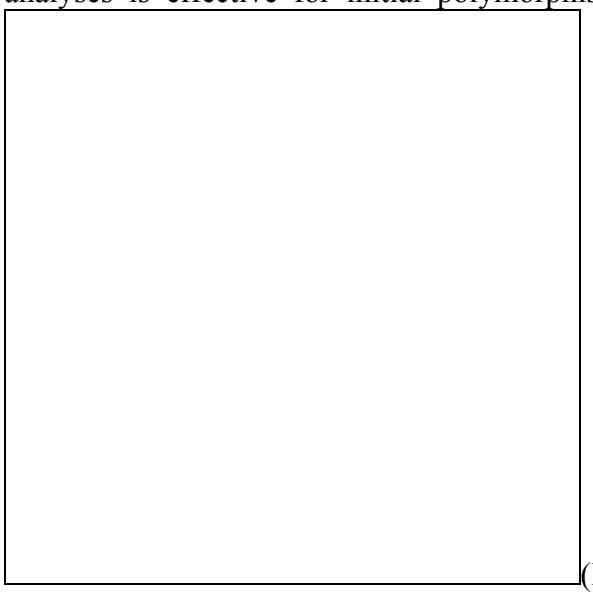


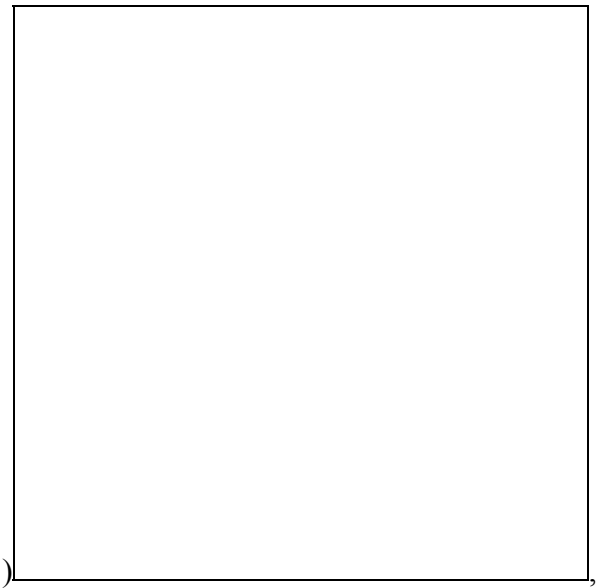
(Perez de la Rosa *et al.*, 1995; Rafalski *et al.*,



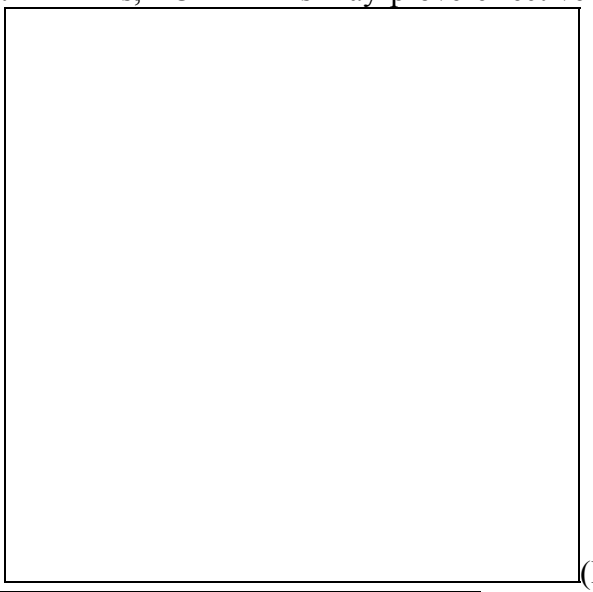
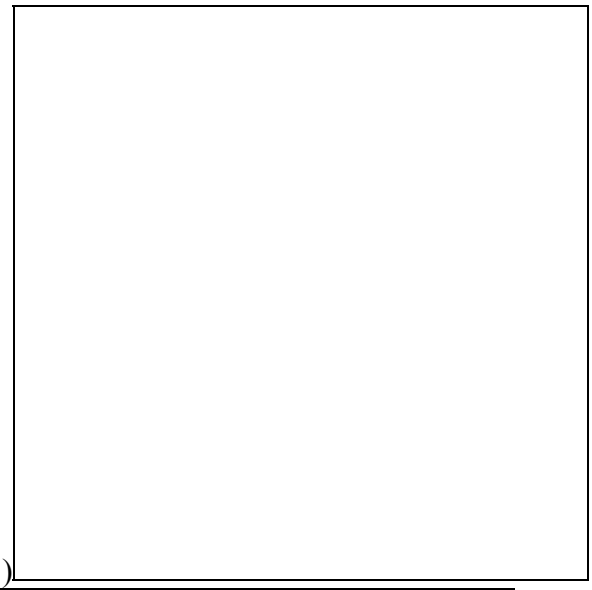
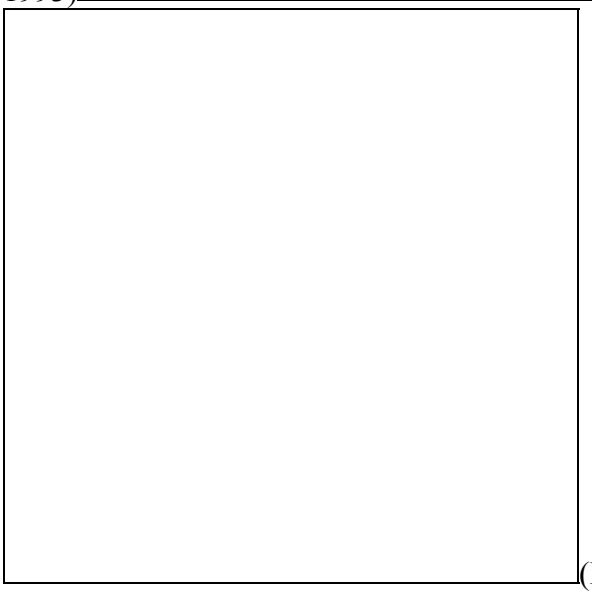
1997). Suitable primers may be designed from sequence databases, analysis of low copy number random clones and universal cpDNA, mtDNA and nDNA sequences

 (Demesure *et al.*, 1995; Dumolin-Lapegue *et al.*, 1997; Rafalski *et al.*, 1997; Strand *et al.*, 1997)  . Combining sequence and PCR-RFLP analyses is effective for initial polymorphism identification and subsequent screening

 (Ferris *et al.*,

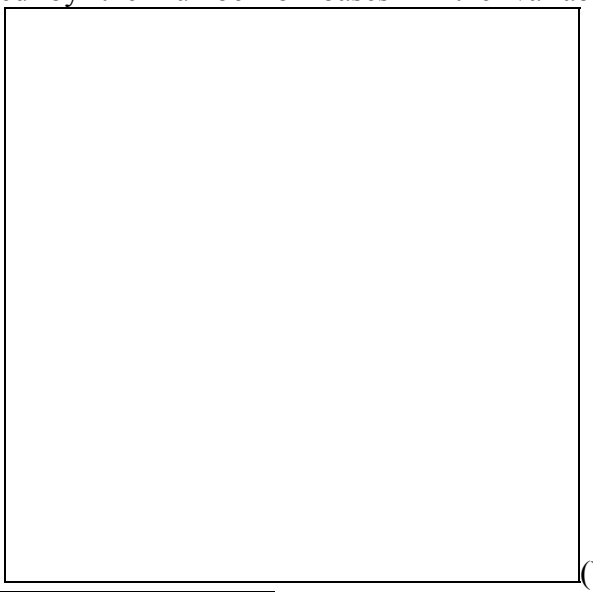
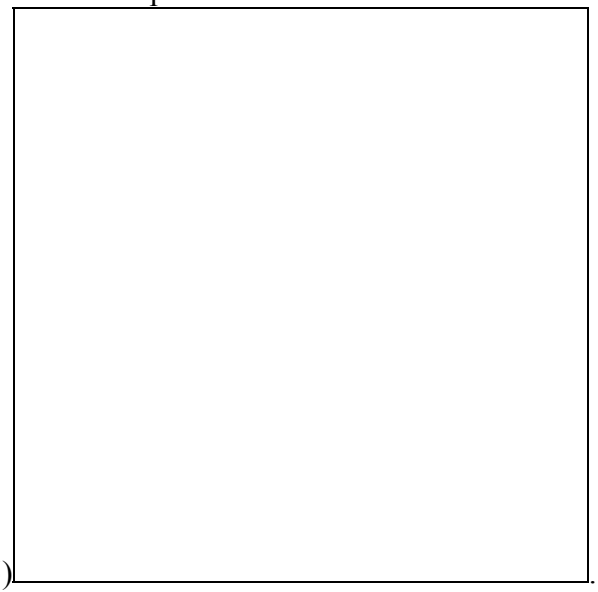


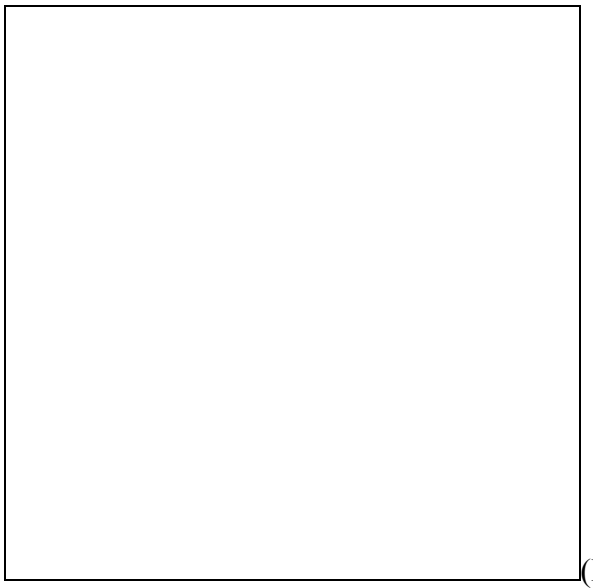
1993), whilst

combined with RAPDs, PCR-RFLPs may prove effective for identification of additional variation  (Paran and Michelmore, 1993)  or confirmation of RAPD band identity  (Rieseberg,

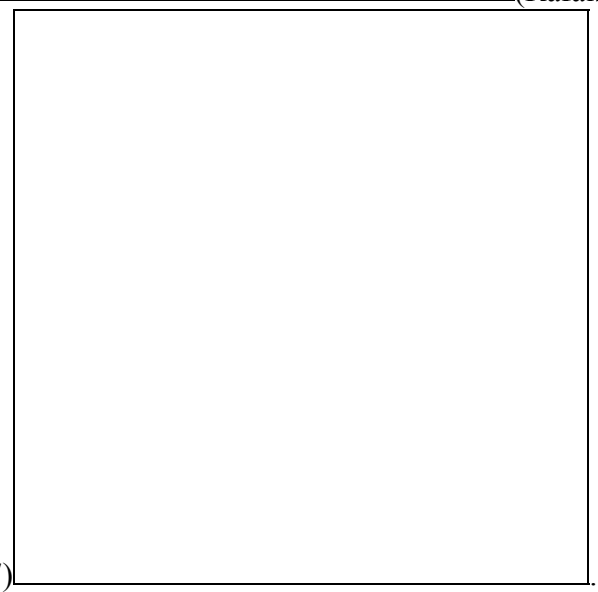
1996).

Amplified fragment length polymorphism (AFLP) analysis involves the selective amplification of an arbitrary subset of restriction fragments generated by single or double RE digestion of DNA (Vos *et al.*, 1995). Prior to amplification fragment ends

are modified by addition of double-stranded adapters, and during amplification pairs of end-labelled primers are used that span the adapter, the restriction site and one to three nucleotides of the fragment. Thus only fragments with ends of similar sequence to the primer's arbitrary sequence will be amplified. The number of bands generated in an AFLP reaction is determined by the number of bases in the variable part of the amplification primer  (Vos *et al.*, 1995)  . AFLPs are expected to be highly polymorphic, either dominant or codominant (although allelic relations may not be immediately obvious) and requires no prior sequence knowledge

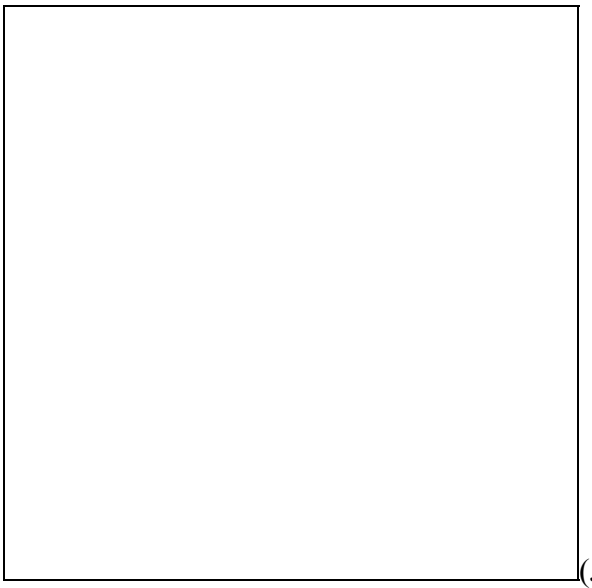
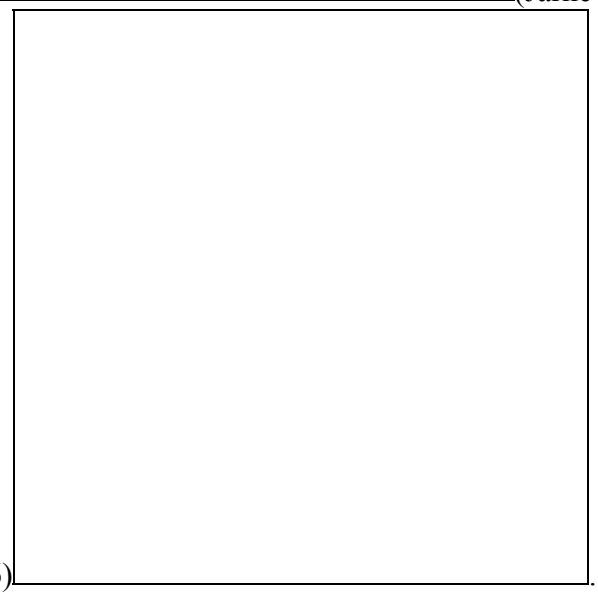
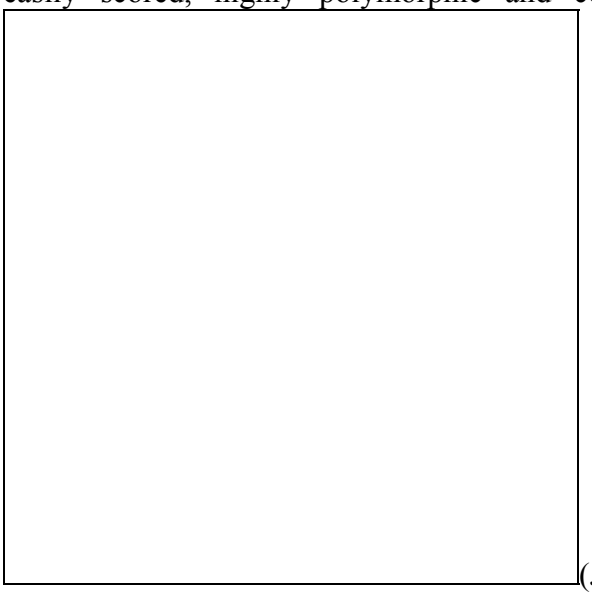


(Rafalski *et al.*,

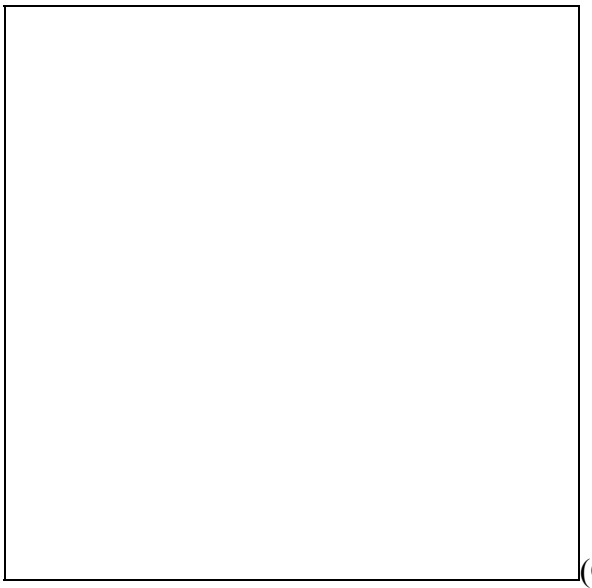
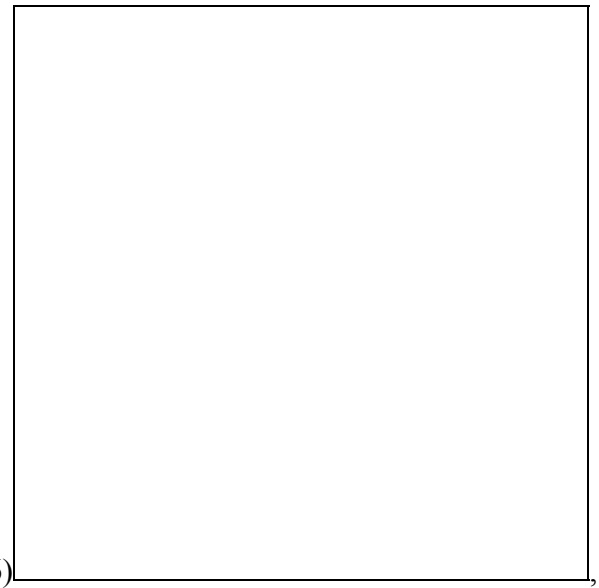
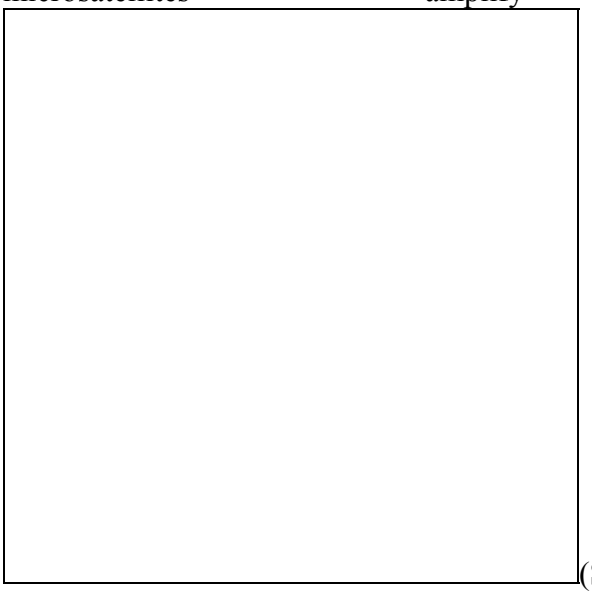


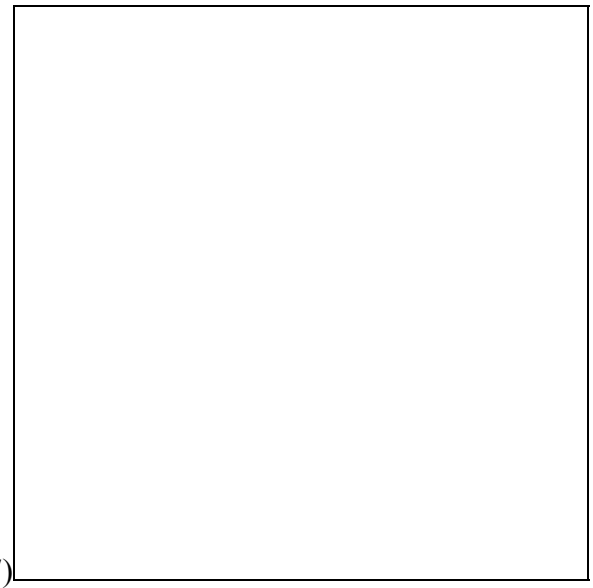
1997). However, the technique requires a high degree of technical skill, large amounts of high quality DNA and methylation insensitive REs.

Microsatellites (simple sequence repeats; SSRs) are short tandem repeats of mono- to tetra-nucleotide repeats which are assumed to be randomly distributed throughout the nDNA, cpDNA and mtDNA and are detected using specifically designed PCR primers


(Jarne and Lagode,

1996). SSRs are simple to detect, as either silver-stained or radio-labelled products, once suitable primers have been designed, are easily scored, highly polymorphic and codominant, in the case of nDNA SSRs

(Jarne and Lagode,

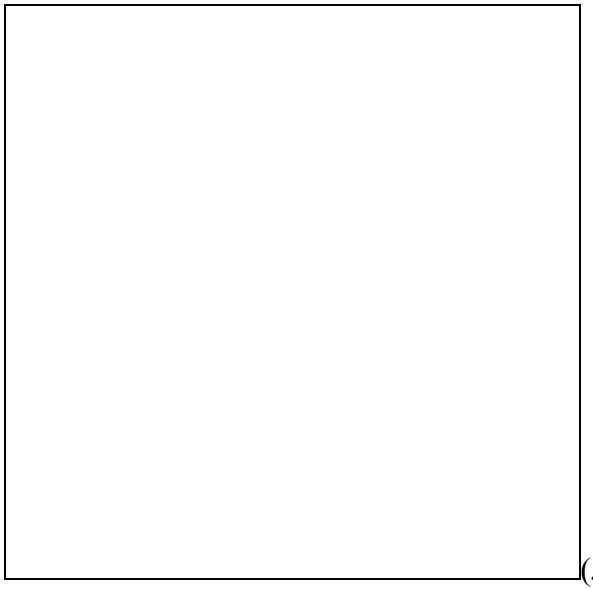
1996). Unfortunately, it is expensive to identify nDNA SSR primers and these do not generally amplify between species, although organelle SSR primers are easily identified from published sequences and appear to amplify many different taxa (Powell *et al.*, 1996). Technical modifications, through the

use of anchored microsatellites  (Charters *et al.*, 1996)  , and evidence that some nDNA microsatellites amplify across species  (Steinkellner *et al.*, 1997; White and Powell,

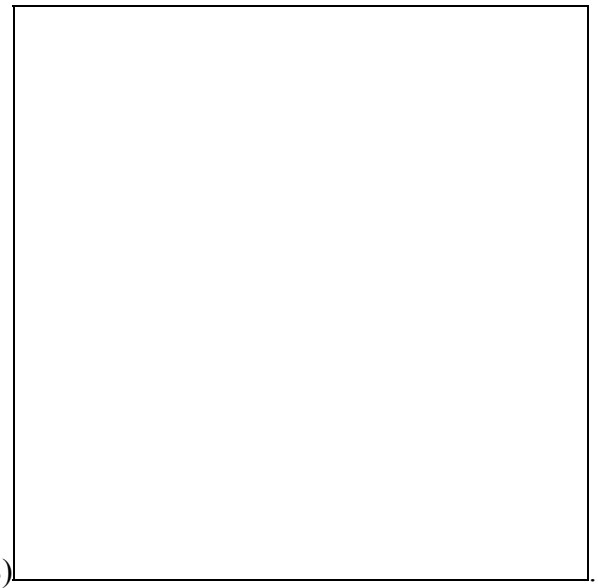
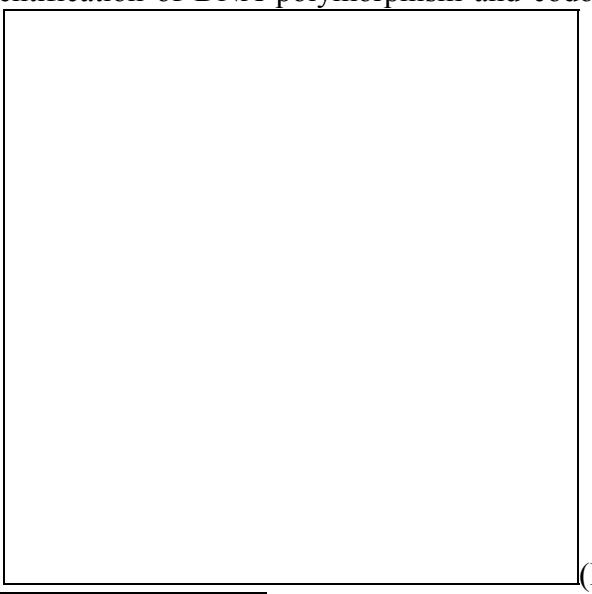
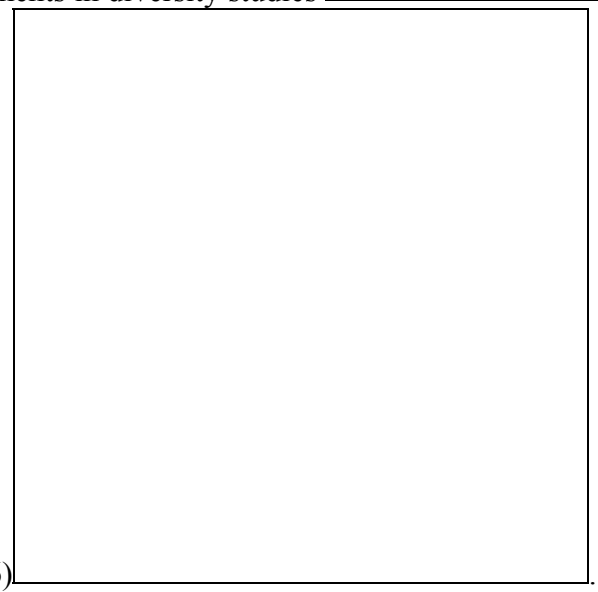


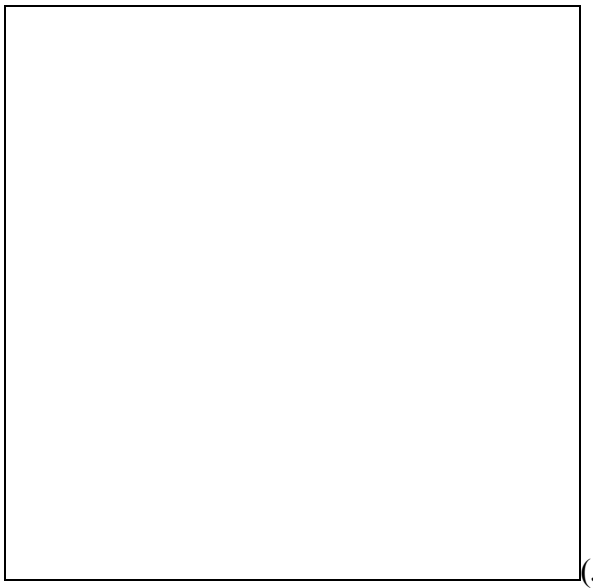
1997) eliminate some of these disadvantages.

Single-stranded conformation polymorphism (SSCP) analysis is based on the principle that single-stranded DNA molecules have specific sequence-based secondary structures under non-denaturing conditions; molecules with one or a few base differences may form conformations that result in different gel mobilities

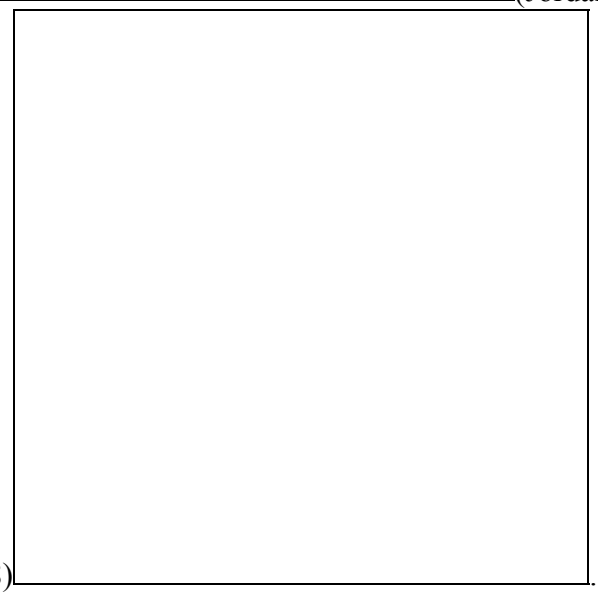


(Jordan *et al.*,

1998)  . The method is quick and simple and has great potential for the identification of DNA polymorphism and codominant nDNA fragments in diversity studies  (Bodénès *et al.*, 1996)  . However, it is necessary to test segregation ratios to validate genetic hypotheses and the methodology is sensitive to both sequence composition and the sequence itself

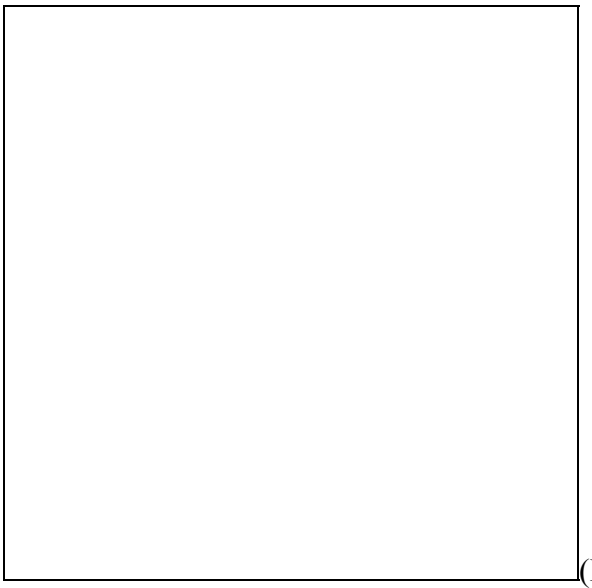


(Jordan *et al.*,

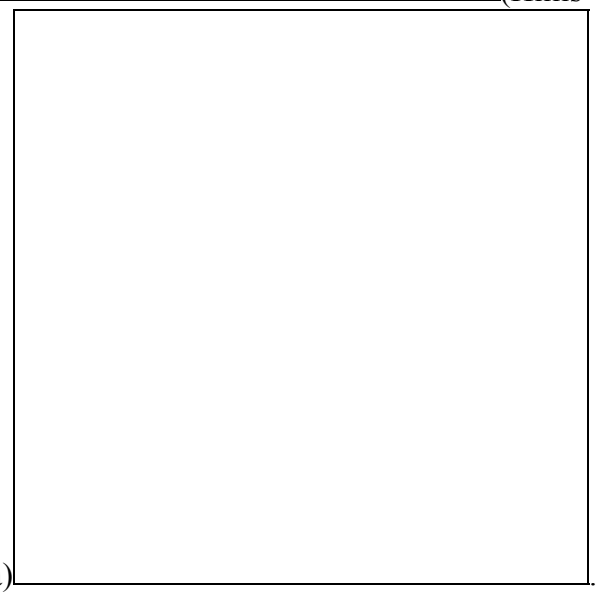


1998).

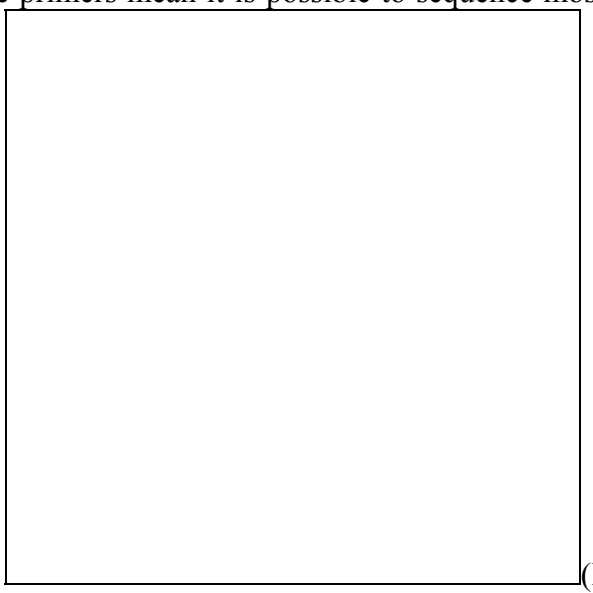
DNA sequence analysis (SA) provides information of nucleotide variation directly, rather than indirectly as other molecular methods do, and with the availability of automated sequencing and high-powered computer facilities SA is likely to becoming increasing important and has become the method of choice for phylogenetic studies



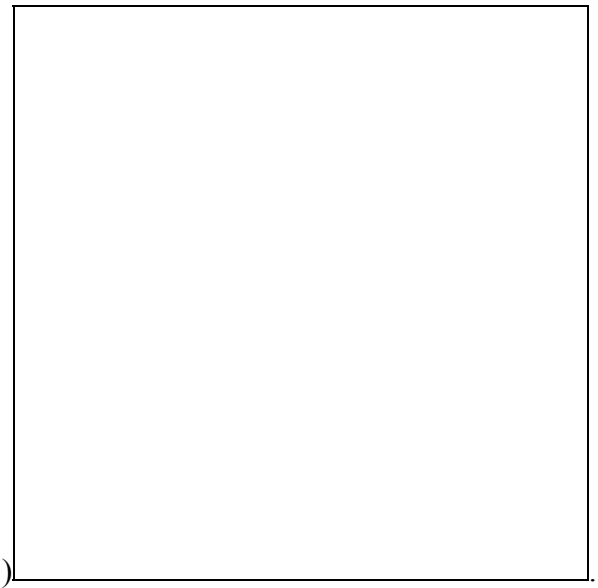
(Hillis *et al.*,



1996a). The method provides very high quality information that may quickly and easily be compared between studies, whilst universal sequence primers mean it is possible to sequence most taxa with no knowledge



of DNA sequence (Baldwin, 1992; Demesure



et al., 1995). The method is, however, labour intensive, expensive for general diversity surveys and loci are screened one at a time.

In general, the technologies for identifying molecular markers are characterised by three features: (i) technique utilisation has progressed with technique development, often in the absence of a rigorous understanding of the basis of a technique; (ii) large numbers of different techniques are available; and (iii) many claims have been made for particular techniques, especially in the early stages of the introduction of a technology.

Table 2. Structure of questionnaire and rationale for individual questions. Comments are in square brackets in italics.

A. Organisation details. *[Basic contact information]*

1. Name: 2. Position: 3. Organisation: 4. Full Postal Address: 5. Fax.:
6. Telephone: 7. e-mail: 8. Web-site:

B. Background information.

9. Does your organisation have any interest in the use of molecular markers? Yes/No.
   [A wide-ranging sample of organisations were contacted and therefore it was possible that some of them had no direct interest in molecular markers. However, it was important to know whether there was any information that might be usefully included within a molecular marker manual that would be of interest to such an organisation]
10. Does your organisation use molecular markers in its research or management programmes? Yes/No.
    [Organisations may have an interest in using molecular markers, but not use them]
11. What molecular markers does your organisation use?
    [An opportunity to indicate the molecular markers that organisations might be currently using].
12. What types of questions does your organisation use these markers to address?
    [An opportunity to indicate how molecular markers were being used to solve particular types of problems]
13. Is this work done within your organisation? If 'Yes' then indicate the number and grade of staff involved. If 'No' then please state where the work is done.
    [An opportunity to indicate whether molecular marker studies were undertaken in-house or contracted-out. In the case of in-house work it was useful to know how many staff were involved]
14. What types of training have your staff received?
    [An opportunity to indicate the training, if any, that organisation staff had received in molecular markers]
15. What books/laboratory manuals do you use as reference?
    [This was designed to determine what literature was being used and how up-to-date this was]
16. What limits your use of molecular marker information?
    [An opportunity for organisations to indicate the main limitations that they had in the use of molecular data].
17. How much knowledge do members of your organisation have about molecular markers? How was this knowledge gained?
    [If organisations had responded 'no' to the question 10, this gave it an opportunity to indicate the amount of knowledge that was present within the organisation and how this knowledge was gained]
18. What types of questions would your organisation be interested in addressing?
    [An opportunity to indicate the type of questions that organisations were interested in addressing if molecular markers were available]
19. Are you intending to use molecular markers within the next five years? If so, for what purposes (please rank in order of priority)?
    [An opportunity for an organisation to state their priorities over a five year time scale]

C. Intentions.

20. Are you intending to install or upgrade a laboratory for handling molecular markers within the next five years? If so, who will provide: (a) capital funding; (b) running costs; (c) training?
    [An opportunity to detail any intentions that organisations had towards the handling of molecular markers, including an indication of funding for capital, running and training costs]

D. Manual information.

21. Do you consider there are roles for molecular markers in tropical forestry? If 'Yes', what are these roles? If 'No', then please give reasons.
    [An opportunity to indicate organisational views on molecular markers in relation to tropical forestry]
22. Is there a need for a manual that details the practical application of molecular markers in forestry biodiversity studies? If 'No', then please give reasons. If 'Yes', then please give reasons.
    [To determine whether there was interest in a molecular marker manual, and to identify the reasons for and against such a enterprise]
23. What areas would be most usefully covered in a manual?
    [An opportunity for organisations to identity those parts of a manual that they might find most useful]
24. What techniques would you like to see covered?
    [Aimed at identifying whether there were any techniques that the organisation had heard of which they would like to see included in a manual]
25. What would be the most convenient format for a manual?
    [Designed to identify the most useful medium for a molecular marker manual]
26. If a manual were to be prepared, would you be willing to comment on a draft of this manual?
    [Included to give an indication of whether organisations would be interested in providing feedback on a draft manual]

2.0 Objectives.

(i) to identify the types of information that are necessary for the effective dissemination and utilisation of molecular information;

(ii) to identify the user groups of such information;

(iii) to assess the potential value of a manual on the application of molecular marker technologies in tropical trees.

3.0 Methodology.

Organisations were selected from mailing lists held by the Oxford Forestry Institute and the IUFRO secretariat. As wide a geographical spread of organisations as possible in developing countries was aimed at and all sectors were covered as far as possible, from NGOs through forestry organisations to research organisations. In addition, the chairpersons of all relevant IUFRO committees were contacted. Following a screen of nearly 4000 names, 168 organisations were targeted (Appendix I). Each organisation was sent a copy of a questionnaire (Appendix II) along with a covering letter (Appendix III) on 5th November 1998. If organisations had not responded by 17th December 1998 a reminder letter (Appendix IV) was sent. The rationale for the components of the questionnaire is shown in Table 2.

4.0 Results and discussion.

4.1 Background information.

29 replies (17.2%; Appendix V) were received from the 168 letters sent. One unsolicited reply was received from Dr Milton Kanashiro (EMBRAPA Amazonia Oriental, Brazil; this was ignored for analysis, except where it made different points to the other replies). The geographical distribution of the requests and the replies showed that there was no significant correlation between the requests sent to and those returned by Institutes from different regions of the world (Table 3). No replies were received from chairpersons of IUFRO Committees.

Table 3. Geographical distribution of questionnaires sent and returned by 31st January 1999. Excludes the questionnaires sent to the chairpersons of IUFRO Committees.

Region	Sent	Returned (percentage)
Latin America	23	3 (10.3)
Africa	57	15 (51.7)
South East Asia	15	4 (13.8)
South Asia	46	3 (10.3)
Pacific Islands	6	2 (6.9)
China	8	2 (6.9)
Caribbean	5	0 (0.0)

Of the 29 replies, 24 (82.8%) of the organisations had an interest in the use of molecular markers, and of these organisations 13 (54.2%) used them in their research or management. It was clear that organisations used a wide variety of different marker systems (Table 4) to address an array of different types of questions (Table 6). The most popular marker systems were RAPDs and allozymes, used by nine (69.2%) and eight (61.5%) of the organisations respectively. Most laboratories used a single technique, either allozymes, RAPDs or PCR-RFLPs (Table 5). If two or more techniques were to be combined then allozymes and RAPDs were the preferred choice. Few organisations used four or more techniques (4; 13.8%; Table 5).

Table 4. Techniques used by organisations in response to questionnaire.

Technique*	Number of organisations (percentage)
Allozymes	8 (61.5)
RAPDs	9 (69.2)
AFLPs	4 (30.8)
PCR-RFLPs	3 (23.1)
RFLPs	4 (30.8)
SSRs	2 (15.4)

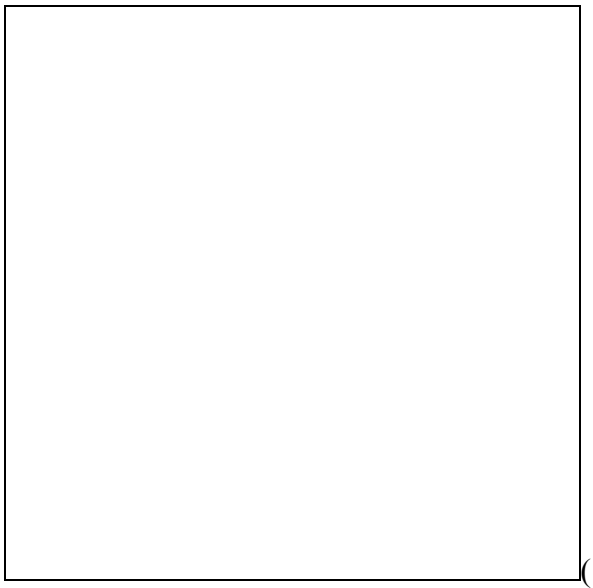
* For details of technique see Section 1.2.

Table 5. Numbers and types of techniques used by organisations in response to questionnaire.

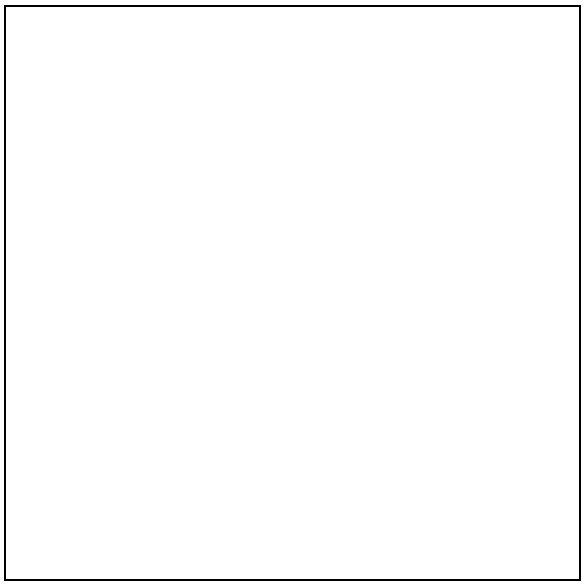
Number of techniques	Number of organisations (percentage)	Technique*	Number using technique
1	6 (46.2)	Allozymes	2
		RAPDs	2
		PCR-RFLPs	2
2	3 (23.1)	Allozymes + RAPDs	2
		RAPDs + PCR-RFLPs	1
3	0 (0.0)	—	0
4	2 (15.4)	Allozymes, RAPDs, AFLPs, RFLPs	2
5	2 (15.4)	Allozymes, RAPDs, AFLPs, RFLPs, SSRs	2

* For details of technique see Section 1.2.

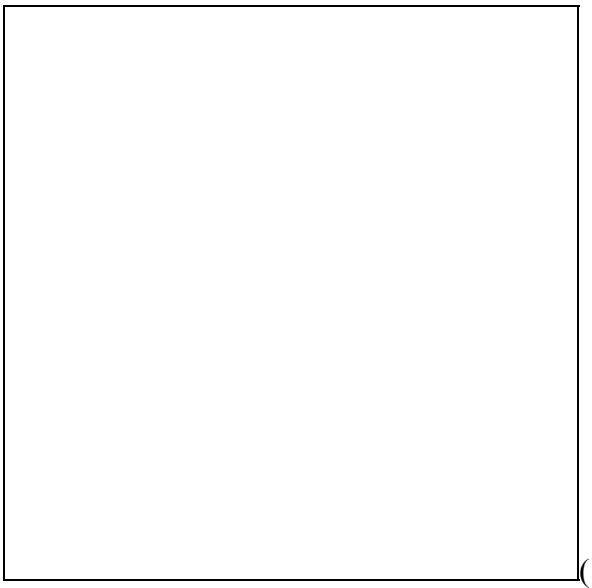
Of the organisations that used molecular markers, 11 (84.6%; Table 6) were interested in the application of markers for the study of genetic diversity. Other uses for the markers were much less frequent and included clone identification (5; 38.5%), systematic studies (4. 30.8%) and marker-aided selection (4, 30.8%; Table 6). Haines



(1994)



considered that the immediate applications of molecular markers were in supportive research for advanced breeding programmes with industrial species (e.g. clone identification, investigation of orchard contamination and 'fingerprinting') and in supportive research on tropical hardwoods and non-industrial species (e.g. taxonomic and mating system studies). Furthermore, Haines



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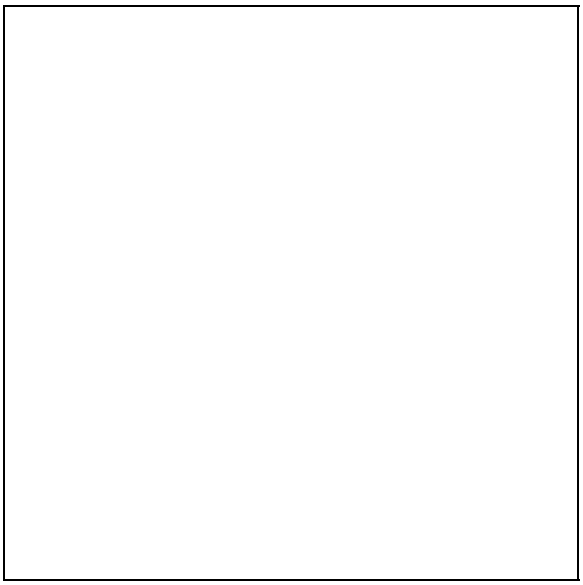


emphasised that marker-aided selection of non-industrial species in the short to medium term was likely to be of very limited value, since much cheaper methods of data generation were needed and such techniques were only likely to be used in advanced breeding programmes. The use of molecular markers is likely to be of greatest value for long-term strategic research, understanding basic genetic mechanisms and understanding genome organisation at the molecular level.

Table 6. Organisational application of molecular markers in response to questionnaire.

Application	Number of organisations (percentage)
Genetic diversity	11 (84.6)
Relatedness	2 (15.4)
Phylogeny	4 (30.8)
Mating systems	3 (23.1)
Marker-aided selection	4 (30.8)
Hybridisation	3 (23.1)
Clone identification	5 (38.5)

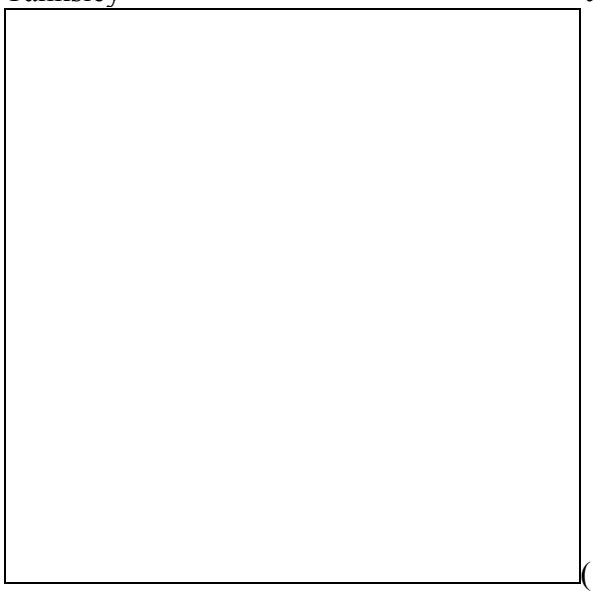
The majority of organisations were interested in single questions (Table 7), whilst only one organisation was interested in more than four types of question; the same organisation (Forest Research Institute Malaysia) that considered there were no limitations on its activities. The numbers of staff employed by each of the organisations in the field of

molecular markers varied from 1 to 16, and ranged from Ph.D. level to technicians. Of the organisations, 46.2% had staff with degrees, 84.6% with staff that had learnt via taught courses and four (30.8%) had staff that had been trained by both degrees and courses.

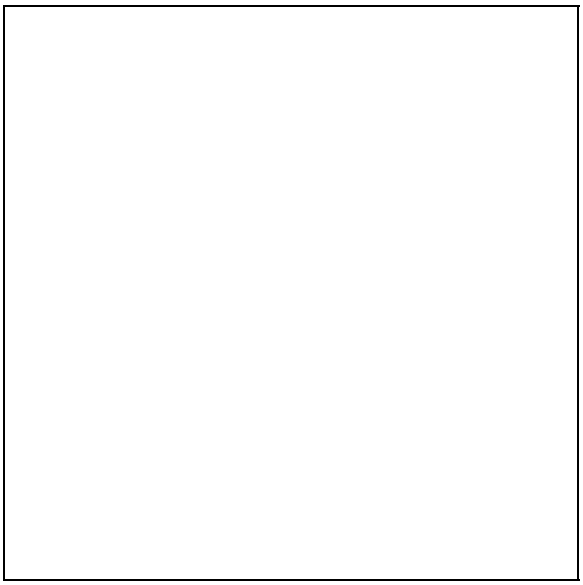
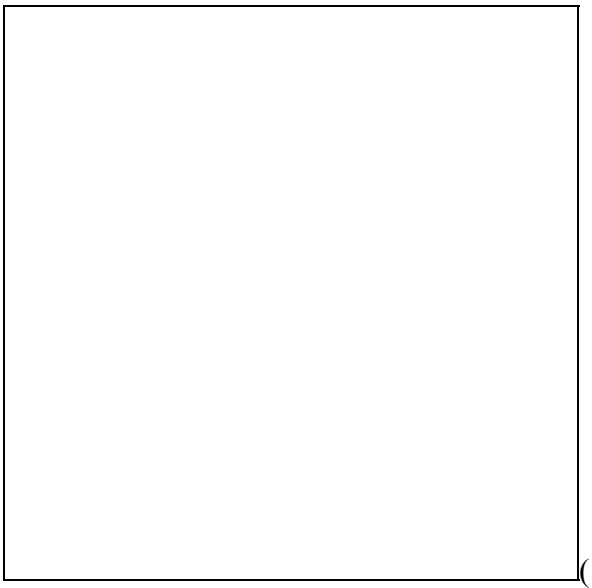
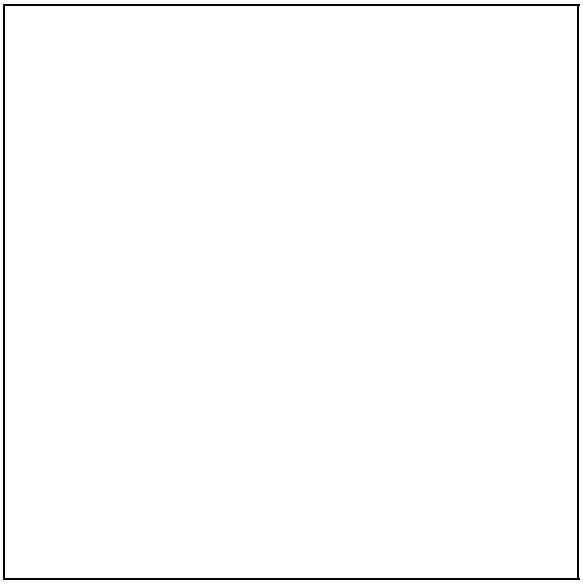
Table 7. Distribution of organisational application of molecular markers in response to questionnaire.

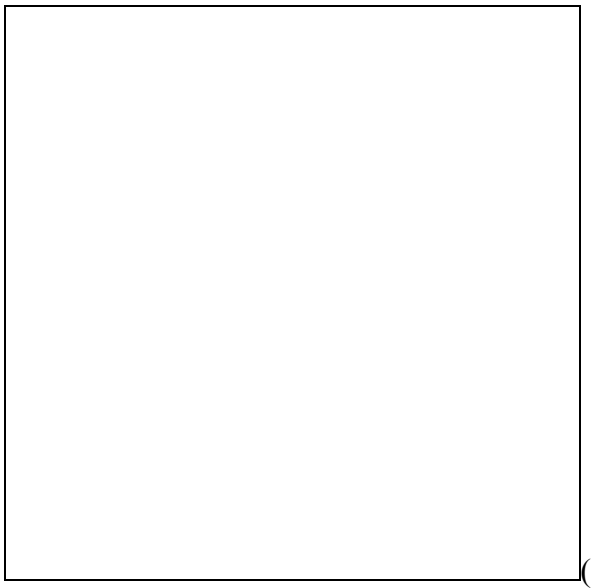
Number of applications	Number of organisations (percentage)	Application	Number using application(s)
1	5 (38.5)	Genetic diversity	4
		Clone identification	1
2	2 (15.4)	Genetic diversity + phylogeny	1
		Genetic diversity + marker-aided selection	1
3	3 (23.1)	Genetic diversity + relatedness + hybridisation	1
		Genetic diversity + phylogeny + hybridisation	1
		Relatedness + clone identification + marker-aided selection	1
4	2 (15.4)	Genetic diversity + mating systems + clone identification + phylogeny	1
		Genetic diversity + mating systems + clone identification + marker-aided selection	
6	1 (7.7)	Genetic diversity + phylogeny + mating systems + hybridisation + clones + marker-aided selection	1

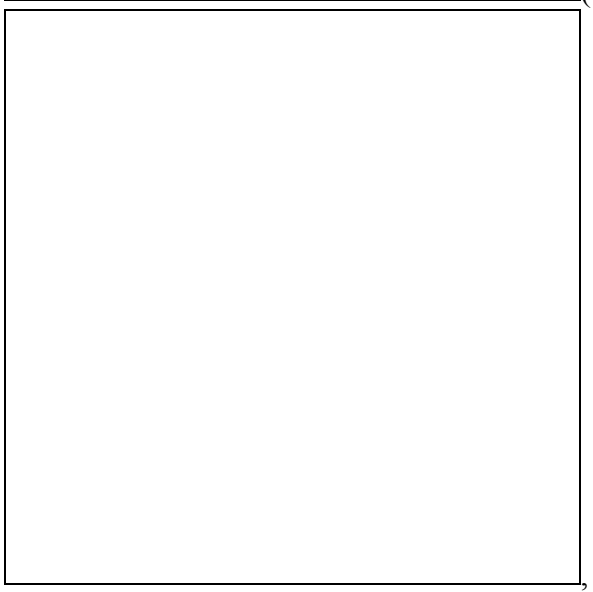
A limited range, of often out-dated manuals are being used by organisations. For example, Tanksley and Orton

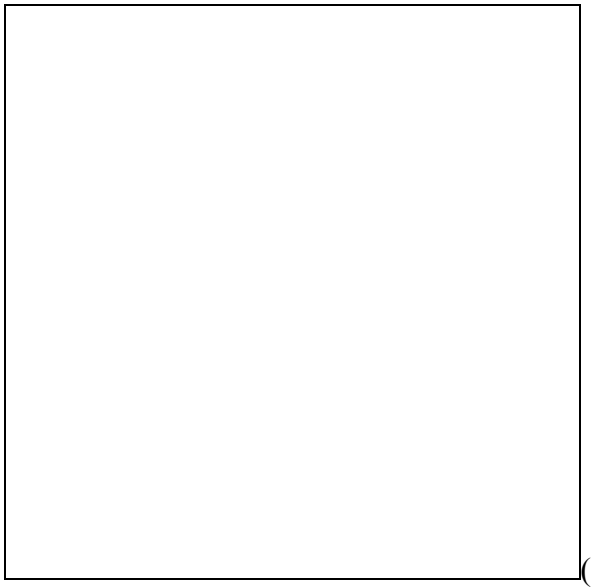


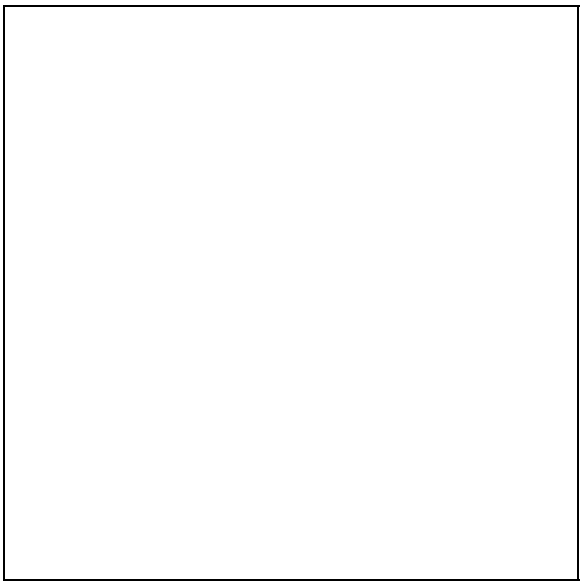
(1983)

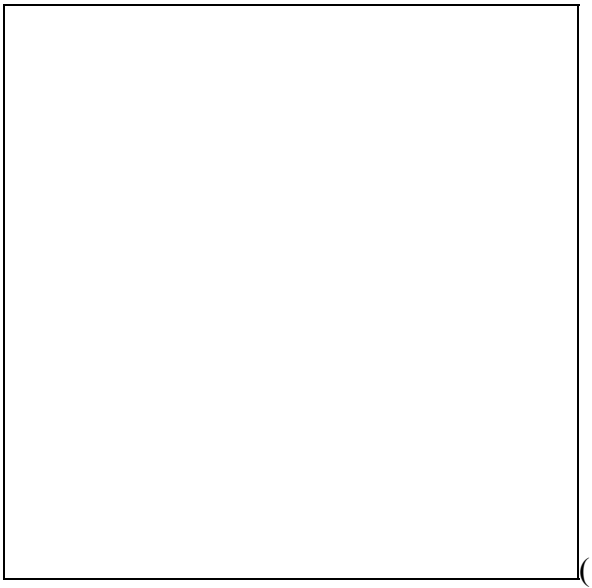

and Cheliak and Pitel

(1984)

have been largely replaced by Soltis and Soltis

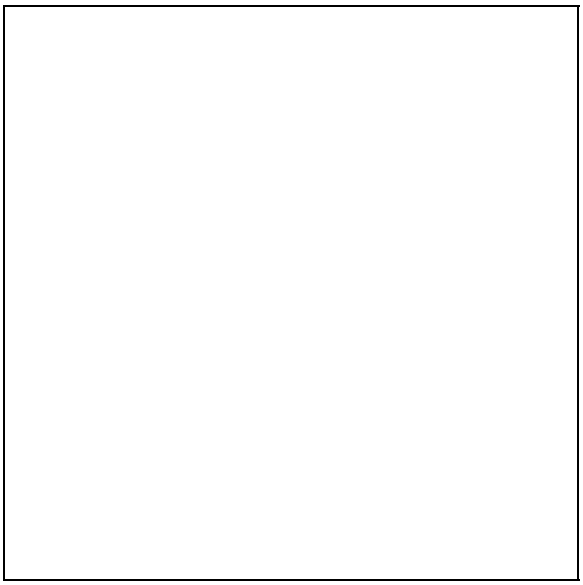
 (1990)

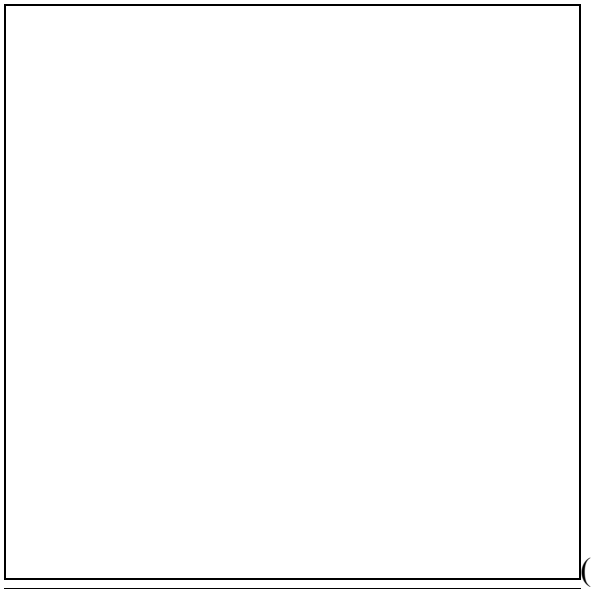
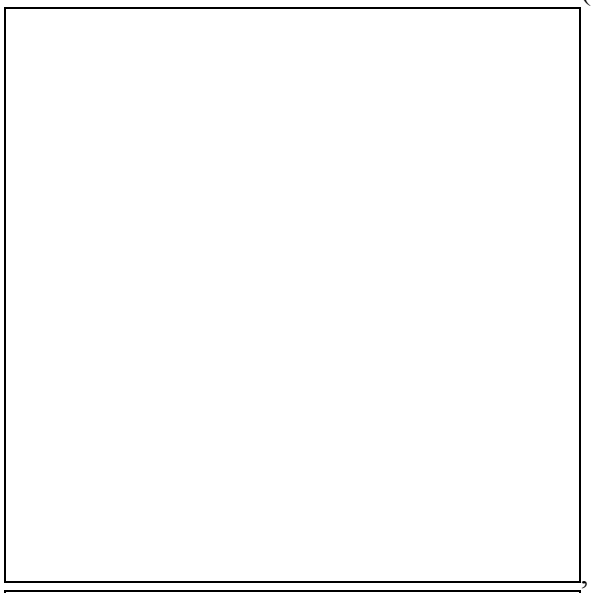
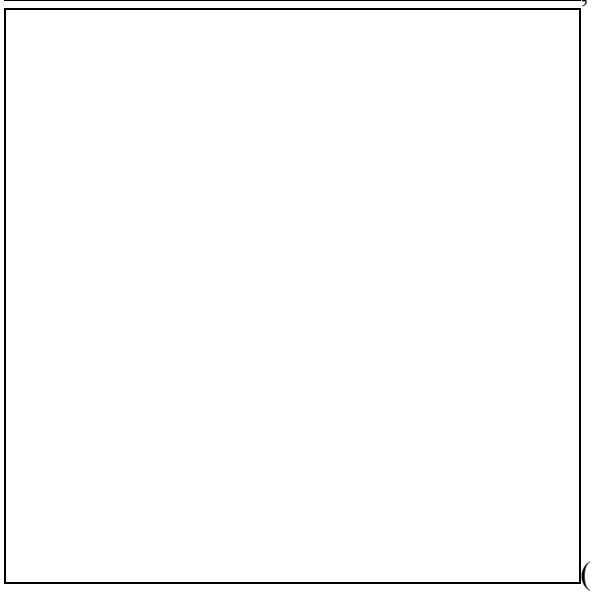
 , a generally more useful text on allozymes. Avise

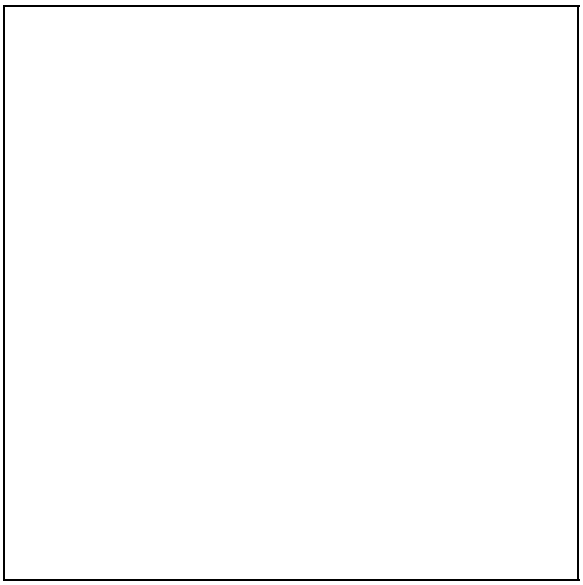
 (1994)


is an excellent introductory text, but does not discuss methodologies very extensively and is directed largely at animal systems. Maniatis *et al.*

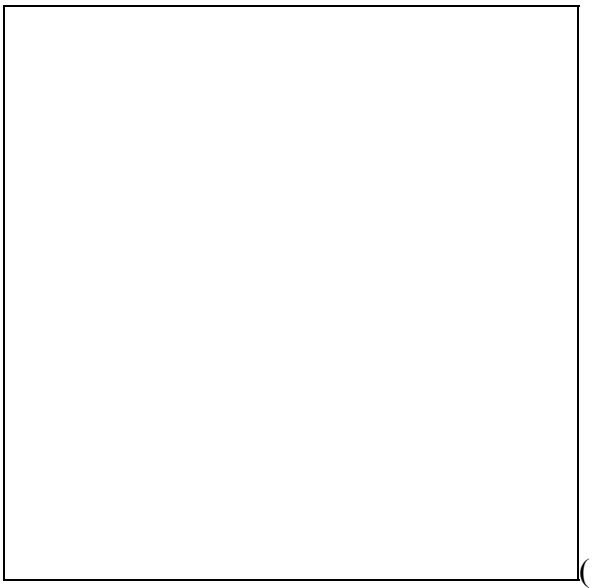

(1982)


is useful for general molecular methods, but is useless for PCR-based methods since it predates the invention of PCR. More relevant

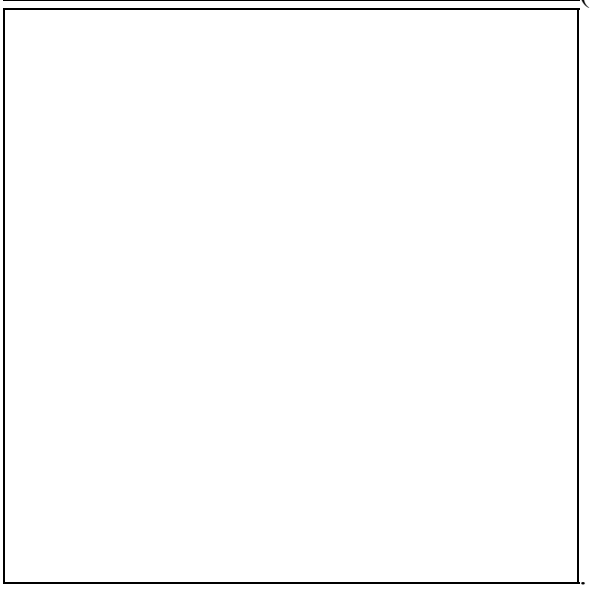
PCR-based books are those by Hillis *et al.*  (1996b)  , Weising *et al.*  (1991)



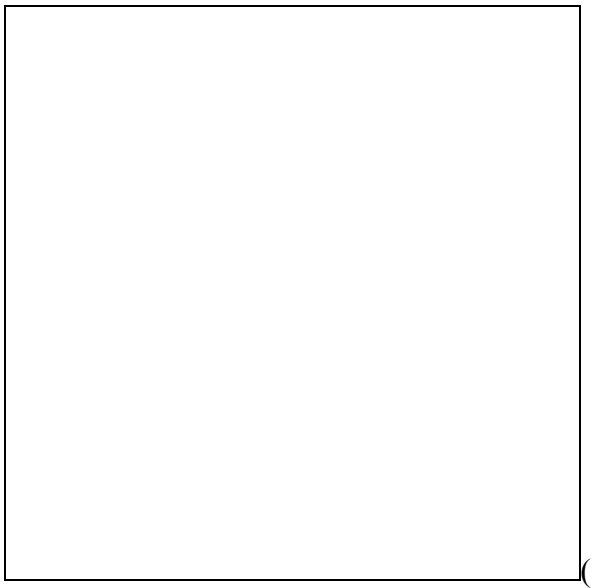
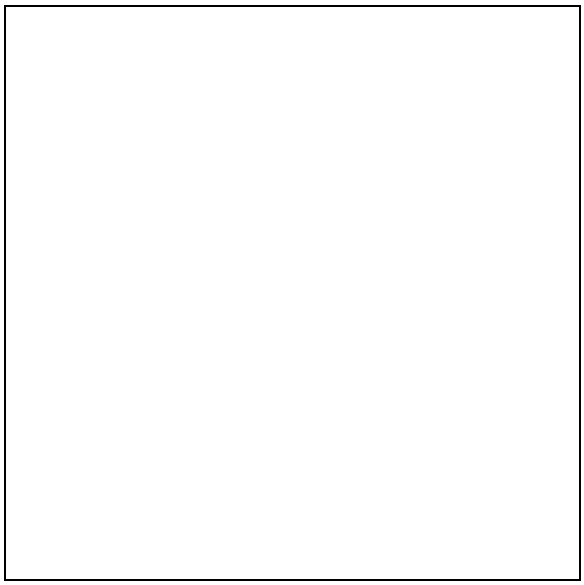
and Erlich

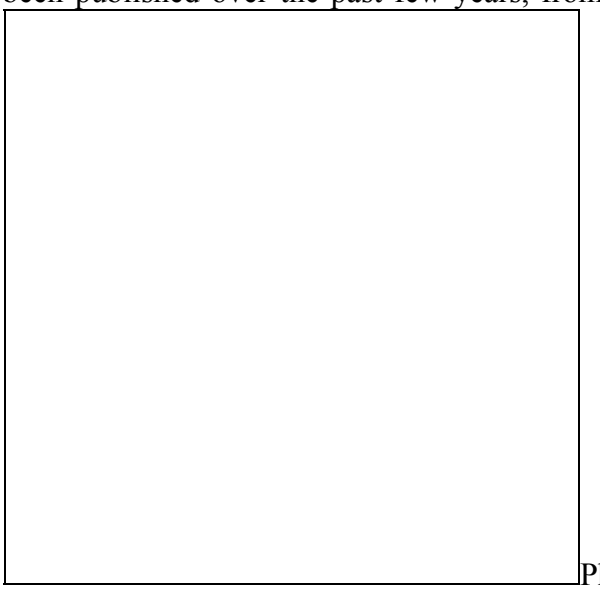


(1989)

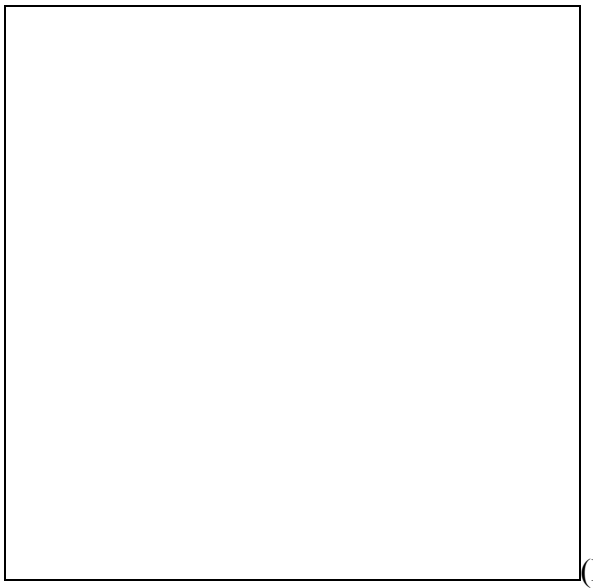


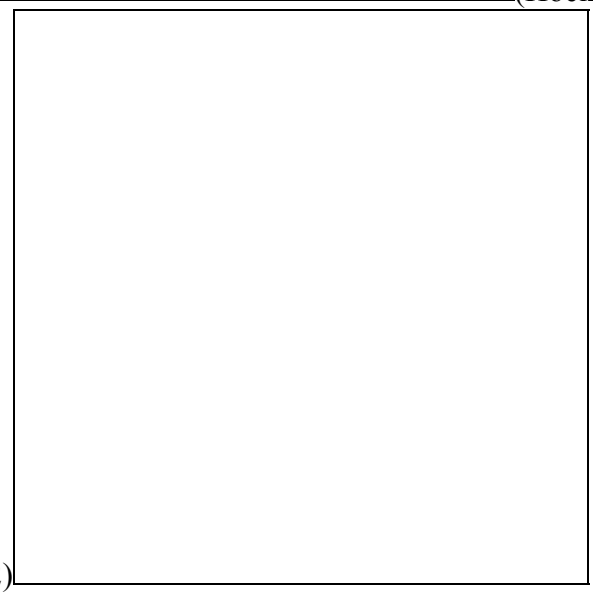
. Ferreira and Grattapaglia

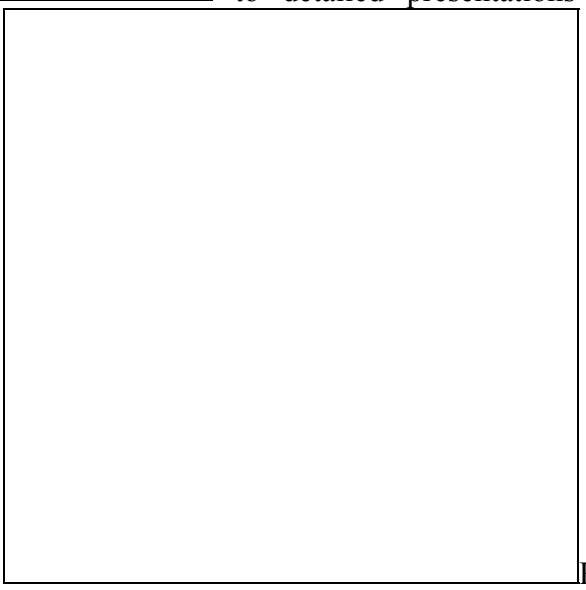
 (1996)
 is an excellent marker manual, directed at crops and the breeding of trees, but is only available in Portuguese.

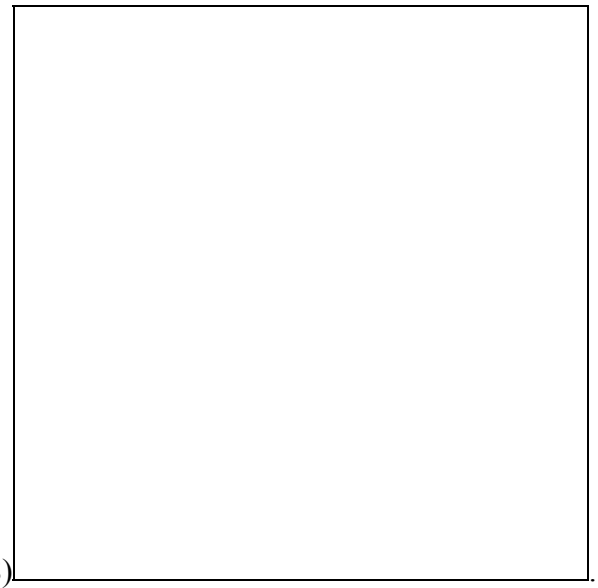
Numerous manuals have been published over the past few years, from those directed at agricultural crops (e.g.  Philips and Vasil,

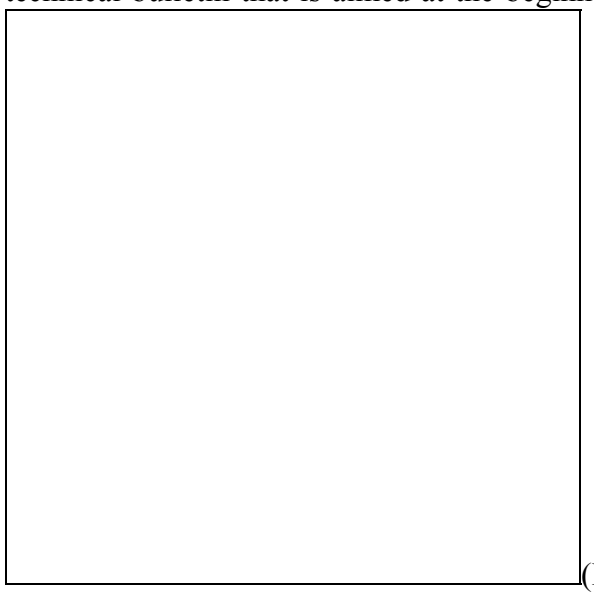
1994) through applications to conservation (e.g. Smith and Wayne, 1996) and population genetics

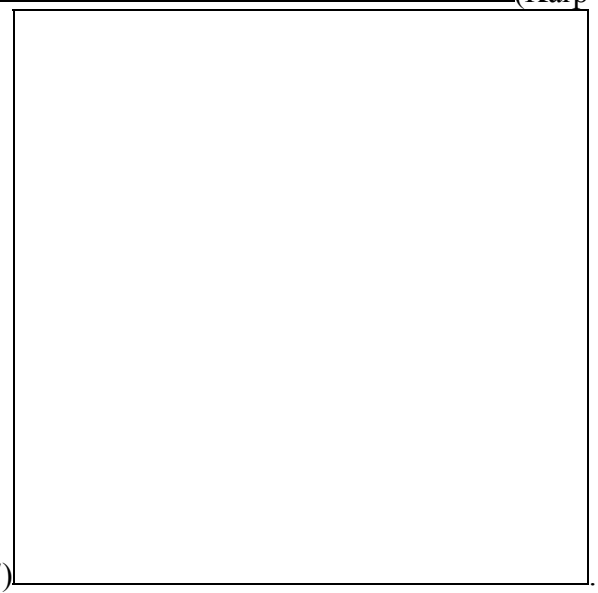
 (Hoelzel,

 1992) to detailed presentations of recent

 experimental methodologies (e.g. Karp *et al.*,

1998)  . Few of these manuals are aimed at beginners in the field, despite claims to the contrary in their introductions. One small technical bulletin that is aimed at the beginner has recently be made available by IPGRI

 (Karp *et al.*,

1997)  . This briefly describes the background to individual methodologies and highlights the importance of the decision-making process

in identifying effective molecular markers for a particular problem.

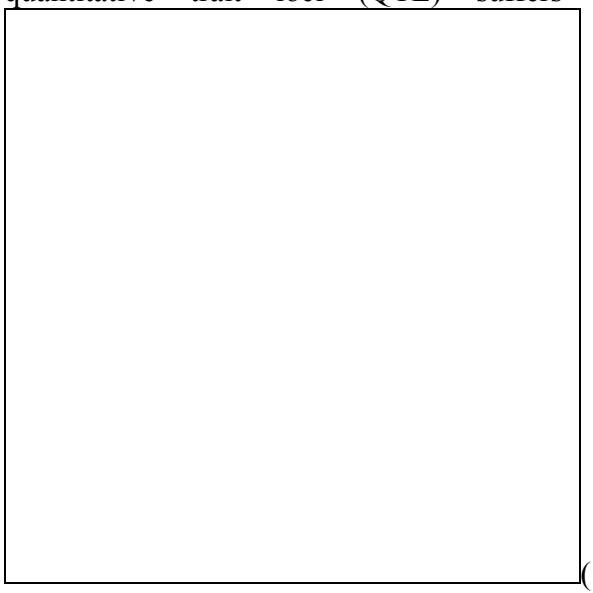
Limitations on the use of molecular markers by those organisation that are currently using them are the result of funding (58.8%), information (30.8%), facilities (23.1%) and personnel (15.4%). One organisation (Forest Research Institute Malaysia) considered that they had no limitations, whilst another organisation (International Centre for Research in Agroforestry, Kenya) considered that the time-scale for field evaluation of trials was a limitation.

Of those organisations that did not use molecular markers, but had an interest in them, 10 (90.9%) considered that they had some knowledge of molecular markers, although this knowledge was apparently confined to a few individuals within the organisation. Only one (9.1%) organisation (Nyabyena Forestry College, Uganda) indicated that they had no knowledge of molecular markers. The knowledge that was available had been obtained almost equally from courses (45.5%) and reading (54.5%).

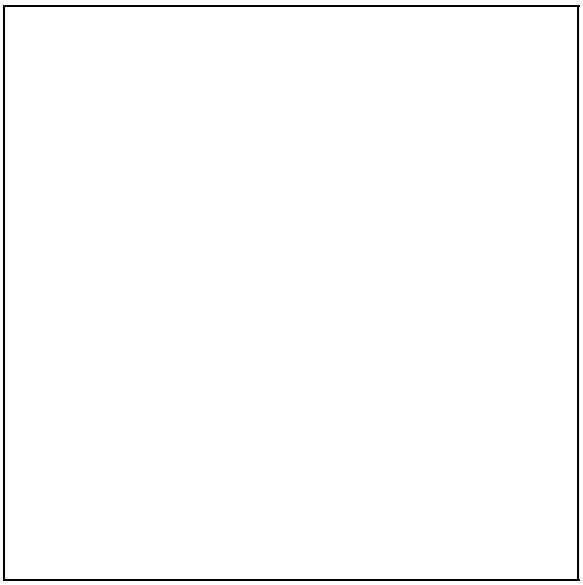
Table 8. Molecular marker applications of interest to organisations not currently using molecular methods.

Application	Number of organisations (percentage)
Genetic diversity	8 (72.7)
Mating systems	1 (9.1)
Management	1 (9.1)
Clone identification	2 (18.2)
Phylogeny	1 (9.1)
Quantitative trait loci	2 (18.2)
Knowledge on applications	1 (9.1)

Eight (72.7%) of the organisations that did not use molecular markers, but had an interest in them, were interested in using markers to look at genetic diversity, whilst all other potential uses had either one or two organisations interested (Table 8). The analysis of quantitative trait loci (QTL) suffers from the same problems as Haines



(1994)



highlighted for marker-aided selection in all but the most commercial of trees.

Six (54.5%) organisations stated that they are planning to use molecular markers in the next five years. One (9.1%) organisation stated that they were not planning on using molecular markers in the next five years and four (36.4%) did not respond to the question. Six organisations indicated that they were planning to analyse genetic diversity and in five of these cases this was ranked as the most important application for molecular markers (Table 9). All other applications of markers were of much lesser significance, both in terms of the numbers that wished to solve problems in the area and the ranking that those problems were given (Table 9). Organisations gave the use of molecular markers in conservation a low priority, although studies of genetic diversity were clearly of great importance. This may indicate a lack of detailed knowledge of the application of the methods to address conservation problems or an indication that the types of conservation problems facing these organisations are not easily resolved using molecular data.

Table 9. Ranking of molecular marker applications, over the next five years, by organisations not currently using molecular methods.

Application	Application ranking by organisations				Total
	1	2	3	4	
Genetic diversity	5	1			6
Mating systems		1	1		2
Clone identification		1			1
Tree improvement			1		1
Phylogeny	1				1
Quantitative trait loci	1				2
Management	1	1			2
Species selection		1			1
Species identification	2				2
Conservation				1	1

4..2 Intentions.

Statements of intentions were received from 22 of the organisations. Ten (45.5%) had no intentions of installing or upgrading laboratory facilities. Two organisations (University of Peradeniya, Sri Lanka; International Centre for Research in Agroforestry, Kenya) had either recently upgraded or were satisfied with their facilities. In the latter case, any increase in technology needed would be met by using external collaborators. Eleven (50%) of the organisations were planning either to install or upgrade laboratories in the

next five years, although two of these institutions (Faculty of Forestry, Gadjah Mada University, Indonesia; Forestry Research Institute of Malawi) had no clear idea of funding sources. Of the remaining nine institutions, five considered that Government sources would provide capital funding, and that projects would provide training and running costs., whilst two others considered that Development Agencies would cover costs. One institution considered that projects would provide both capital and training/running costs.

Although 50% of the organisations were planning on installing or upgrading laboratories it is clear that a diverse array of funding sources are being used for these purposes. Government and institution sources of funding are considered as the primary opportunity to secure financial resources. From the questionnaire responses it is unclear whether organisations have a clear idea of the full capital costs associated with the establishment of laboratory facilities, including the training of staff, the costs of equipment repair and up-grading and the safe disposal of the many toxic chemicals used in molecular procedures.

One organisation (International Centre for Research in Agroforestry, Kenya) considered that any increased sophistication in the techniques that they used would be undertaken through external collaboration. Other organisations appeared to be less realistic, considering that project funding could cover capital costs and associated staff training.

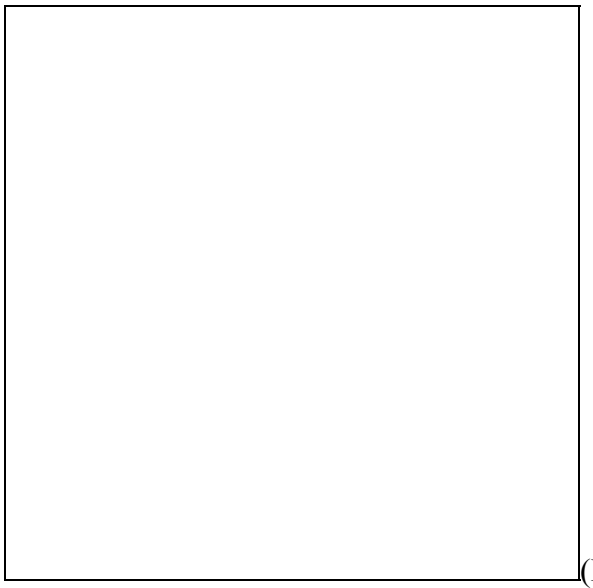
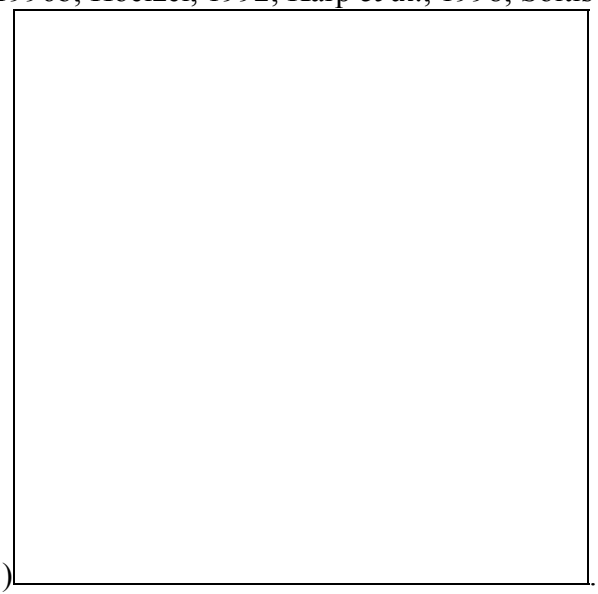
4.3 Manual information.

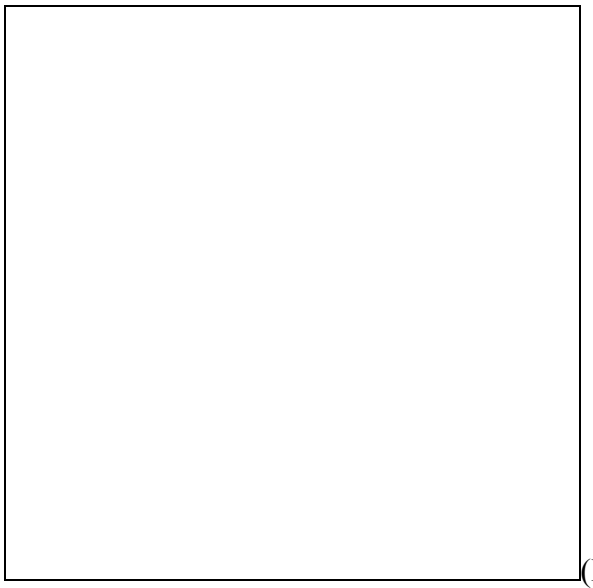
Molecular markers were considered to have a useful role in tropical forestry by 27 (93.1%) of the organisations. Two organisations (Pakistan Forest Institute, Peshawer; United Nations Environment Programme, Kenya) indicated that molecular markers either had a limited role at the present time or that they were unsure of their role. The reasons for the limited role were associated with the lack of expertise, the difficulty of equipment procurement and the availability of funds.

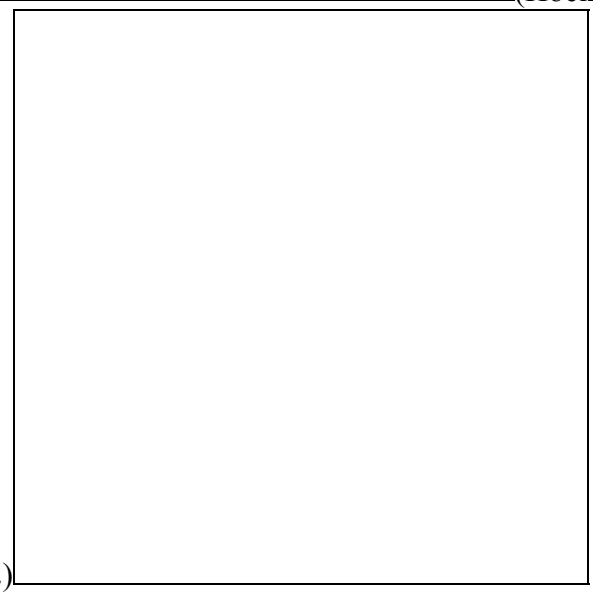
21 (77.8%) organisations identified molecular markers as having an important role in understanding diversity and differentiation of tropical trees, whilst nine (33.3%) and six (22.2%) organisations identified mating systems and systematics respectively as important roles for molecular markers. Clone identification was identified only by one (3.7%) organisation as a role, despite 21.7% of organisations currently using molecular markers in clone identification. Conservation (51.9%) and tree improvement (37.0%) were identified as benefiting from the application of molecular markers, whilst management (22.2%) was also considered important.

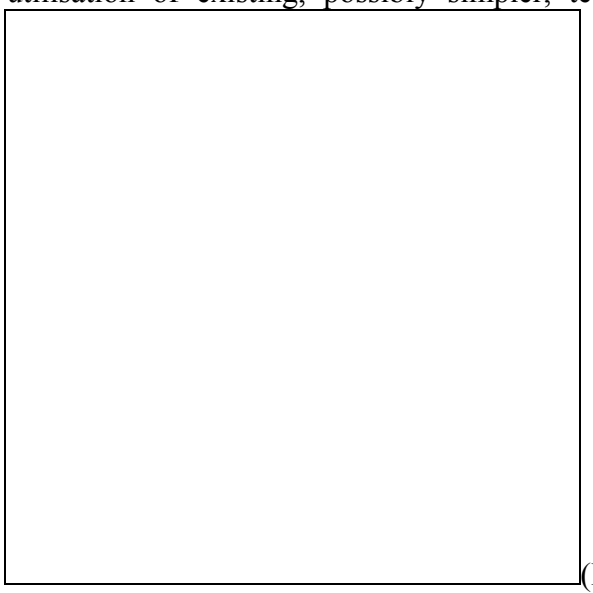
26 (89.7%) organisations considered that a manual was useful as a means of providing standard recipes for non-specialists and in order to be used for teaching and research purposes. Three organisations either questioned the need for a manual (Ian Dawson, International Centre for Agroforestry, Kenya; Prof. H.N.B. Gopalon, United Nations Environment Programme) or did not have enough experience to provide a considered opinion (Jonathan Timberlake, Biodiversity Foundation for Africa, Bulawayo). Prof. Gopalon's comment questioned whether molecular markers were already in use, and whether standard ‘cook book’ approaches in molecular biology manuals were sufficient. A similar issue was raised by Dr. Dawson in response to the need for a manual, that is, how are trees different to any other organism from a molecular view point.

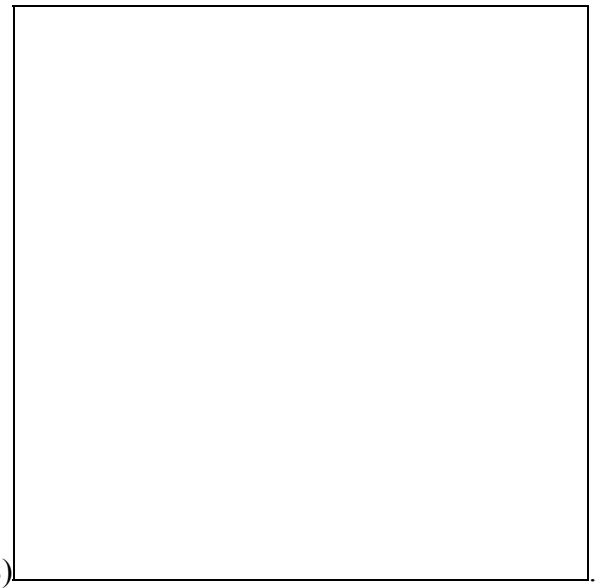
Molecular marker manuals are widely available in the developed world from many range

sources  (Ferreira and Grattapaglia, 1996; Hillis *et al.*, 1996b; Hoelzel, 1992; Karp *et al.*, 1998; Soltis and Soltis, 1990; Tanksley and Orton, 1983)  . However, many of these manuals deal with applications in agricultural species that may be unrealistic in often poorly researched tropical trees. Manuals may appear too simple and give the impression that molecular data can be resolved easily from the technical point of view, yet fail to include adequate provision for the interpretation/analysis of data (e.g.

 (Hoelzel,

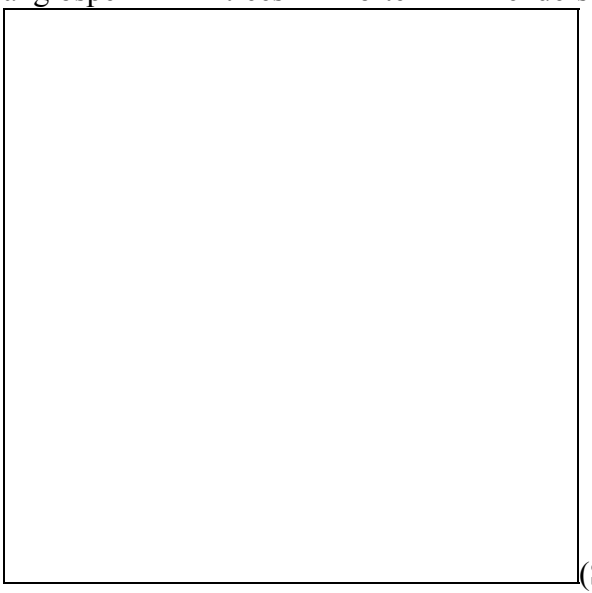
 1992) Alternatively, published manuals are too complex and concentrate on the development of technologies, rather than on the application and utilisation of existing, possibly simpler, technological solutions to a

 problem (Karp *et al.*,



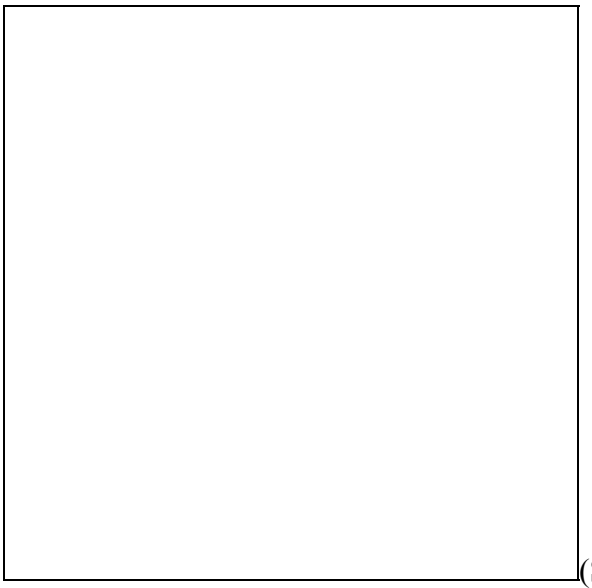
1998).

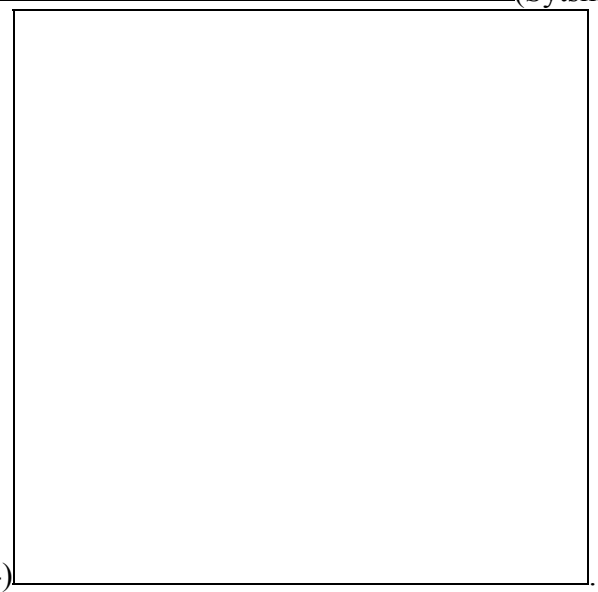
Trees are significantly different systems with which to work compared to the data derived from agricultural species. In the first instance the high level of polyploidy in many angiosperm trees often renders data difficult to interpret



(Soltis and Soltis,

1993), whilst the easy access to haploid tissue in gymnosperms means that data are easily generated for detailed analyses of population structure (Cheliak and Pitel, 1984). Trees are also chemically diverse and hence the isolation of DNA in the first instance may be a significant barrier; such information are either only available in obscure publications or through experience

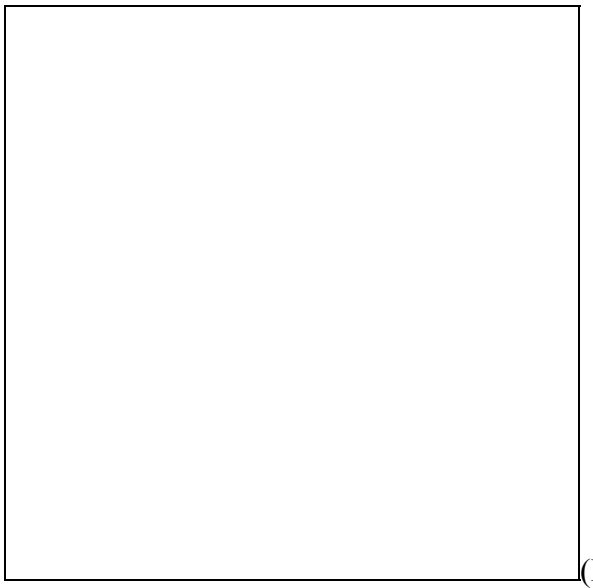
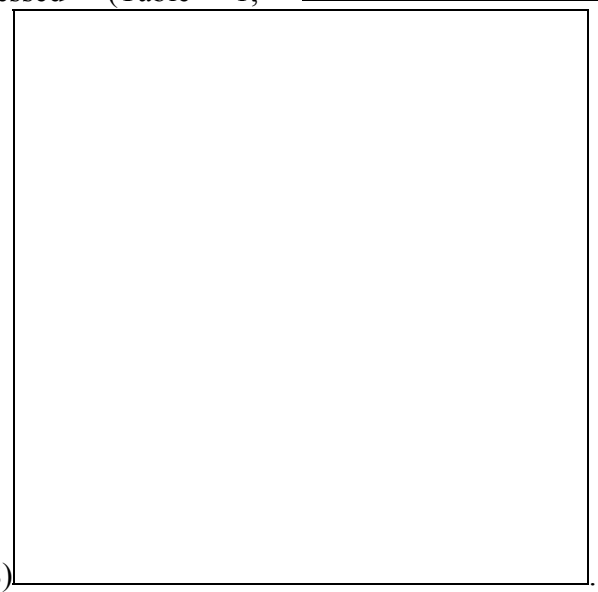
 (Sytsma,

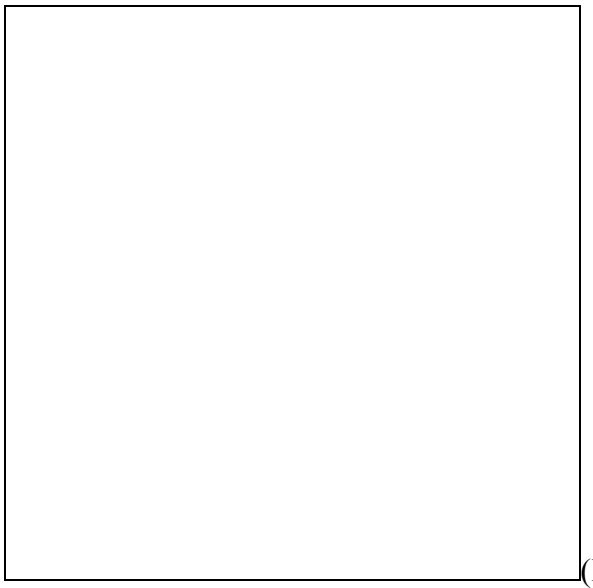
 1994). There is a need for a text that would enable much of this information to be located in a single place. Molecular markers must also be placed into the context of the sources of information derived from field work, e.g. distribution information and pollinator behaviour, and fruiting/flowering phenology. 'Tried-and-tested' technologies are the most effective approaches to understanding the biology of tropical organisms in the context of developing countries; there is little value in incorporating a expensive technological infrastructure for which there are no funds for training, equipment replacement and running costs.

Three areas were identified as being particularly important for inclusion within a molecular marker manual; methodology (19; 65.5%), interpretation (19; 65.5%) and analysis (24; 82.8%). Dr. Dawson (International Centre for Agroforestry, Kenya) highlighted the importance of the inclusion of methodologies specific to trees (e.g. sampling strategies) and that the focus of a manual should be on analysis and case studies. 14 (48.3%) of the organisations considered that case studies were an important component, whilst inclusion of information on appropriate markers (7; 24.1%) and the types of questions that might be addressed (8; 27.6%) were also considered to be important. Information on laboratory and equipment provisions was considered important by one organisation. The specific issue of sampling was raised by Dr. Dawson (International Centre for Agroforestry, Kenya), since most sampling guidelines for

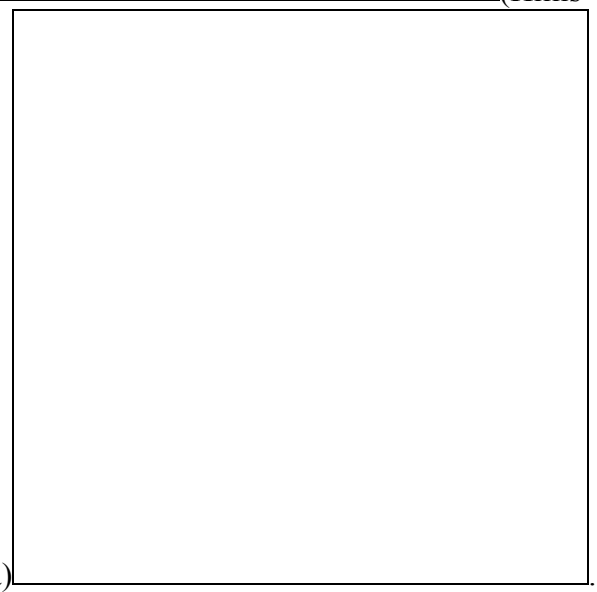
molecular studies have been generated by extrapolation from agricultural situations (Frankel *et al.*, 1995); concentration in any manual on this issue would be of great value.

Techniques that would appear to be of greatest interest for coverage are the DNA-based techniques of RAPDs (9; 31.0%), AFLPs (8; 27.6%) and microsatellites (7; 24.1%) and allozyme (6; 20.7%) analysis. RFLPs (3; 10.3%) and DNA sequencing (2; 6.9%) were mentioned to a much lesser degree. These may reflect either the techniques that particular institutions are using or techniques that have been mentioned in the literature. However, the techniques being used may not be the most appropriate for the questions being

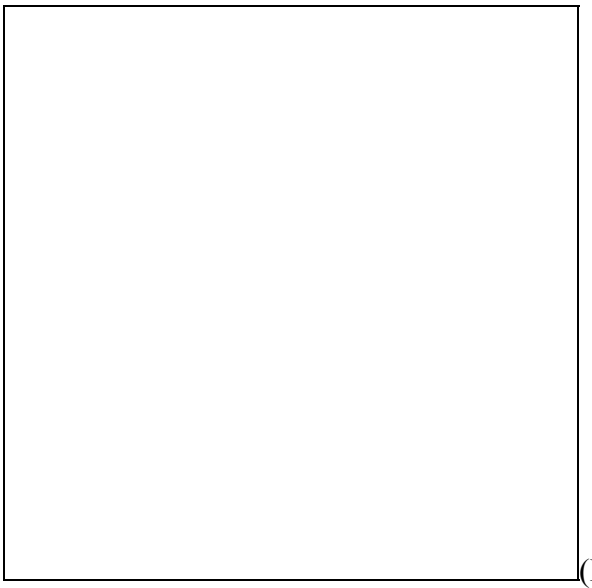
addressed (Table 1;  (Karp *et al.*,  1998). The choice of techniques will be governed by two factors, the facilities available and the questions being addressed. DNA sequence analysis appears to be impractical for the majority of organisations at the present time given the level of support that they have, the exception to this would appear to be Forest Research Institute Malaysia. Any manual must contain marker systems that have been 'tried-and-tested', are simple to use and generate high quality data quickly. DNA sequence analysis is not a practical technique in most developing countries since it requires access to automated DNA sequence facilities; manual methods could be used but these do not generate data cost efficiently



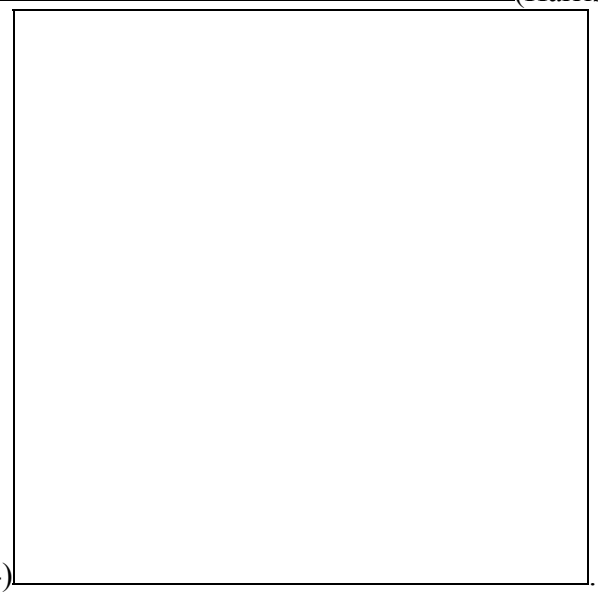
(Hillis *et al.*,



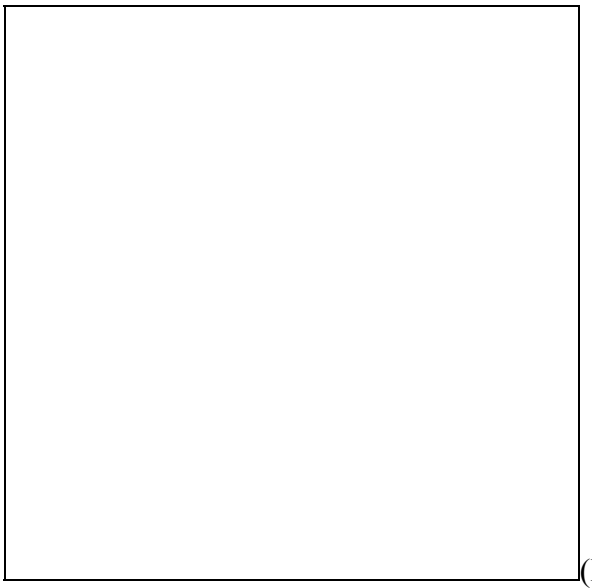
1996a). Given the range of interests that most organisations have, the most useful methods of analysis would be those associated with allozymes, PCR-RFLPs; RAPDs, AFLPs and microsatellites. As has already been highlighted all methods of data generation have problems associated with them (Section 1.2). Whilst RAPDs has been criticised as an approach to getting detailed information on population structure, it does allow the rapid generation of data



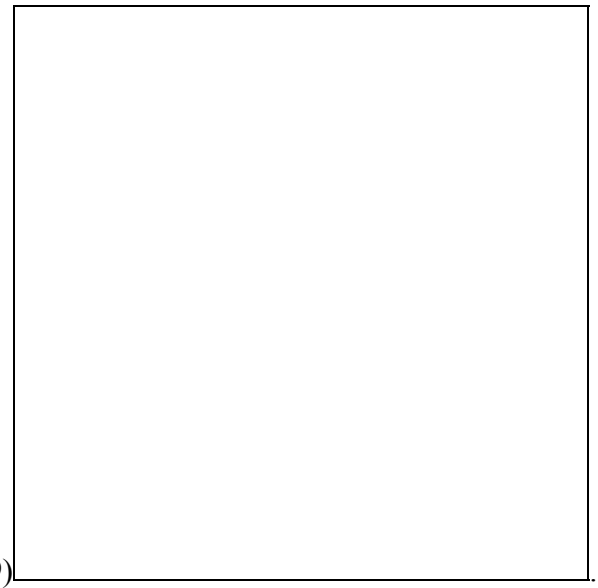
(Harris, in press-b; Lynch and Milligan,



1994). The degree to which generated data contains information needs to be carefully considered within the context of a cost-benefit analysis of the technique. Such analyses have not been attempted within the context of tropical biodiversity studies. Such an analysis will have to take into account the problem of the resource availability and, most importantly, the type of question being addressed. For example, what are optional sample sizes? How many populations should be studied? How many markers need to be studied? What is the influence of different marker combinations? Data is available for such analyses in at least one multipurpose tree,



Faidherbia albida (Rendell,



1999).

The majority of users (21 vs. 14 in all other media) preferred paper as the medium for a manual. This would be advantageous but a parallel web-site/list-server would allow the up-dating of the manual and integration of either new methods of data analysis or approaches to data interpretation. In addition, 17 (58.6%) of the organisations expressed an interest in commenting on an early draft of the manual.

5.0 Conclusions.

A picture emerges of the type of manual that would be preferred by a potential end-user community that is primarily composed of researchers; few forest managers/decision makers appear appreciate the potential of such marker systems. The manual should: (i) be paper-based and cheap; (ii) self-contained, exploring methodology, interpretation and analysis of data and 'trouble-shooting'; (iii) contain basic, 'tried-and-tested' techniques; (iv) detailed discussions of advantages and disadvantages with appropriate references; (v)

include worked examples of interpretation and analysis; (vi) include detailed case studies linked to real development situations; (vii) be written for the non-specialist, under the assumption of no previous knowledge; (viii) be heavily illustrated and referenced.

Such a manual could be approached in a number of different ways. The major source of interest is in genetic diversity studies, although other studies are of interest. Issues associated with marker-aided selection are probably inappropriate for the majority of tropical tree species (Haines, 1994). A manual would have two major user groups; researchers and decision makers/managers. In the case of researchers it is necessary to highlight how studies are undertaken with particular marker systems and provide detailed methods for data generation, interpretation and analysis. Decision makers/managers do not need to have detailed knowledge of methodology, but they do need to know how to interpret molecular data and to have a realistic expectation of the outcome from these data sources; the fact that data is derived from molecular sources does not make it good data. An understanding of the role of molecular data within the context of other data sources, for example, demography, reproductive biology and taxon distribution, is crucial.

Within any manual there are two problematic situations: (i) initial training; and (ii) up-dating the manual. Training can be approached through the release of the manual in a

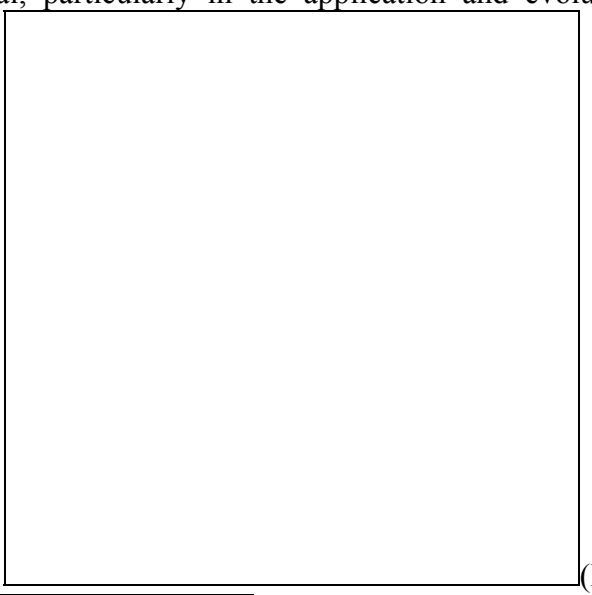
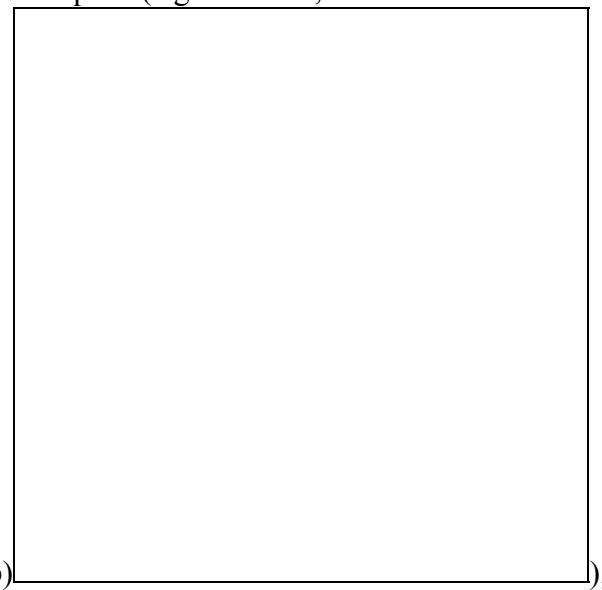
form that would give instructors a source of material, especially since the majority of organisations have well-qualified staff associated with their molecular programmes. In order to up date a manual, particularly in the application and evolution of different techniques (e.g. RAPDs;  (Harris, in press-b) ), it would be useful to have either a web-site or list-server for such up-dates. A manual should provide information that will enable users to: (i) ask the correct questions; (ii) determine the genome to analyse; (iii) identify the suitable techniques available; (iv) determine which part of the genome to use. An outline for such a manual is shown in Table 10.

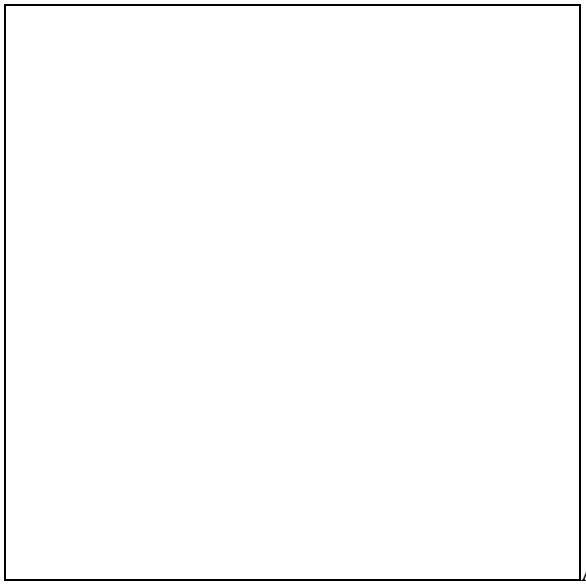
Table 10. Outline of molecular marker manual based on responses to questionnaires.

- Chapter 1. Molecular markers in tropical forestry.
    - What are molecular markers?
    - Types of molecular markers.
    - Value of molecular markers in tropical forestry.
    - Basic concepts of genetic diversity measurement and partitioning.
    - Sampling strategies.
- Chapter 2. Setting-up a molecular marker laboratory.
    - Safety: personal and environmental.
    - Basic equipment and facilities.
- Chapter 3. Allozymes.
    - Introduction to allozymes and the basis of the technique.
    - Methodologies for starch gel electrophoresis and allozyme staining.
    - Guidelines for the interpretation of allozyme gels.
    - Guidelines for the analysis of allozyme data.
    - Types of problems that allozymes have been used to resolve.
    - Troubleshooting.
- Chapter 4. Randomly amplified polymorphic DNA (RAPDs).
    - Introduction to RAPDs and the basis of the technique.
    - Methodologies for RAPD generation.
    - Guidelines for the interpretation of RAPDs.
    - Guidelines for the analysis of RAPD data.
    - Types of problems that RAPDs may be used to resolve.
    - Troubleshooting.
- Chapter 5. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLPs).
    - Introduction to PCR-RFLPs and the basis of the technique.
    - Methodologies for PCR-RFLPs generation.
    - Guideline to the sources of PCR-RFLPs primers.
    - Guidelines for the interpretation of PCR-RFLPs.
    - Guidelines for the analysis of PCR-RFLPs.
    - Types of problems that PCR-RFLPs may be used to resolve.
    - Troubleshooting.
- Chapter 6. Microsatellites (SSR).
    - Introduction to SSRs and the basis of the technique.
    - Methodologies for SSR generation.
    - Guideline to the sources of SSR primers.
    - Guidelines for the interpretation of SSRs.
    - Guidelines for the analysis of SSRs.
    - Types of problems that SSRs may be used to resolve.
    - Troubleshooting.
- Chapter 7. Arbitrary fragment length polymorphisms (AFLPs).
    - Introduction to AFLPs and the basis of the technique.
    - Methodologies for AFLP generation.
    - Guidelines for the interpretation of AFLPs.
    - Guidelines for the analysis of AFLPs.
    - Types of problems that AFLPs may be used to resolve.
    - Troubleshooting.
- Chapter 8. Molecular markers in tropical forestry.
    - Advantages and disadvantages of different types of molecular markers.
    - Which technique for which problem? - A decision matrix.
    - Patterns of genetic diversity: *Faidherbia albida* and *Calliandra calothyrsus*.
    - Mating system studies: *Cordia alliodora* and *Pithecellobium*.
    - Fragmentation: *Swietenia humilis*.
    - Hybridisation and its consequences: *Leucaena*.
    - Genetic resource management: *Pinus kesiya*.
- Glossary
- References.
- Index.

6.0 Acknowledgements.

I would like to thank Martin Billingham for all of his hard work in dealing with the questionnairres and for Prof. J. Burley and Dr P. Bacon for permission to screen Oxford Forestry Institute mailing lists.

7.0 References.



Allendorf, F.W. and Leary, R.F., 1986, Heterozygosity and fitness in natural populations of animals., in Soule, M.E. (Ed.): *Conservation biology.*, pp. 57-76, Sunderland, Massachusetts: Sinauer Associates.

Avise, J.C., 1994, *Molecular markers, natural history and evolution.*, London: Chapman & Hall.

Baldwin, B.G., 1992, Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: an example from the Compositae., *Molecular Phylogenetics and Evolution*, **1**, 3-16.

Birky, C.W., Fuest, P. and Maruyama, T., 1989, Organelle gene diversity under migration, mutation and drift: equilibrium expectations, approaches to equilibrium, effects of heteroplasmic cells and comparison of nuclear genes., *Genetics*, **121**, 613-627.

Bodénès, C., Laigret, F. and Kremer, A., 1996, Inheritance and molecular variations of PCR-SSCP fragments in pedunculate oak (*Quercus robur* L.)., *Theor. Appl. Genet.*, **93**, 348-354.

Butlin, R.K. and Tregenza, T., 1998, Levels of genetic polymorphism: marker loci versus quantitative traits., *Phil. Trans. R. Soc. Lond. B*, **353**, 187-198.

Charters, Y.M., Robertson, A., Wilkinson, M.J. and Ramsay, G., 1996, PCR analysis of oilseed rape cultivars (*Brassica napus* L. ssp. *oleifera*) using 5'-anchored simple sequence repeat (SSR) primers., *Theor. Appl. Genet.*, **92**, 442-447.

Cheliak, W.M. and Pitel, J.A.: Techniques for starch gel electrophoresis of enzymes from forest tree species. Petawawa National Forestry Institute. (1984)

Crawford, D.J., 1990, *Plant Molecular Systematics: Macromolecular Approaches.*, London: John Wiley & Sons.

Demesure, B., Sodzi, N. and Petit, R.J., 1995, A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in

plants., *Mol. Ecol.*, **4**, 129-131.
Dowling, T.E., Moritz, C., Palmer, J.D. and Rieseberg, L.H., 1996, Nucleic acids III: Analysis of fragments and restriction sites., in Hillis, D.M., Moritz, C. and Mable, B.K. (Ed.): *Molecular systematics*, pp. 249-320, Sunderland, Mass.: Sinauer Associates.
Dumolin-Lapegue, S., Peminge, M.-H. and Petit, R.J., 1997, An enlarged set of consensus primers for the study of organelle DNA in plants., *Mol. Ecol.*, **6**, 393-397.
Erlich, H.A., 1989, *PCR technology: Principles and applications for DNA amplification.*, London: Stockton Press.
Falk, D.A. and Holsinger, K.E., 1991, *Genetics and conservation of rare plants.*, Oxford: Oxford University Press.
Ferreira, M.E. and Grattapaglia, D., 1996, *Introdução ao uso de marcadores moleculares em análise genética.*, Brasîlia.: EMBRAPA-CENARGEN.
Ferris, C., Oliver, R.P., Davy, A.J. and Hewitt, G.M., 1993, Native oak chloroplast reveal an ancient divide across Europe., *Molecular Ecology*, **2**, 337-344.
Frankel, O.H., Brown, A.H. and Burdon, J.J., 1995, *The conservation of plant biodiversity.*, Cambridge: Cambridge University Press.
Furman, B.J., Grattapaglia, D., Dvorak, W.S. and O'Malley, D.M., 1997, Analysis of genetic relationships of Central American and Mexican pines using RAPD markers that distinguish species., *Mol. Ecol.*, **6**, 321-331.
Haines, R., 1994, *Biotechnology in forest tree improvement.*, Rome: FAO.
Hamrick, J.L. and Godt, M.J.W., 1996, Conservation genetics of endemic plant species., in Avise, J.C. and Hamrick, J.L. (Ed.): *Conservation genetics. Case histories from nature.*, pp. 281-304, London: Chapman & Hall.
Harris, S.A., in press-a, Molecular approaches to assessing plant diversity., in E., B.E. (Ed.): *Plant Conservation Biotechnology*, pp. London: Taylor & Francis.
Harris, S.A., in press-b, RAPDs in systematics – A useful methodology?., in Hollingsworth, P., Bateman, R. and Gornall, R.J. (Ed.): *Advances in Molecular Systematics.*, pp. London: Taylor & Francis.
Hillis, D.M., Mable, B.K., Larson, A., Davis, S.K. and Zimmer, E.A., 1996a, Nucleic acids IV: sequencing and cloning., in Hillis, D.M., Moritz, C. and Mable, B.K. (Ed.): *Molecular systematics.*, pp. 321-381, Sunderland, Massachusetts: Sinauer Associates.
Hillis, D.M., Moritz, C. and Mable, B.K., 1996b, *Molecular systematics.*, Sunderland, Massachusetts: Sinauer Associates.
Hoelzel, A.R., 1992, *Molecular genetic analysis of populations. A practical approach.*, Oxford: IRL Press.
Jarne, P. and Lagode, P.J.L., 1996, Microsatellites, from molecules to populations and back., *Trends Ecol. Evol.*, **11**, 424-429.
Jones, C.J., Edwards, K.J., Castaglione, S., Winfield, W.O., Sala, F., van de Wiel, C., Bredmeijer, G., Vosman, B., Matthes, M., Daly, A., Brettschneider, R., Bettini, P., Buiatti, M., Maestri, E., Malcevschii, A., Marmiroli, N., Aert, R., Volckaert, G., Rueda, J., Linacero, R., Vazquez, A. and Karp, A., 1997, Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories., *Molecular Breeding*, **3**, 381-390.
Jordan, W.C., Foley, K. and Bruford, M.W., 1998, Single-stranded conformation polymorphism (SSCP) analysis., in Karp, A., Isaac, P.G. and Ingram, D.S. (Ed.): *Molecular tools for screening biodiversity.*, pp. 152-156, London: Chapman & Hall.
Karp, A., Isaac, P.G. and Ingram, D.S., 1998, *Molecular tools for screening biodiversity.*, London: Chapman & Hall.
Karp, A., Kresovich, S., Bhat, K.V., Ayad, W.G. and Hodgkin, T.: Molecular tools in

plant resources conservation: a guide to the technologies. IPGRI, Rome. (1997)
Konieczny, A. and Ausubel, F.M., 1993, A procedure for mapping *Arabidopsis* mutations using co-dominant ecotype-specific PCR-based markers., *Plant J.*, **4**, 403-410.
Kunin, W.E. and Lawton, J.H., 1996, Does biodiversity matter? Evaluating the case for conserving species., in Gaston, K.J. (Ed.): *Biodiversity. A biology of numbers and difference.*, pp. 283-308, London: Blackwell Science.
Lande, R., 1988, Genetics and demography in biological conservation., *Science*, **241**, 1455-1460.
Lynch, M. and Milligan, B.G., 1994, Analysis of population genetic structure with RAPD markers., *Molecular Ecology*, **3**, 91-99.
Maniatis, T., Fritsch, E.F. and Sambrook, J., 1982, *Molecular cloning: a laboratory manual.*, Cold Spring Harbor Laboratory.
Matthes, M.C., Daly, A. and Edwards, K.J., 1997, Amplified fragment length polymorphism (AFLP)., in Karp, A., Isaac, P.G. and Ingram, D.S. (Ed.): *Molecular tools for screening biodiversity.*, pp. 183-190, London: Chapman & Hall.
Maxted, N., Ford-Lloyd, B.V. and Hawkes, J.G., 1997, *Plant genetic conservation. The in situ approach.*, London: Chapman & Hall.
Morgante, M., Pfeiffer, A., Jurman, I., Paglia, G. and Olivieri, A.M., 1998, Isolation of microsatellite markers in plants., in Karp, A., Isaac, P.G. and Ingram, D.S. (Ed.): *Molecular tools for screening biodiversity.*, pp. 288-296, London: Chapman & Hall.
Nei, M., 1987, *Molecular evolutionary genetics.*, New York: Columbia University Press.
Newbury, H.J. and Ford-Lloyd, B.V., 1993, The use of RAPD for assessing variation in plants., *Plant Growth Regulation*, **12**, 43-51.
Paran, I. and Michelmore, R.W., 1993, Development of reliable PCR based markers linked to downy mildew resistance genes in lettuce., *Theor. Appl. Genet.*, **85**, 989-993.
Perez de la Rosa, J., Harris, S.A. and Farjon, A., 1995, Noncoding chloroplast DNA variation in Mexican pines., *Theor. Appl. Genet.*, **91**, 1101-1106.
Philips, R.L. and Vasil, I.K., 1994, *DNA-based markers in plants.*, London: Dordrecht.
Powell, W., Machray, G.C. and Provan, J., 1996, Polymorphism revealed by simple sequence repeats., *Trends Pl. Sci.*, **1**, 215-222.
Rafalski, J.A., Vogel, J.M., Morgante, M., Powell, W., Andre, C. and Tingey, S.V., 1997, Generating and using DNA markers in plants., in Birren, B. and Lai, E. (Ed.): *Non-mammalian genomic analysis: a practical guide.*, pp. 75-134, New York: Academic Press.
Rendell, S., 1999; Population genetic structure of *Faidherbia albida* (Del.) A. Chev. (Leguminosae, Mimosoideae). — D.Phil., Oxford University.
Rieseberg, L.H., 1996, Homology among RAPD fragments in interspecific comparisons., *Molecular Ecology*, **5**, 99-105.
Schemske, D.W., Husband, B.C., Ruckelhaus, M.H., Goodwillie, C., Parker, I.M. and Bishop, J.G., 1994, Evaluating approaches to the conservation of rare and endangered plants., *Ecology*, **75**, 574-606.
Sharp, P.J., Desai, S. and Gale, M.D., 1988, Isozyme variation and RFLPs at the ß-amylase loci in wheat., *Theor. Appl. Genet.*, **76**, 691-699.
Smith, T.B. and Wayne, R.K., 1996, *Molecular genetic approaches in conservation.*, Oxford: Oxford Univeristy Press.
Soltis, D.E. and Soltis, P.S., 1990, *Isozymes in plant biology*, London: Chapman and Hall.
Soltis, D.E. and Soltis, P.S., 1993, Molecular data and the dynamic nature of polyploidy., *Crit. Rev. Plant Sci.*, **12**, 243-273.
Steinkellner, H., Lexer, C., Turetschek, E. and Glössl, J., 1997, Conservation of (GA)n microsatellite loci between *Quercus* species., *Mol. Ecol.*, **6**, 1189-1194.

Strand, A.E., Leebens-Mack, J. and Milligan, B.G., 1997, Nuclear DNA-based markers for plant evolutionary biology., *Mol. Ecol.*, **6**, 113-118.

Sytsma, K.J., 1994, DNA extraction from recalcitrant plants: Long, pure, and simple?, in Adams, R.P., Miller, J.S., Golenberg, E.M. and Adams, J.E. (Ed.): *Conservation of plant genes II: Utilization of ancient and modern DNA.*, pp. 69-82, Missouri: Missouri Botanical Garden.

Szmidt, A.E. and Wang, X.-R., 1991, DNA markers in forest genetics., in Müller-Stark, G. and Zieke, G. (Ed.): *Genetic variation in European populations of forest trees.*, pp. 79-94, Frankfurt-am-Main: J. D. Sauerländer's Verlag.

Tanksley, S.D. and Orton, T.J., 1983, *Isozymes in plant genetics and breeding, Parts A & B.*, Amsterdam: Elsevier Science Publishers B. V.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. and Zabeau, M., 1995, AFLP: A new technique for DNA fingerprinting., *Nucl. Acid Res.*, **23**, 4407-4414.

Weeden, N.F. and Wendel, J.F., 1990, Genetics of plant isozymes., in Soltis, D.E. and Soltis, P.S. (Ed.): *Isozymes in plant biology*, pp. 46-72, London: Chapman and Hall.

Weising, K., Ramser, J., Kaemmer, D., Kahl, G. and Epplen, J. T., 1991, Oligonucleotide fingerprinting in plants and fungi., in (Ed.): *DNA fingerprinting: Approaches and applications.*, pp. Basel,

Weising, K., Nybom, H., Wolff, K. and Meyer, W., 1995, *DNA fingerprinting in plants and fungi.*, London: CRC Press.

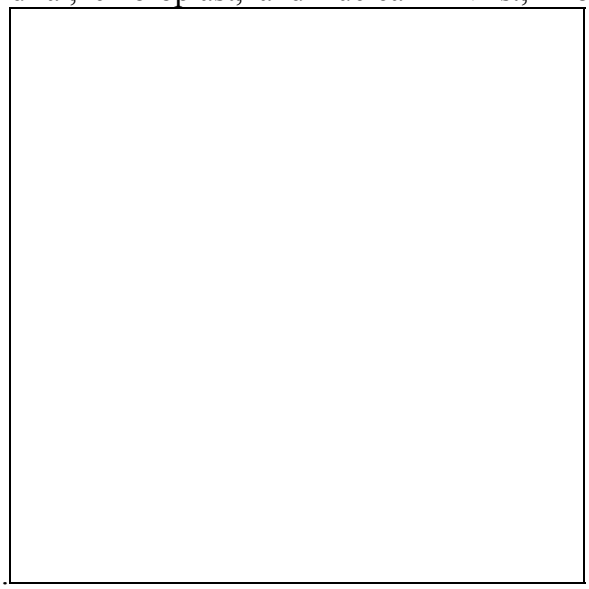
Wendel, J.F. and Weeden, N.F., 1990, Visualisation and interpretation of plant isozymes., in Soltis, D.E. and Soltis, P.S. (Ed.): *Isozymes in plant biology*, pp. 5-45, London: Chapman and Hall.

White, G. and Powell, W., 1997, Cross-species amplification of SSR loci in the Meliaceae family., *Mol. Ecol.*, **6**, 1195-1197.

Williams, J.G.K., Hanafey, M.K., Rafalski, J.A. and Tingey, S.V., 1993, Genetic analysis using random amplified polymorphic DNA markers., *Methods Enzymol.*, **218**, 704-740.

Wilson, E.O., 1992, *The diversity of life*, London: Penguin.

Wolfe, K.H., Li, W.-H. and Sharp, P.M., 1987, Rates of nucleotide substitution vary greatly among plant mitochondrial, chloroplast, and nuclear DNAs., *Proc. Natl. Acad. Sci. USA*, **84**, 9054-9058.



Appendices

Appendix I. Organisations and individuals to whom the questionnaire was sent.

Appendix II. Questionnaire.

Appendix III Covering letter.

Appendix IV. Reminder letter.

Appendix V. Questionnaire responses.

Appendix I. Organisations and individuals to whom the questionnaire was sent.

Prof. Claudio Balocchi,
BIO Forest S. A.,
Edificio Prales, Oficina 410, 4th Floor,
Casilla 347,
Valdivia,
Chile

Dr. John Beer,
CATIE,
Dept. de Agroforesteria,
7170 Turrialba,
Cartago,
Costa Rica

Prof. Jean-Pierre Bouillet,
URPPI,
ECO S. A., Congo CIRAD,
B. P. 1291,
Pointe- Noire,
Congo

Prof. Chen Chaw- Ming,
National Taiwan University,
College of Agriculture,
Dept. of Forestry,
Num. 1, Sec. 4, Roosevelt Road,
Taipei, Taiwan 10764
China

Prof Coert Geldenhuys,
Forestwood,
PO Box 228 ,
La Montagne,
Pretoria,
0184
South Africa

Prof. Eko Hardiyanto,
Uni. Gadjah Mada,
Fakultas Kehutanan,
Bulaksumur,
Yogyakarta,
55281
Indonesia

Prof. Wenyue Hsiung,
Nanjing Forestry University,
Shao Shan Road,
Nanjing,
210037
China

Prof. Roberto Ipinza,
Cooperativa Mejoramiento Genetico,
PO Box 853,
Valdivia,
Chile

Prof. Shams R. Khan,
Pakistan Forest Institute,
PO Forest Institute,
Peshawar,
Pakistan

Prof. F. Thomas Ledig,
USDA Forest Service,
Institute of Forest Genetics,
PO Box 245,
Berkeley, California,
94701
USA

Prof. M. Manokoran,
Institut Penyelidikan Perhutanan Malaysia,
52190 Kuala Lumpar,
Kepong,
Malaysia

Prof. Olivier Monteuuis,
CIRAD- Foret/ICSB,
PO Box 60793,
91017 Tawan,
Sabah,
Malaysia

Prof. Okoro Otusi,
Uni. of Agriculture,
Dept. of Forestry, Wildlife and Range Management,
PO Box 2373,
Makurdi,
Benue State
Nigeria

Prof. Peter Roberts,
University of Natal,
ICFR,
PO Box 100281,
Scottsville,
3200
South Africa

Prof. Outi Savolainen,
Oulun Yliopisto,
PO Box 333,
FIN-90571,
Oulu,
Finland

Prof. Natalino Silva,
Empera Brasileira de
Pesquisas Agropecuaria,
CPATU,
CP 48,
66095-100 Belem,
Parana Brazil

Prof. Terry Stanger,
SAPPI Forests,
Navorsing,
PO Box 473,
Howick,
Natal 3291
South Africa

Prof. Hubertus J. Van Hensbergen,
Uni. of Stellenbosch,
Dept. of Nature Conservation,
PO Box X1,
Matieland,
7602
South Africa

Prof. Huoran Wang,
Chinese Academy of Forestry,
Wan Shou Shan,
Beijing,
100091
China

Prof. Francis C. Yeh,
University of Alberta,
Dept. of Renewable Resources,
8-55 General Servives Building,
Edmonton, Alberta,
T6G 2P5
Canada

Professor Brian Bredenkamp,
University of Stellenbosch,
Department of Forest Science,
Private Bag X1,
Matieland,
7602
South Africa

Dr Jose Joaquin Campos,
CATIE,
Turrialba,
7170
Costa Rica

Dr Bjerne Ditlevsen,
Danish Tree Seed Centre,
Krogerupvej 3A,
Humlebaek,
Copenhagen,
DK-3050
Denmark

Professor Victor Gerding,
Instituto de Silvicultura,
Universidad Austral de Chile,
Casilla,
Valdivia,
567
Chile

Mr Felix Girard,
Guyana Natural Resources Agency,
UNDP-GEF Assistance to Iwokrama Rainforest Prgm,
41 Brickdam and Boyle Place,
Stabroek,
Georgetown,
Guyana

Mr Affonso Augusto Guidao Gomes,
Academia Brasileira de Ciencias,
Rua Anfilofio de Carvalho, 29 3 andar,
Rio de Janeiro,
20030 060
Brazil

Dr R Gunasekaran,
Government of Tamilnadu,
Forest Department,
Corporation Complex,
Tatabad,
Coimbatore,
641 012
India

Dr David A Harcharik,
FAO,
Forestry Department,
Viale delle Terme di Caracalla,
Rome,
100
Italy

Dr Lew Wing Hing,
ASEAN Timber Technology Centre,
1st Floor, Wisma DNP,
Jalan Ampang,
PO Box 10887,
Kuala Lumpur,
50728
Malaysia

Dr S D Verryn,
CSIR,
Environmentek,
PO Box 395,
Pretoria,
0001
South Africa

Prof Don K Lee,
Seoul National University,
Department of Forest Resources,
College of Agriculture and Life Sciences,
103 Seoodoondong,
Suwon,
441-744
Korea

Mr Ian Napier,
SEADD,
c/o British Embassy,
Wireless Road,
Bangkok,
10330
Thailand

Dr J A Odera,
KEFRI,
PO Box 20412,
Nairobi,
Kenya

Dr Abdou-Salam Ouedraogo,
IPGRI,
Via delle Sette Chiese 142,
Rome,
145
Italy

Mr A G Raddi,
Maharashtra Forestry Project,
New PMT Building, M Floor,
Swargate, Shankar Sheth Road,
Pune,
411042
India

Prof Youxu Jiang,
Chinese Academy of Forestry,
Institute of Ecology &
Environmental Sciences,
Wan Shou Shan,
Beijing,
100091
China

Mr Shwe Kyaw,
Ministry of Forestry,
Forest Department,
Bayintnaung Road,
West Gyogone,
Insein,
Yangon,
Myanmar

Mr Duncan Macqueen,
EMBRAPA/CPATU,
Projecto ODA,
CP 48,
CEP 66 095-100,
Belem,
Para,
Brazil

Dr Nguyen Hoang Nghia,
Forest Science Institute of Vietnam,
Ministry of Agriculture and
Rural Development,
Chem,
Tu Liem,
Hanoi,
Vietnam

Dr Albert Ofosu-Asiedu,
Forestry Research Institute of Ghana,
University of Science and Technology,
PO Box 63,
Kumasi,
Ghana

Dr Ernesto G Ponce,
CONSEFORH,
Apartado 45,
Siquatepeque,
Honduras

Dr Lee Soon Leong,
Forest Research Institute of Malaysia,
Kepong,
Kuala Lumpur,
52109
Malaysia

Dr Lucrecio L Rebugio,
College of Forestry,
University of the Philippines Los Banos,
College,
Laguna,
4031
Philippines

Professor P J Roberts,
Institute for Commercial Forestry Research,
PO Box 100281,
Scottsville,
3209
South Africa

Mr Tomas Schlichter,
Instituto Nacional de Tecnologia Agropecuaria (INTA),
National Forestry Research Programme,
Casilla de Correo 277,
Bariloche,
8400
Argentina

Dr El Hadji Sene,
FAO,
Forest Resources Division,
Via delle Terme di Caracalla,
Rome,
100
Italy

Dr A J Simons,
ICRAF,
PO Box 30677,
Nairobi,
Kenya

Dr V Strakhov,
Federal Forest Service of Russia,
Information Centre for Forest Resources,
69-a Novocheriomushkinskaya Str.,
Moscow,
117877
Russia

Dr H N B Gopalan,
United Nations Environment Programme,
PO Box 30552,
Nairobi,
Kenya

Dr Robert Mwase,
Forestry Association of Zimbabwe,
PO Box 660,
Mutare,
Zimbabwe

Chief Conservator of Forests,
Bangladesh Forest College,
Bangladesh Forest Directorate,
Sholashaher,
Chittagong ,
4211
Bangladesh

Chief Conservator of Forests,
Forest Department,
PO Box 70228,
Ndola,
Zambia

Chief Conservator of Forests,
Forest Development Corporation,
Forest & Environment, Government of Bihar,
Bihar State Forest Development Corporation Ltd,
13 Patliputra Colony,
Patna,
Bihar,
800 013 India

Chief Conservator of Forests,
Indian Forest Service,
Kerala Forest Department,
Vazhuthakad,
Trivandrum,
Kerala,
695 001
India

Chief Conservator of Forests,
Indian Forestry Service,
Rajasthan Forest Department,
Jalebi Chowk,
Jaipur Rajasthan,
302 001
India

Chief Conservator of Forests,
Indian Forestry Service,
Nagaland Forest Department,
Kohima Nagaland State,
797 001
India

Chief Conservator of Forests,
Indian Forestry Service,
Assam Forest Department,
Rehabari PO,
Gauhati,
Assam,
781 008
India

Chief Conservator of Forests,
Indian Forestry Service,
Bihar Forest Department,
PO Hinoo,
Ranchi,
Bihar,
834 002
India

Chief Conservator of Forests,
Indian Forestry Service,
West Bengal Forest Department,
p-16 India Exchange Place (Extn),
3rd Floor C.I.T. Building,
Calcutta,
West Bengal,
700 073 India

Chief Conservator of Forests,
Indian Forestry Service,
Orissa Forest Department,
Old Secretariat,
Cantonment Road,
Cuttack Orissa,
753 001
India

Chief Conservator of Forests,
Indian Forestry Service,
Manipur Forest Department,
Sanjenthong,
Imphal ,
Manipur State,
795 001
India

Chief Conservator of Forests,
Indian Forestry Service,
Jammu and Kashmir Forest Department,
Office of the Chief Conservator of Forests,
Srinigar,
Kashmir,
India

Chief Conservator of Forests,
Indian Forestry Service,
Meghalaya Forest Department,
Additional Secretariat Building,
Shillong,
Meghalaya,
793 001
India

Chief Conservator of Forests,
Indian Forestry Service,
Tripura Forest Department,
PO Kunjaban,
Agartal Tripura,
799 006
India

Chief Conservator of Forests,
Indian Forestry Service,
Himachal Pradesh Forest Department,
Talland,
Simla,
Himachal Pradesh,
171 001
India

Chief Conservator of Forests,
Ministry of Agriculture, Natural Resources & Forestry,
Forestry Division,
Youyi Building,
Brookfields,
Freetown,
Sierra Leone

Chief Forest and Lands Officer,
Ministry of Agriculture,
Department of Forestry,
Castries,
St Lucia

Chief Forest Officer,
Ministry of Agriculture,
Division of Forestry and Range Ecology,
Private Bag 003,
Gaborone,
Botswana

Chief Forest Officer,
Ministry of Agriculture & Cooperatives,
Forestry Section,
PO Box 162,
Mbabane,
Swaziland

Chief Forest Research Officer,
Forestry Research,
PO Box 22099,
Kitwe,
Zambia

Chief Forestry Officer,
Department of Forestry,
PO Box 30048,
Lilongwe,
Malawi

Chief Forestry Officer,
Ministry of Agriculture, Cooperatives & Marketing,
Conservation and Forestry Department,
Forestry Division,
PO Box 774,
Maseru,
100
Lesotho

Conservator of Forests,
Forest Department,
PO Box 509,
Colombo 02,
Sri Lanka

Conservator of Forests,
Indian Forestry Service,
Goa, Darman & Diu Forest Department,
Janta House (3rd Floor),
Panaji,
Goa Daman & Diu,
403 001
India

Director,
ASEAN/Canada Forest Tree Seed Centre Project,
Muak Lek,
Saraburi,
18180
Thailand

Director,
Forest Department,
173 Constant Spring Road,
Kingston 8,
Jamaica

Director,
Forestry Association of Botswana,
PO Box 2088,
Gaborone,
Botswana

Prof. J S Owonubi,
Forestry Research Institute of Nigeria,
PMB 5054,
Jericho,
Ibadan,
Oyo State,
Nigeria

Chief Forestry Officer,
Ministry of Agriculture, Lands, Fisheries & Co-operati
Forests & Lands Department,
Castries,
Saint Lucia,
West Indies

Dr P Haripensand,
Forestry Commission,
PO Box 1017,
1 Water Street,
Kingston,
Georgetown,
Guyana

Conservator of Forests,
Ministry of Agriculture, Natural Resources & Environm
Forestry Service,
Botanic Gardens Street,
Curepipe,
Mauritius

Conservator of Forests,
Ministry of Forests,
Forestry Department,
PO Box 2218,
Government Buildings,
Suva,
Fiji

Director,
Bangladesh Forest School,
P.O. Sylhet Cadet College,
Sylhet,
3101
Bangladesh

Director,
Forest Research Institute of Nigeria,
PMB 1039,
Samaru,
Zaria,
Nigeria

Director,
Forestry Commission,
PO Box 8111,
Causeway,
Harare,
Zimbabwe

Director,
Institute of Science and Technology,
University of Science and Technology,
Private Mail Bag,
Kumasi,
Ghana

Director,
Kerala Forest Research Institute,
Peechi,
Kerala,
Trichur District,
680 653
India

Director,
Bangladesh Forest Research Institute,
Ministry of Forests,
PO Box 273,
Chittagong,
4000
Bangladesh

Director,
Tanzania Forestry Research Institute,
PO Box 1854,
Morogoro,
Tanzania

Director,
Forestek Forestry Research Centre,
Private Bag X5011,
Jonkershoek,
Stellenbosch,
7600
South Africa

Director,
Ministry of Agriculture, Water & Rural Development,
Directorate of Forestry,
Private Bag 13184,
Windhoek,
Namibia

Director General,
ICFRE,
Forest Research Institute,
Dehra Dun,
248 006
India

Director of Forestry,
Forestry Department,
Ministry of Industry & Primary Resources,
Bandar Seri Begawan,
2067
Brunei Darussalam

Director of Forestry,
Ministry of Environment & Natural Resources,
Forest Department,
PO Box 30513,
Nairobi,
Kenya

Director,
Ministry of Agriculture, Trade & Tourism,
Forestry Division,
Botanical Gardens,
Roseau,
Dominica

Director,
National Research Council,
Post Bag 388,
Capital City,
Lilongwe 3,
Malawi

Director,
Forestek Forestry Research Centre,
Private Bag X11227,
Nelspruit,
1200
South Africa

Director,
Division of Forest Science & Technology,
CSIR PO Box 395,
Pretoria,
1
South Africa

Director,
National Agriculture Research Organization,
Forest Research Institute,
PO Box 1752,
Kampala,
Uganda

Director General of Forestry,
Forestry Department Peninsular Malaysia,
Jalan Mahameru,
Kuala Lumpur,
50660
Malaysia

Director of Forestry,
Forestry Division,
Private Bag,
Long Circular Road,
Port of Spain,
Trinidad and Tobago

Director of Forestry,
Ministry of Agriculture & Natural Resources,
Forestry Department,
5 Marina Parade,
Banjul,
Gambia

Director of Forestry,
Sabah Forestry Department,
Extension and Education,
PO Box 311,
Sandakan,
Sabah,
90007
Malaysia

Director of Forests,
Sarawak Forest Department,
STIDC Building Complex,
Petra Jaya,
Kuching,
93503
Malaysia

General Manager,
Fiji Pine Commission,
PO Box 521,
Lautoka,
Fiji

Head,
Forest Research Institute and Colleges,
Forest Research Centre,
Burnihat,
Assam,
India

Head of Department,
Department of Forest Resources Management,
University of Ibadan,
Ibadan,
Nigeria

Head of Department,
Papua New Guinea University of Technology,
Department of Forestry,
Private Mail Bag,
Lae,
Papua New Guinea

Head, Forestry Department,
Moi University,
Faculty of Forets Resources & Wildlife Management,
PO Box 3900,
Eldoret,
Kenya

President,
Forestry Association of Nigeria,
c/o Department of Forest Resources Management,
PO Box 4185,
Ibadan,
Nigeria

Principal,
Forest Research Institute and Colleges,
Forest Rangers College,
Balaghat,
Madhya Pradesh,
India

Principal,
Forest Research Institute and Colleges,
Southern Forestry Rangers College,
Coimbatore,
India

Principal,
Forest Research Institute and Colleges,
Eastern Forest Rangers College,
PO St. Mary's Hill,
Kurseong District P,
Darjeeling,
734 220
India

Principal,
Forest Research Institute and Colleges,
Northern Forest Rangers College,
PO New Forest,
Dehra Dun,
Uttar Pradesh,
248 006
India

Principal,
Forest Research Institute and Colleges,
Central Forest Rangers College,
Maharashtra,
Chandrapur,
442 401
India

Principal,
Indian Forestry Service,
Environment and Forest Department,
Aizawal,
Mizoram,
796 001
India

Dr G O Okajia,
Nyabyeya Forestry College,
Private Bag,
Masindi,
Uganda

Principal Chief Conservator of Forests,
Gujarat Forest Department,
Gujarat State, Kothi Annexe,
Vadodara Gujarat,
390 001
India

Principal Chief Conservator of Forests,
Indian Forest Service,
Karnataka Forest Department,
Aranya Bhavan, 7th Floor,
18th Cross, Malleswaram,
Bangalore,
Karnataka,
560 003 India

Principal Chief Conservator of Forests,
Indian Forest Service,
Uttar Pradesh Forest Department,
17 Rana Pratap Marg,
Lucknow,
Uttar Pradesh,
226 001
India

Principal Chief Conservator of Forests,
Indian Forest Service,
Harayana Forest Department,
30 Bays Building, Sector 17,
Chandigarh,
Harayana,
160 017
India

Principal Chief Conservator of Forests,
Indian Forestry Service,
Andra Pradesh Forest Department,
Hyderabad,
Andhra Pradesh,
500 004
India

Principal Chief Conservator of Forests,
Indian Forestry Service,
Madhya Pradesh Forest Department,
Ground Floor, Satpura Bhawan,
Bhopal,
Madhya Pradesh,
462 004
India

Principal Chief Conservator of Forests,
Indian Forestry Service,
Andaman & Nicobar Islands Forest Department,
PO Chatlam,
Port Blair,
Andaman & Nicobar Islands,
744 102
India

Regional Coordinator,
FAO/RAPA Regional Forestry Project,
UN Building,
Pulchowk,
Kathmandu,
Nepal

Secretary,
Department of Forests,
PO Box 5055,
Boroko,
Papua New Guinea

Dr Leonardo Areil Gallo,
Instituto Nacional de Tecnologia Agropecuaria,
CC 277,
San Carlos de Bariloche,
8400
Argentina

Mr Juan Francisco Goya,
Facultad de Ciencias Agraria y Forestales,
Universidad nacional de la Plata,
Calle 60 Esquina 119,
La Plata,
1900
Argentina

Dr Shyamal Roy,
Department of Botany,
Institute of Life Sciences,
Jahangirnagar University,
Savar,
Dhaka,
Bangladesh

Dr Alcides Leao,
Departmento de Ciencias Ambientais,
UNESP - FCA,
CP237,
Botucatu SP,
CEP 18603-970
Brazil

Dr Giorgini Venturieri,
Inst Nac de Pesquisas da Amazonia,
Coord de Pesquisas em Cien Agrarias,
CP 478,
Manaus,
69086-000
Brazil

Mr Tiby Guissou,
Laboratoire de Microbiologie Forestiere,
Institut de Recherche en Biologie et Ecologie Tropicale,
03 BP 7047,
Ouagadougou,
3
Burkina Faso

Mr Boukary Diallo,
Recherche Forestiere,
Institut de Recherche en Biologie et Ecologie Tropicale,
03 BP 7047,
Ouagadougou 03,
Burkina Faso

Dr Ngong Fronweban,
Department of Forestry,
Faculty of Agronomy,
Univerisity of Dschang,
PO Box 222,
Dschang,
Cameroon

Mr Yun Wang,
Department of Botany,
Institute of Forestry and Soil Science,
Academia Sinica,
PO Box 417,
Shenyang,
China

Dr Zhongze Zhang,
Institute of Forestry and Soil Science,
Academia Sinica,
PO Box 417,
Shenyang,
China

Ruben Guevara,
Director General,
CATIE,
Turrialba,
Costa Rica

Mr Jesus Mba,
Biologist,
ECOFAC Equatorial Guinea,
BP 317,
Bata,
Equatorial Guinea

Mr Clayton Hall,
Guyanan Forestry Commission,
1 Water St,
Kingston,
Georgetown,
Guyana

Dr R Yasodha,
Institute of Forest Genetics and Tree Breeding,
PO Box 1061,
Coimbatore 641 002,
India

Mr Pierre Youmesi,
Chief of Bureau,
Prov. Deleg. of Env. and Forestry,
PMB 10,
Buea,
SW Province
Cameroon

Dr Margarita Caru,
Departamento de Ciencias Ecologicas,
Facultad de Ciencias,
Universidad de Chile,
AP 653,
Santiago,
Chile

Dr Longqing Chen,
Department of Forestry,
Institute of Floriculture,
Huazhong Agricultural University,
Wuhan,
Hubei 430070
China

Mr Rodrigo Bernal,
Instituto de Ciencias naturales,
Universidad nacional de Colombia,
AP 7495,
Bogota,
Colombia

Mr Guillermo Arias,
Proyecto COSEFORMA,
APDO 8-4190,
1000 San Jose,
Costa Rica

Mr Abu-Juam Musah,
Head of Botany Unit,
Department of Forestry,
Planning Branch,
PO Box 1457,
Kumasi,
Ghana

Dr Arvind Khare,
Conservation Director,
WWF for Nature India,
172-B Lodi Estate,
New Delhi,
11003
India

Mr Rajive Kumar,
Divisional Director,
Forest Department,
24 Civil Lines,
Bareilly,
Uttar Pradesh,
India

Jeff Sayer,
Director General,
CIFOR,
PO Box 6596,
JKPWB,
Jakarta 10065,
Indonesia

Dr Phanuel Oballa,
Kenya Forestry Research Institute,
POBox 20412,
Nairobi,
Kenya

Mr Fadiala Dembele,
Programme Ressources Forestiere,
centre de Sotuba,
BP 1704,
Bamako,
Mali

Mr T Nen,
Director,
Papua New Guinea Forest Research Dist.,
National Forest Service,
PO Box 5055,
Boroko,
National Capital District,
Papua New Guinea

Dr Rogelio Baggayan,
Department of Environment and
Natural Resources Region 7,
Ecosystems Research and development Service,
Bureau of Mines Building,
Baniland,
Mandaue City 6014,
Philippines

Mr Mohamed Imam Bakarr,
Department of Biological Sciences,
Tiwai Island Field Research Station,
University of Sierra Leone,
Private Mail Bag,
Freetown,
Sierra Leone

Mr Sam Thabethe,
Deputy Director,
Mpumalanga Parks Board,
PO Box 1990,
Nelspruit,
1200
South Africa

Dr E Kireger,
Department of Forestry,
Moi University,
PO Box 1125,
Eldoret,
Kenya

Mr J P Gowela,
Forestry Research Officer,
University of Malawi,
Chancellor College,
PO Box 280,
Zomba,
Malawi

Dr Jose Navar,
Department of Agroforestry,
Facultad de Ciencias Forestales,
Universidad Autonoma de Nuevo Leon,
AP136,
Linares,
NL67700
Mexico

Dr Phillip Siaguru,
Senior Lecturer,
PNG University of Technology,
Forestry department,
Private Mail Bag,
Lae,
Papua New Guinea

Mr Samba Ndao Sylla,
Departement de Biologie Vegetale,
faculte des Sciences et Techniques,
Universite Cheik Anta Diop,
BP 5005,
Dakar-Fann,
Senegal

Dr Graham Kerley,
Director,
Terrestrial Ecology,
University of Port Elizabeth,
Dept of Zoology and Entomology,
PO Box 1600,
Port Elizabeth,
6000 South Africa

Dr D K Pushpakumara,
University of Peradeniya,
Office of the Dean,
Peradeniya,
Sri Lanka

Ms Maureen Playfair,
Suriname Forest Service,
Cornelis Jongbawstraat 10,
Paramaribo,
Suriname

Dr Abwoli Banana,
Department of Forestry,
Makerere University,
PO Box 7062,
Kampala,
Uganda

Prof Bruce Campbell,
Director,
Institute of Environmental Studies,
P.O.box MP 167,
Mount Pleasant,
Harare,
Zimbabwe

Dr Kouami Kokou,
Departement de Botanique,
Universite du Benin,
BP1515,
Lome,
Togo

Mr Vo Dai Hai,
Forest Science Institute of Viet Nam,
Chem,
Tu-Liem,
Hanoi,
Viet Nam

Mr J Timberlake,
Biodiversity Foundation for Africa,
PO Box FM730,
Famona,
Bulawayo,
Zimbabwe

Appendix II. Questionnaire

Survey of interest in a manual on molecular markers in tropical forestry.

Aims.

- To identify the information necessary for effective dissemination and utilisation of molecular information on tropical trees.
- To identify the user groups of molecular information on tropical trees.
- To assess the need for a practical manual on the application of molecular technologies to tropical trees.

Introduction

Biodiversity is most evident at the level of the community, although it can also be measured at the species, population and gene levels. In order to measure biodiversity within an area answers are needed to the questions:

- What species are there?
- How many species are there?
- How are species distributed?
- How are species related?
- What are the effects of environmental change?

The efficient utilisation, improvement and conservation of a taxon and its wild relatives must be based on a sound understanding of:

- The amount and distribution of genetic variation
- The effective design of sampling strategies
- The biology of the species concerned.
- Species relationships

Crucial to the success of long-term management of a taxon is an understanding of its genetics and demography; enabling biologically sound management strategies to be designed, including integrated conservation strategies that combine population and species management with *in-situ* and *ex-situ* conservation.

Such issues can be addressed at many levels in the biodiversity debate, but require the interpretation and integration of data sets from many different sources. One such data source, that has been promoted in recent years, is molecular markers. Access to information regarding molecular marker technologies may be limited, either through expensive books or through the absence of suitable books. For example, manuals are very useful if one is familiar with molecular marker technologies. However, in the absence of such familiarity there are no books that deal specifically with tropical trees and describe what are needed with respect to laboratory facilities, training and solving technical problems.

This survey is funded by the United Kingdom's Department for International Development's Renewable Natural Resources Research Strategy Forestry Research Programme and coordinated by Dr Stephen Harris at the address below. The purpose of this survey is to identify the information needs of users of molecular marker technology in tropical forestry.

Please return the completed questionnaire by 30th January 1999 to: Mr M. Billingham, Oxford Forestry Institute, Department of Plant Sciences, University of Oxford, South Parks Road, Oxford, OX1 3RB, UK. e-mail: <Stephen.Harris@Plant-sciences.oxford.ac.uk>. Fax: (01865) 275074.

If you require an electronic version of this questionnaire then please contact Dr S. A. Harris. at the address below. Please feel free to continue answers to any of the questions on additional sheets.

A. Organisation details.	
1. Name:	
2. Position:	
3. Organisation:	
4. Full Postal Address:	
5. Fax.:	6. Telephone:
7. e-mail:	8. Web-site:

B. Background information.
9. Does your organisation have any interest in the use of molecular markers? Yes/No. If 'No' then please go to Question 21.
10. Does your organisation use molecular markers in its research or management programmes? Yes/No. If 'No' then please go to Question 17.
11. What molecular markers does your organisation use? e.g. isozymes, randomly amplified polymorphic DNA (RAPDs), amplified fragment length polymorphisms (AFLPs).
12. What types of questions do your organisation use these markers to address? e.g. assessment of genetic variation, identification of clones.
13. Is this work done within your organisation? If 'Yes' then indicate the number and grade of staff involved. If 'No' then please state where the work is done.

B. Background information continued.
14. What types of training have your staff received? e.g. taught courses, research degrees.
15. What books/laboratory manuals do you use as reference?
16. What limits your use of molecular marker information? **Please go to Question 20**.
17. How much knowledge do members of your organisation have about molecular markers? How was this knowledge gained?
18. What types of questions would your organisation be interested in addressing?
19. Are you intending to use molecular markers within the next five years? If so, for what purposes (please rank in order of priority)?

C. Intentions.
20. Are you intending to install or upgrade a laboratory for handling molecular markers within the next five years? If so, who will provide: (a) capital funding; (b) running costs; (c) training?

D. Manual information.
21. Do you consider there are roles for molecular markers in tropical forestry? If 'Yes', what are these roles? If 'No', then please give reasons.
22. Is there a need for a manual that details the practical application of molecular markers in forestry biodiversity studies? If 'No', then please give reasons. If 'Yes', then please give reasons.
23. What areas would be most usefully covered in a manual? e.g. methodology, interpretation, analysis, case studies.
24. What techniques would you like to see covered?
25. What would be the most convenient format for a manual? e.g. CD-ROM, Web-site, paper.
26. If a manual were to be prepared, would you be willing to comment on a draft of this manual? Yes/No.
If you have any additional comments that you think would be useful then please add them here.

Thank you for taking the time to complete this questionnaire.

Deadline for return of questionnaire: 30th January 1999

Please return the completed questionnaire to: Mr M. Billingham, Oxford Forestry Institute, Department of Plant Sciences, University of Oxford, South Parks Road, Oxford, OX1 3RB, UK. e-mail: <Stephen.Harris@Plant-sciences.oxford.ac.uk>. Fax: (01865) 275074.

Appendix III Covering letter.

5th November 1998

Dear Sir,

The United Kingdom's Department for International Development's Renewable Natural Resources Research Strategy Forestry Research Programme has funded a project to: (i) identify the information necessary for effective dissemination and utilisation of molecular information on tropical trees; (ii) identify the user groups of molecular information on tropical trees; and (iii) assess the need for a practical manual on the application of molecular technologies to tropical trees.

As part of this project I would be grateful if you could complete the enclosed survey form which attempts to identify the background and intentions of organisations regarding molecular markers in tropical forestry and the best format that a molecular marker manual would take.

I would like to take the opportunity of thanking you for your time to complete the questionnaire.

Yours sincerely,

Stephen A. Harris (Dr)

Appendix IV. Reminder letter.

17th December 1998

Dear Sir,

Survey of interest in a manual on molecular markers in tropical forestry.

A few months ago a copy of the above survey, funded by the United Kingdom's Department for International Development's Renewable Natural Resources Research Strategy Forestry Research Programme, was sent to you. This survey aims to:

- To identify the information necessary for effective dissemination and utilisation of molecular information on tropical trees.
- To identify the user groups of molecular information on tropical trees.
- To assess the need for a practical manual on the application of molecular technologies to tropical trees.

To date I appear not to have had your survey form returned to me. If you are planning on returning the form could you please do so as soon as possible since the deadline for the submission of the report about this survey is 28th February 1999. It is possible that you did not receive a copy of the survey, in which case you can obtain one by e-mailing Martin Billingham on Martin.Billingham@Plant-sciences.oxford.ac.uk or writing to him at the above address.

Thank you for your time in completing this survey.

Yours faithfully,

Stephen A. Harris

Appendix V. Questionnaire responses.

1	Mr D Macqueen	EMBRAPA Amazonia Oriental	Brazil
2	Dr G O Okojia	Nyabyeya Forestry College	Uganda
3	Dr R Mwase	Forestry Association of Zimbabwe	Zimbabwe
4	Mr J Timberlake	Biodiversity Foundation for Africa	Zimbabwe
5	Prof. E Hardiyanto	Uni. Gadjah Mada	Indonesia
6	Dr M Kanashiro	EMBRAPA Amazonia Oriental	Brazil
7	Mr T Nen	Papua New Guinea Forest Authority	PNG
8	Ms R J K Hangula	National Forest Research Centre	Namibia
9	Dr N H Nghia	Forest Science Institute of Vietnam	Vietnam
10	Mr O G Dangasuk	Moi University	Kenya
11	Dr L Soon Leong	Forest Research Institute of Malaysia	Malaysia
12	Dr L Nshubzuki	Tanzania Forestry Research Institute	Tanzania
13	Prof. H Wang	Chinese Academy of Forestry	China
14	Dr H N B Gopalan	United Nations Environment Programme	Kenya
15	Dr K Kokou	Universite du Benin	Togo
16	Dr R Yasodha	Institute of Forest Genetics and Tree Breeding	India
17	Dr S D Verryn	CSIR	South Africa
18	Mr S N Sylla	Universite Cheik Anta Diop	Senegal
19	Dr R Baggayan	Bureau of Mines Building	Philippines
20	Dr L Chen	Institute of Floriculture	China
21	Dr P Haripensand	Forestry Commission	Guyana
22	Mr J P Gowela	Forestry Research Officer	Malawi
23	Prof. S R Khan	Pakistan Forest Institute	Pakistan
24	Prof. J S Owonubi	Forestry Research Institute of Nigeria	Nigeria
25	Dr D K Pushpakumara	University of Peradeniya	Sri Lanka
26	Dr E Kireger	Moi University	Kenya
27	Dr P Oballa	KEFRI	Kenya
28	Dr E M Shumba	Forestry Commission	Zimbabwe
29	Dr I Dawson	ICRAF	Kenya
30	Dr D S Hammond	Iuokrama International Centre	Guyana