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Genetic variation of *Prunus cerasoides* D. Don, a framework tree species in northern Thailand

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Abstract. *Prunus cerasoides* D. Don has been identified as an excellent 'framework tree species' for restoring evergreen forest in seasonally dry tropical forestlands. Here we describe the level of microsatellite variation in *P. cerasoides* trees within and among three National Parks in northern Thailand: Doi Suthep-Pui, Doi Inthanon and Doi Ang Khang, using published primers developed for peach, sweet cherry and sour cherry. The five microsatellite loci employed detected a total of 41 alleles, with the average number of alleles per locus per study site ranging from 2.7 to 8.0 ($n = 82$). The value of F_{ST} over the three sites was 0.115, indicating that while the majority of genetic diversity may be contained within sites, they should be considered as genetically distinct. The implications of this for seed collection of this species for forest restoration are discussed.

Introduction

Deforestation remains the most serious environmental problem in Thailand, causing floods and droughts, biodiversity loss and worsening rural poverty. If Thailand is to retain any significant natural forest cover in the long-term, stricter forest protection must be complemented with forest restoration on degraded sites, especially within protected areas (FORRU 1998). One method of forest restoration, which is proving successful in the highlands of northern Thailand, is the so-called 'framework species method' (Blakesley et al. 2000; Elliott et al. 2002). First developed in Queensland, Australia (Goosem and Tucker 1995; Lamb et al. 1997; Tucker and Murphy 1997), the method involves planting 20–30 indigenous tree species to rapidly restore basic forest structure and function, while relying on wildlife to bring about biodiversity recovery.

Initial planting trials that tested 39 potential framework tree species in deforested sites within Doi Suthep-Pui National Park identified *Prunus cerasoides* D. Don (Rosaceae) as meeting all the criteria of a framework species (Elliott et al. 2001). *P. cerasoides* is distributed throughout the mountains of the

Himalayas, Yunnan, Myanmar, northern Thailand and northern Indo-China, where it occurs in evergreen and deciduous forest, evergreen forest, and evergreen and pine forest, particularly in disturbed areas, at elevations of 1040–1700 m (FORRU 2000). The tree is a popular ornamental in northern Thailand and seeds have been collected from the wild and planted along roadsides for about 20 years. However, no studies have been carried out on genetic diversity within *P. cerasoides*, or on seed collection strategies for forest restoration programmes. The Convention on Biological Biodiversity (Rio de Janeiro, Brazil, 1992) emphasises the importance of maintaining intraspecific genetic diversity and evolutionary potential. Consequently, adaptability and the maintenance of a broad genetic base must be ensured. Genetic variation in a founding population is critical, particularly if restored areas are far from pollen sources. The collection of plant material from a few individuals can result in low effective population size, severe inbreeding depression and a decrease in the evolutionary adaptive potential of the population (Barrett and Kohn 1991). General guidelines for seed collection to maintain maximum genetic diversity have been published (e.g. Brown and Marshall 1995; Guarino 1995; Schmidt 2000). Molecular techniques however provide a valuable tool for measuring the genetic diversity of trees, thus contributing to decisions to enable better genetic management for forest restoration.

Nuclear microsatellites, which consist of tandemly repeated, short DNA sequence motifs are highly informative, PCR based molecular markers. They are believed to detect selectively neutral variation (Nauta and Weissing 1996), and should therefore be used with caution in conservation genetics (Hedrick 2001). However, microsatellites have been used to assess genetic variation in tropical tree species in threatened forests and forest fragments (Aldrich et al. 1998). The development of microsatellites can be time consuming and costly due to library generation and sequencing, even before primers can be identified, optimised and tested for polymorphism. Consequently the use of published primers from closely related species could be very useful, particularly for programmes where precise, practical answers, such as those posed above, are sought, as part of much broader, non-molecular programmes.

Microsatellites primers from *P. avium* L. (sweet cherry), *P. persica* (L.) Batsch (peach) and *P. cerasus* L. (sour cherry) (all Rosaceae) have been shown to be informative in *P. serotina* Ehrh., a temperate cherry of the New World (Downey and Iezzoni 2000). In particular, *P. persica* microsatellite primers have a high potential for cross-species amplification within the Rosaceae (Cipriani et al. 1999; Sosinski et al. 2000). The aims of this study were, therefore; to investigate the conservation of 11 microsatellite loci which were developed in peach, sweet cherry and sour cherry (Cipriani et al. 1999; Downey and Iezzoni 2000; Joobeur et al. 2000; Sosinski et al. 2000) and to assess molecular genetic diversity of *P. cerasoides* within and among three national parks in the north of Thailand; and to consider how this genetic information might contribute to local seed collection strategies for forest restoration.

Materials and methods

Study sites

Plant material was collected from three sites in northern Thailand. Doi Suthep-Pui National Park (18°43′–19°08′N, 98°48′–98°58′E) covers an area of 262.5 km^2 and rises from an elevation of 330–1,685 m. Doi Inthanon National Park (18°24′–18°41′N, 98°21′–98°38′E), includes the highest mountain in Thailand, rising to 2565 m elevation. It covers an area of 272 km^2 and is part of the Thanon Thongchai Range located south of Doi Suthep-Pui National Park. Doi Ang Khang (19°50′–19°57′N, 99°01′–99°06′E) covers an area of 91.4 km^2 and rises to an elevation of 1800 m. *P. cerasoides* buds were collected in March 2000 from 20 trees in Doi Inthanon National Park, 20 trees in Doi Ang Khang and 42 trees in Doi Suthep-Pui National Park. Trees selected were at least 100 m apart. Many trees sampled from each site were close to the roadside forest edge, suggesting that some may have been dispersed locally (collected and germinated or transplanted) by Forestry Department Officials. However, nineteen trees sampled in Doi Suthep-Pui were certainly naturally dispersed, due to their age and location, deep in the forest.

DNA isolation and microsatellite analysis

Total genomic DNA was extracted from *P. cerasoides* buds using the hexadecyltrimethyl ammonium bromide (CTAB) method described by Murray and Thompson (1980), with the following adaptations. Fresh buds (0.2 g) were ground into a fine powder in liquid nitrogen using a mortar and pestle and transferred to a 2 ml extraction tube. To this was added 800 µl of CTAB buffer (2% (w/v) CTAB, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris (pH 8), 2% PVP 40 (polyvinylpolypyrrolidone; mol wt. 40,000) and 1% 2-mercaptoethanol), pre-heated to 65 °C. The homogenates were mixed well by inverting, and incubated in a 65 °C water-bath for 3 min. The samples were then mixed again, extracted with an equal volume of chloroform:isoamyl alcohol (24:1) and centrifuged at 13,000 rpm for 10 min. If the solution appeared cloudy the chloroform:isoamyl extraction was repeated. The supernatant was then transferred to a new tube and the DNA was precipitated by adding 500 µl cold isopropanol, mixed by inversion, and centrifuged at 13,000 rpm for 5 min. The DNA pellet was washed in 500 µl of 70% ethanol, air dried and resuspended in 50 µl TE (10 mM Tris–HCl, 0.1 mM EDTA, pH 8) containing 10 µg μl^{-1} RNAse. The samples were incubated at 37 °C for 30 min to remove RNA prior to storage at 4 °C, or longer term storage at −20 °C. The DNA was estimated against lambda standards on agarose gels stained with ethidium bromide.

Microsatellite primers reported to display a high degree of polymorphism in other cultivated *Prunus* species were selected for the amplification reactions. These included five primers from peach (*Prunus persica* L. Batsch) (Cipriani

et al. 1999), two primers from sour cherry (*Prunus cerasus* L.) (Downey and Iezzoni 2000), and four primers from sweet cherry (*Prunus avium*) (Sosinski et al. 2000; Joobeur et al. 2000). Eighty two trees were screened with the above 11 primers. PCR amplification was performed in a 12.5 µl reaction mix containing 10 ng DNA, 10 mM Tris–HCl (pH 8), 1.5 mM $MgCl_2$, 50 mM KCl, 0.2 mM of each dNTP, 0.2 µM of reverse primer, 0.2 µM of forward primer labelled with γ^{33}P, and 0.025 units of Taq polymerase (Gibco-BRL). The following conditions were used: an initial denaturation step at 94 °C for 3 min, followed by 35 cycles of 94 °C for 30 s, annealing temperature for 1 min, 72°C for 1 min 30 s, and a final extension at 72 °C for 8 min.

One volume of 80% formamide containing 0.5% bromophenol blue and xylene cyanol was added to the PCR products. The microsatellites were denatured at 90 °C for 3 min and separated on a 6% denaturing sequencing gel in 1×TBE buffer at a constant 65 W for 2.5 h (Sambrook et al. 1989). The gel was dried onto Whatman 3MM paper and the microsatellites visualised following autoradiography (Kodak Biomax MR). The size of each fragment was estimated with reference to a size marker of 10 bp DNA ladder standard (GIBCO, BRL).

Genetic diversity

The mean observed heterozygosity (H_O, direct count estimate) and Hardy–Weinberg expected heterozygosity (H_E), and inbreeding coefficients (F_{IS}, F_{IT} and F_{ST}) (Wright 1965) were computed using GENEPOP version 3.3 (Raymond and Rousset 1995) for each locus and averaged over all loci. A bilateral test for departure from the Hardy–Weinberg equilibrium was also performed using GENEPOP. The distribution of alleles in the different populations was classified in two frequency classes, common ($P \geq 0.05$) or rare ($P < 0.05$), as suggested by Marshall and Brown (1975).

Results

Genetic diversity across the three sites

Three primer pairs from *P. avium* (PS12A02, PS05CO3, PS09FO8), two from *P. cerasus* (PceGA34, PceGA77) but only one of the five from *P. persica* (UDP98-409) amplified polymorphic loci with *P. ceraosides* (Table 1). Five of these primers were used for this study, as PceGA77 was difficult to score. The remaining five microsatellites were believed to be monomorphic. No more than two fragments per loci were amplified for each tree/primer pair combination.

Four to 12 alleles were detected at the five polymorphic loci, with a total of 41 alleles identified among the 82 trees collected from the three sites. The allele

Table 1. Summary of primer pair characteristics when screened with 82 *P. cerasoides* trees

Species	Primer	Primer source	Repeat motif	Annealing temp (°C)	Size range of products (bp)	No. of alleles
Sour cherry	PceGA34	Downey and Iezzoni 2000	$(GA)_{25}$	57.0	148–170	4
	PceGA77	Downey and Iezzoni 2000	$(AG)_{13}$	51.0	160–163	3
Sweet cherry	PS12A02	Sosinski et al. 2000	$(GA)_{22}$	55.0	151–177	11
	PS08E08	Sosinski et al. 2000	$(CAA)_7$	58.0	172	1
	PS05CO3	Sosinski et al. 2000	$(GA)_{30}$	50.0	108–136	10
	PS09F08	Joobeur et al. 2000	$(GA)_{17}$	50.0	138–148	4
Peach	UDP98-406	Cipriani et al. 1999	$(AG)_{15}$	50.0	105	1
	UDP98-405	Cipriani et al. 1999	$(AG)_9$	55.0	105	1
	UDP96-003	Cipriani et al. 1999	$(CT)_{11}(CA)_{28}$	55.0	87	1
	UDP98-409	Cipriani et al. 1999	$(AG)_{19}$	55.0	150–184	12
	UDP96-018	Cipriani et al. 1999	$(AC)_{21}$	56.4	250	1

Table 2. Allele frequencies of five microsatellite loci in trees collected in; Doi Inthanon; Doi Ang Khang; and in Doi Suthep-Pui (total, and split into two sub-groups based on dispersal)

Locus	Allele (bp)	Frequency				
		Doi	Doi Ang	Doi	Doi Suthep-Pui	
		Inthanon	Khang	Suthep-Pui	Unknown dispersal	Natural dispersal
PceGA34	148	0.875	0.9	0.622	0.7	0.529
	150	0.075	0	0	0	0
	154	0.025	0.1	0.378	0.3	0.471
	170	0.025	0	0	0	0
UDP98-409	150	0.05	0.125	0.15	0.167	0.132
	154	0.075	0.125	0.013	0.024	0
	156	0.2	0.175	0.575	0.476	0.684
	162	0.05	0.4	0.138	0.167	0.105
	166	0.2	0	0	0	0
	170	0.175	0.075	0.062	0.119	0
	172	0.15	0.025	0.013	0.024	0
	174	0.05	0	0	0	0
	180	0.05	0	0	0	0
	184	0	0.075	0	0	0
	152	0	0	0.013	0.024	0
	160	0	0	0.038	0	0.079
PS05C03	108	0	0	0.013	0.022	0
	110	0	0.075	0	0	0
	116	0.05	0	0.013	0.022	0
	118	0.175	0.3	0.312	0.391	0.206
	120	0.375	0.2	0.2	0.217	0.176
	124	0	0.025	0	0	0
	126	0.225	0.175	0.062	0.109	0
	128	0.125	0.15	0.4	0.239	0.618
	130	0.025	0.075	0	0	0
	136	0.025	0	0	0	0
PS09F08	138	0.775	0.6	0.308	0.348	0.25
	140	0.1	0.25	0.397	0.304	0.531
	142	0.125	0	0.051	0.022	0.094
	148	0	0.15	0.244	0.326	0.125
PS12A02	151	0.5	0	0	0	0
	153	0.05	0.15	0.103	0.13	0.063
	155	0.2	0.05	0.256	0.13	0.438
	157	0	0.125	0.013	0.022	0
	159	0.175	0.125	0.013	0.022	0
	161	0	0.05	0	0	0
	163	0	0.125	0.192	0.304	0.031
	167	0.05	0.075	0.308	0.348	0.25
	171	0	0.075	0	0	0
	177	0.025	0.2	0.115	0.043	0.219
	152	0	0.025	0	0	0

Table 3. Descriptive statistics for the five microsatellite loci studied over all sites

Locus	Total number of alleles	Mean A[a]	H_E	H_O
PceGA34	4	2.667	0.292	0.250
PS12A02	11	8.000	0.689	0.420***
PS05CO3	10	6.667	0.623	0.580*
PS09F08	4	3.333	0.509	0.390***
UDP98-409	12	5.667	0.636	0.490***

[a] Mean no alleles per locus per population.
Significant departure from Hardy–Weinberg equilibrium (*$P < 0.05$; ***$P < 0.001$).

Table 4. F-Statistic analysis estimates of the parameters F_{IS}, F_{IT}, and F_{ST}

Locus	F_{IS}	F_{IT}	F_{ST}
PceGA34	0.042	0.192	0.156
PS12A02	0.332	0.421	0.133
PS05CO3	0.040	0.086	0.049
PS09F08	0.158	0.274	0.137
UDP98-409	0.163	0.265	0.122
All	0.162	0.259	0.115

Table 5. Measure of microsatellite DNA genetic diversity in the three sites

	Doi Suthep-Pui	Doi Ang Khang	Doi Inthanon	Total
No. of trees	42	20	20	82
Allele number over five loci	27	29	29	41
Unique alleles	3	6	7	16
Alleles common to all populations	19	19	19	19
Mean no. of alleles per loci	5.4	5.8	5.8	8.2
H_E	0.662	0.655	0.595	0.697
H_O	0.574*	0.530***	0.480*	0.539***
F_{IS}	0.133	0.192	0.193	0.162

Significant departure from Hardy–Weinberg equilibrium (*$P < 0.05$; ***$P < 0.001$).

frequencies are presented in Table 2. The average number of alleles per locus per site was 2.7 for PceGA34, 8 for PS12A02, 6.7 for PS05CO3, 3.3 for PS09F08 and 5.7 for UDP98-409 (Table 3). The observed heterozygosity (H_O) for each locus ranged from 0.250 to 0.580, and deviated significantly from Hardy–Weinberg expectations for locus PS12A02, UDP98-409, PS09F08 (heterozygote deficiency, $P < 0.001$) and PS05CO3 (heterozygote deficiency, $P < 0.05$). Based on Wright's F_{IS} values (Table 4) the deviations are due to an excess of homozygotes (positive F_{IS} values).

The allelic richness and the mean number of alleles per locus were very similar between the three sites (Table 5). For each locus, the number of unique alleles was two for PceGA34, four for PS12A02, four for PS05CO3, six for

Table 6. Distribution of 41 alleles in allele frequency classes for three sites

Allele frequency class	Number of alleles (% of total number of alleles)		
	Doi Suthep-Pui	Doi Ang Khang	Doi Inthanon
Total no. alleles	27	29	29
Common ($P \geq 0.05$)	18 (0.667)	26 (0.897)	24 (0.828)
Rare ($P < 0.05$)	9 (0.333)	3 (0.103)	5 (0.172)

UDP98-409 and none for PS09F08 (Table 2). In total, seven of the alleles present in the Doi Inthanon sample were unique, in comparison to six in Doi Ang Khang and three in Doi Suthep-Pui. When alleles were classified as rare or common (Table 6), the majority of the alleles were distributed in the common class (66.7–89.7% across the three sites). All of the trees had a unique set of alleles, and consequently could be distinguished from each other.

For each site, over all the loci, the expected heterozygosity (H_E) was significantly higher than the observed heterozygosity (H_O) ($P < 0.05$), leading to positive inbreeding coefficients (F_{IS}). For each site, the observed heterozygosity ranged from 0.480 to 0.574, with an average heterozygosity of 0.539 (Table 5), indicating considerable genetic diversity within the sites. The levels of inbreeding for each population ranged from 0.133 to 0.192, with an average inbreeding coefficient of 0.162 (Table 5), indicating a low level of inbreeding. To assess the degree of population differentiation between the three sites, the infinite alleles theoretical model (F_{ST}) was used. Over the three sites, *F*-statistic analysis showed positive levels of F_{IS}, F_{IT}, and F_{ST} (0.162, 0.259 and 0.115 respectively) (Table 4). The overall F_{ST} for the three populations (significantly higher than zero, $P < 0.001$) indicated that 11.5% of the variation was attributable to differentiation among the populations.

Genetic diversity within Doi Suthep-Pui

Within the 42 trees selected in Doi Suthep-Pui, 19 were believed to be naturally dispersed, due to their size and location, deep in the forest. Consequently, splitting the Doi Suthep-Pui site into two sub-locations allowed a further comparison to be carried out between trees believed to be naturally dispersed with those of unknown dispersal (Table 7). Of the 27 alleles detected in Doi Suthep-Pui, 26 were found in unknown dispersed trees, and only 18 in the naturally dispersed trees, which represents a 31% reduction in allelic richness. The one unique allele found in the naturally dispersed trees (locus UDP98-409) was also unique among the three sites (Tables 2 and 7). The mean number of alleles ranged from 5.2 (unknown dispersal) to 3.6 (naturally dispersed). The observed heterozygosity of the naturally dispersed trees (0.491), was lower than that of the trees of unknown dispersal (0.636). In the trees of unknown dispersal the expected heterozygosity (H_E) was significantly higher ($P < 0.001$) than the observed heterozygosity (H_O), leading to a positive inbreeding coefficient (F_{IS}).

Table 7. Measure of microsatellite DNA genetic diversity within Doi Suthep-Pui National Park, when split into two subpopulations; comparing trees believed to be naturally dispersed with those of unknown dispersal

	Dispersal	
	Unknown	Natural
No. of trees	23	19
Allele number over five loci	26	18
Unique alleles	9	1
Alleles common to both populations	17	17
Mean no. of alleles per loci	5.2	3.6
H_E	0.678	0.589
H_O	0.636	0.491***
F_{IS}	0.061	0.162

Significant departure from Hardy–Weinberg equilibrium (***$P < 0.001$).

Discussion

This is the first report concerning genetic diversity in *P. cerasoides*. The cross species amplification of sour cherry, sweet cherry and peach microsatellite primers reported here was expected, as similar transportability has been demonstrated in other *Prunus* spp. (Cipriani et al. 1999). The five microsatellite loci employed in this study detected a total of 41 alleles, with the average number of alleles per locus per study site ranging from 2.7 to 8.0 ($n = 82$). There is no similar study of *P. cerasoides* with allozyme loci for comparison. The informativeness of the microsatellite loci varied from three to 12 alleles, with an average of 8.2 alleles found over all loci. This accords closely with the mean number of alleles per locus of 10.7 in *P. cerasus* ($n = 59$) (Cantini et al. 2001), 6.7 in *P. mahaleb* ($n = 182$) (Godoy and Jordano 2001) and 12.1 in *Malus* × *domestica* ($n = 66$) (Hokanson et al. 1998). The longest repeat, PS05CO3, a $(GA)_{30}$ repeat detected 10 alleles, although the more informative UDP98-409, a $(AG)_{19}$ repeat is much smaller. There was no direct relationship between allele number and the average number of repeats, as was also observed by Weber (1990). The range of heterozygosity was broad (0.250–0.580) across the three sites, averaging 0.539 over all loci. The levels of heterozygosity detected for each site over all loci indicated that each collection of trees exhibited a high level of genetic variation. Comparison between the trees of unknown and natural dispersion in Doi Suthep-Pui demonstrated a considerable difference in terms of allelic richness, although only a single allele was found amongst the naturally dispersed trees which was unique. This could be due to the lower sample size of naturally dispersed trees, or to the relatedness of the parents of these trees.

We have applied Wright's *F*-values to the dataset, but believe that the results should be viewed with some caution, until further evidence is gathered from more trees known to be naturally dispersed. The F_{IS} values indicate that the

samples of trees in the three sites were outbreeding, with little inbreeding. If there was a considerable amount of inbreeding, it might be expected to be evident across all of the loci, which was not the case here. It is possible that the deviations from Hardy–Weinberg and the homozygote excesses, which we observed with four loci, were due to the presence of null alleles (Weber and May 1989; Pemberton et al. 1995). The value of F_{ST} over the three sites was 0.115, indicating that whilst the majority of genetic diversity may be contained within the sites, they should be considered as genetically distinct. No similar studies of genetic diversity have been undertaken with other *Prunus* spp. using nuclear microsatellites. However, using RAPD markers, Jordano and Godoy (2000) found high levels of within population genetic diversity with *Prunus mahaleb* populations extending over a relatively restricted area of approximately 100 km^2. A significant, but low among-population variation of 16.46% was very similar to that reported here. Studies of wild *P. avium* populations in France using isoenzymes (Santi 1988; Frascaria et al. 1993; Mariette et al. 1997) showed little differentiation among populations and a comparatively high level of genetic diversity.

Microsatellite markers present an extremely good overall measure of the level of neutral genetic diversity that is present in a population. Although the mutation rate is high in microsatellite regions, it is likely that high microsatellite allelic diversity indicates a high level of 'general' genetic variation. High neutral variation might also indicate the potential for significant adaptive variation (Hedrick 2001). Here, a relatively simple microsatellite study of genetic diversity of mother trees has contributed to a more informed selection of seed trees in our practical forest restoration programme. Firstly, the F_{ST} value indicated that the three populations are genetically distinct, consequently, seeds should be collected locally, and not moved between the three National Parks. There are large areas of degraded forestland outside the National Parks, which are devoid of local sources of seed. Seed could be supplied to these areas from the closest source of parent trees. Secondly, the data suggests a large amount of genetic diversity in *P. cerasoides* because of the high number of low-frequency microsatellite alleles. It is important that, in a forest restoration programme such as ours, some consideration is given to the conservation of genetic diversity. Our data suggests that seeds should be collected from as many trees as possible, certainly within, or close to the FAO recommendation of 25–50 individuals per population (FAO Forest Resources Division 1995).

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References

Aldrich P.R., Hamrick J.L., Chavarriaga P. and Kochert G. 1998. Microsatellite analysis of demographic genetic structure in fragmented populations of the tropical tree *Symphonia globulifera*. Mol. Ecol. 7: 933–944.

Barrett S.C.H. and Kohn J.R. 1991. Genetic and evolutionary consequences of small population size in plants: implications for conservation. In: Falk D.A. and Holsinger K.E. (eds) Genetics and Conservation of Rare Plants. Oxford University Press, Oxford, pp. 3–30.

Blakesley D., Anusarnsunthorn V., Kerby J., Navakitbumrung P., Kuarak C., Zangkum S., Hardwick K. and Elliott S., 2000. Nursery technology and tree species selection for restoring forest biodiversity in northern Thailand. In: Elliott S., Kerby J., Blakesley D., Hardwick K., Woods K. and Anusarnsunthorn V. (eds) Forest Restoration for Wildlife Conservation. International Tropical Timber Organisation and The Forest Restoration Research Unit, Chiang Mai University, Thailand, pp. 207–220.

Brown A.H.D. and Marshall D.R. 1995. A basic sampling strategy: theory and practice. In: Guarino L., Ramanath Rao V. and Reid R. (eds) Collecting Plant Genetic Diversity (Technical Guidelines). CAB International, Wallingford, 748 pp.

Cantini C., Iezzoni A.F., Lamboy W.F., Boritzki M. and Struss D. 2001. DNA fingerprinting of tetraploid cherry germplasm using simple sequence repeats. J. Am. Soc. Hort. Sci. 126: 205–209.

Cipriani G., Lot G., Huang W.-G., Marrazzo M.T., Peterlunger E. and Testolin R. 1999. AC/GT and AG/CT microsatellite repeats in peach [*Prunus persica* (L.) Batsch]: isolation, characterisation and cross-species amplification in *Prunus*. Theor. Appl. Gen. 99: 65–72.

Downey S.L. and Iezzoni A.F. 2000. Polymorphic DNA markers in black cherry (*Prunus serotina*) are identified using sequences from sweet cherry, peach and sour cherry J. Am. Soc. Hort. Sci. 125: 76–80.

Elliott S., Navakitbumrung P., Kuarak C., Zangkum S., Blakesley D. and Anusarnsunthorn V. 2001. Testing framework tree species for restoring biodiversity on degraded forestland in northern Thailand. Proceedings of the tropical forest symposium: the art and practice of conservation planting.

Elliott S., Kuarak C., Navakitbumrung P., Zangkum S., Anusarnsunthorn V. and Blakesley D., 2002. Propagating framework trees to restore seasonally dry tropical forest in northern Thailand. New Forest. 23: 63–70.

FAO. 1995. State of the world's forests.

Forest Restoration Research Unit. 1998. Forests for the future: growing and planting native trees for restoring forest ecosystems. Elliott S., Anusarnsunthorn V. and Blakesley D. (eds), 60 pp.

Forest Restoration Research Unit. 2000. Tree seeds and seedlings for restoring forests in northern Thailand. Kerby J., Elliott S., Blakesley D. and Anusarnsunthorn V. (eds) Biology Department, Science Faculty, Chiang Mai University, Thailand, 152 pp.

Frascaria N., Santi F. and Gouyon P.H. 1993. Genetic differentiation within and among populations of chestnut (*Castanea sativa* Mill.) and wild cherry (*Prunus avium* L.). Heredity 70: 634–641.

Godoy J.A. and Jordano P. 2001. Seed dispersal by animals: exact identification of source trees with endocarp DNA microsatellites. Mol. Ecol. 10: 2275–2283.

Goosem S.P. and Tucker N.I.J. 1995. Repairing the rainforest – theory and practice of rainforest reestablishment in north Queensland's wet tropics. Wet Tropics Management Authority, Cairns, 71 pp.

Guarino L. 1995. Assessing the threat of genetic erosion. In: Guarino L., Ramanath R.V. and Reid R. (eds) Collecting plant genetic diversity (Technical guidelines). CAB International, Wallingford, 748 pp.

Hedrick P.W. 2001. Conservation genetics: where are we now? Trends Ecol. Evol. 16: 629–636.

Hokanson S.C., Szewc-McFadden A., Lamboy W. and McFerson J. 1998. Microsatellite (SSR) markers reveal genetic identities, genetic diversity and relationships in a *Malus* × *domestica* Borkh. core subset collection. Theor. Appl. Genet. 97: 671–683.

Joobeur T., Periam N., de Vicente M.C., King G.J. and Arús P., 2000. Development of a second generation linkage map for almond using RAPD and SSR markers. Genome 43: 649–655.

Jordano P. and Godoy J.A. 2000. RAPD variation and population genetic structure in *Prunus mahaleb* (Rosaceae), an animal dispersed tree. Mol. Ecol. 9: 1293–1305.

Lamb D., Parrotta J., Keenan R. and Tucker N.I.J. 1997. Rejoining habitat remnants: restoring degraded rainforest lands. In: Laurence W.F. and Bierrgaard Jr. R.O. (eds) Tropical forest remnants: ecology, management and conservation of fragmented communities. University of Chicago Press, Chicago, IL, pp. 366–385.

Mariette S., Lefranc M., Legrand L., Taneyhill D., Frascaria-Lacoste N. and Machon N. 1997. Genetic variability in wild cherry populations in France. Effects of colonizing processes. Theor. Appl. Genet. 94: 904–908.

Marshall D.R. and Brown A.H.D. 1975. Optimum sampling strategies in genetic conservation. In: Frankel O.H. and Hawkes J.G. (eds) Crop genetic resources for today and tomorrow. Cambridge University Press, London, pp. 53–80.

Murray M.G. and Thompson W.F. 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acid Res. 8: 4321–4325.

Nauta M.J. and Weissing F.J. 1996. Constraints on allele size at microsatellite loci: implications for genetic differentiation. Genetics 143: 1021–1032.

Pemberton J.M., Slate J., Bancroft D.R. and Barrett J.A. 1995. Non-amplifying alleles at microsatellite loci: caution for parentage studies. Mol. Ecol. 4: 249–252.

Raymond M. and Rousset F. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J. Heredity 86: 248–249.

Sambrook J., Fritsch E.F. and Manniatis J. 1989. Molecular cloning. A laboratory manual. Cold Spring Harbour Laboratory Press, New York.

Santi F. 1988. Variabilité génétique intra et interpopulations chez le Mersier (*Prunus avium* L.). Thèse Docteur de l'Institut National Agronomique Paris-Grignon, INA-PG.

Schmidt L. 2000. Guide to handling of tropical and subtropical forest seed. Danida Forest Tree Centre, Denmark.

Sosinski B., Gannavarapu M., Hager L.D., Beck L.E., King G.J., Ryder C.D., Rajapakse S., Baird W.V., Ballard R.E. and Abbott A.G. 2000. Characterisation of microsatellite markers in peach [*Prunus persica* (l.) Batsch]. Theor. Appl. Genet. 101: 421–428.

Tucker N.I.J. and Murphy T.M. 1997. The effects of ecological rehabilitation on vegetation recruitment: some observation from the wet tropics of north Queensland. For. Ecol. Manage. 99: 133–152.

Weber J.L. 1990. Informativeness of human $(cC\text{-}dA)_n$ $(dG\text{-}dT)_n$ polymorphisms. Genomics 7: 524–530.

Weber J.L. and May P.E. 1989. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am. J. Human Gen. 44: 388–396.

Wright S. 1965. The interpretation of a population structure by *F*-statistics with special regard to systems of mating. Evolution 19: 395–420.