New Forests
DOI 10.1007/s11056-007-9059-2

Genetic variation and gene flow among *Prunus cerasoides* D. Don populations in northern Thailand: analysis of a rehabilitated site and adjacent intact forest

Greuk Pakkad · Suad Al Mazrooei · David Blakesley · Celia James · Stephen Elliott · Tapio Luoma-Aho · Jarkko Koskela

Received: 28 September 2006 / Accepted: 10 June 2007

Abstract This study describes the level of genetic variation and gene flow within and among populations of *Prunus cerasoides* in rehabilitated sites and adjacent intact forest. The seven microsatellite loci employed detected a total of 75 alleles (n = 401). Polymorphic information content (PIC) varied from 0.34 to 0.83. Between the adult populations there was moderate genetic differentiation with an F_{ST} value of 0.0575, which suggests that the restoration plots had a similar genetic composition to that of the natural population. The gene flow assessment provides some interesting insights into the genetic diversity of *P. cerasoides*. In the 16 naturally occurring trees over 83% of the genotyped seed were fathered by unidentified trees whereas in restoration plot A only about 32% of the pollen came from an unidentified father. This proportion was even less in Plot C where 25% of the pollen parents were unidentified. The naturally occurring trees within Doi Suthep were surrounded by planted trees, which were contributing to the paternity of the seed crop. This result demonstrates that ''fill in'' planting should consist of locally sourced material if it is considered important to conserve the genetic integrity of the local populations.

G. Pakkad (✉) · S. Elliott
Forest Restoration Research Unit, Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand
e-mail: greuk@hotmail.com

S. Al Mazrooei
Department of Biological Sciences, Faculty of Science, Kuwait University, P.O. Box 5969, Safat 13060, Kuwait

D. Blakesley
Wildlife Landscape, Bearsted, Kent, UK

C. James
East Malling Research, New Road, East Malling, Kent ME19 6BJ, UK

T. Luoma-Aho · J. Koskela
Bioversity International – Headquarters, Via dei Tre Denari, 472a, Maccarese, Rome 00057, Italy

Keywords Forest restoration · Tropical trees · Genetic diversity · Microsatellites · Paternity analysis

Introduction

The most serious environmental problem in Thailand today is deforestation as it leads to increased floods and droughts, loss of biodiversity and a worsening of rural poverty (Elliott et al. 1995). Despite the introduction in 1989 of a ban on commercial logging on state-owned forestland, deforestation continues at an annual rate of approximately 2.6%, the highest in continental SE Asia (FAO 1997). Even the establishment of an extensive system of protected areas (covering more than 15% of the country) has failed to prevent forest cover from dwindling to less than 20% of Thailand's land area (Leungaramsri and Rajesh 1992). It is increasingly clear that, if Thailand is to retain any significant natural forest cover in the long-term, stricter forest protection and management must be complemented by forest restoration on degraded sites (FORRU 1998, http://www.unep-wcmc.org/forest/restoration/docs/Thailand.pdf).

In many circumstances where there is large-scale deforestation it is appropriate to practice active forest restoration to aid conservation of the remaining biodiversity (UNCBD, Articles 7, 8, 10 (http://www.biodiv.org/convention/articles.asp)). The project reported here concerns restoration of the seasonally dry tropical forests, which are characteristic of South East Asia region. These forests grow in regions, which have a wet season between May and October when most of the annual rain of between 167 cm and 209 cm falls. This is followed by a dry season between the months of November and April. These seasonally dry forests are now considered to be even more endangered than tropical rainforests (Blakesley et al. 2000) and therefore this project is both timely, and important. Although countries are now addressing the problem of deforestation by protecting remaining forest, such forests are often too degraded to meet the criteria of a healthy, natural forest. Habitat restoration is now considered to be an important approach in conservation biology and this approach is likely to be increasingly adopted in the future (FAO 2001). There is, therefore, an urgent need to obtain sound genetic data to assist our understanding of this process so that useful policies can be developed.

The Forest Restoration Research Unit (FORRU) was established in November 1994 to tackle some of the technical problems involved in re-establishing natural forest ecosystems on degraded sites within national parks and wildlife sanctuaries in Northern Thailand (Elliott et al. 1995). FORRU has adopted the so-called ''framework species method'' and found it to be successful in the highlands of northern Thailand (Blakesley et al. 2000). First conceived in Queensland, Australia (Goosem and Tucker 1995; Lamb et al. 1997; Tucker and Murphy 1997), the method involves the planting of 20–30 native tree species to bring about rapid restoration of basic forest structure and function. Framework tree species are selected for their capability to survive well, grow fast, shade out herbaceous weeds and attract seed-dispersing wildlife into planted areas with fruit, nectar or nest sites for birds whilst relying on wildlife to bring about more generalised biodiversity recovery.

FORRU now operates a tree-planting programme which utilises local labour to prevent further loss of forest cover and to bring about biodiversity recovery (UNCBD, Article 8). However, seedlings produced by FORRU may be the product of seed collected from a

limited number of mother trees and this may mean that the new populations in reforestation sites have a rather narrow genetic base. The Convention on Biological Biodiversity emphasises the importance of maintaining intra-specific genetic diversity so that populations have the capacity to evolve and adapt to future environmental changes. Consequently, the maintenance of a broad genetic base must be ensured in order to retain adaptability. Genetic variation in a founding population is critical, particularly if restored areas are far from pollen sources (Blakesley and Pakkad 2004). Framework species planted in degraded areas will need to be able to adapt to the ''new'' environment of the restoration plots. They may also become sources of seed for other degraded areas, both through local natural dispersal, and through seed collection for planting. Data for pollination distances are difficult to obtain and are not available for the species used in the restoration programmes. It therefore remains unknown how far pollen travels and how far restoration plots can be from natural forest and still retain genetic contact via pollen flow.

Microsatellite markers are powerful tools for studying genetic diversity and gene flow in natural plant populations (Chase et al. 1996; Dow et al. 1995; Dayanandan et al. 1997; Streiff et al. 1998; Ueno et al. 2000) because of their high allelic diversity, and co-dominant inheritance. One potential drawback of microsatellite marker is the presence of null alleles. This is often seen as elevated levels of homozygosity, as the heterozygote with an amplifying and a non-amplifying allele is scored as a homozygote (Dakin and Avise 2004).

Prunus cerasoides has been identified as an excellent ''framework tree species'' for restoring evergreen forest in seasonally dry tropical forestlands in Thailand (Elliott et al. 2002). *P. cerasoides* is distributed from the Himalayas and South China to Myanmar and North Indochina, where it occurs in evergreen and deciduous forest, evergreen forest, and evergreen and pine forest, particularly in disturbed areas, at elevations of 1,040–1,700 m (FORRU 2000). Pakkad et al. (2004) examined genetic diversity of *P. cerasoides* within and between three National Parks in northern Thailand: Doi Suthep-Pui; Doi Inthanon and Doi Ang Khang, using published primers developed for *Prunus persica* (Cipriani et al. 1999), *Prunus cerasus* (Downey and Iezzoni 2000) and *Prunus avium* (Sosinski et al. 2000). The five microsatellite loci employed detected a total 41 alleles, with the average number of alleles per locus per study site ranging from 2.7 to 8.0. 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, the sites could be considered to be genetically distinct (Muluvi et al. 1999; Bussell et al. 2006). This suggested that seed should be collected locally, and not moved between the three National Parks, and should be collected from much higher numbers of trees than is currently practiced, to maintain genetic diversity. However, there are some important questions which were not addressed by this study; firstly, four of the five microsatellites used in the previous study showed significant deviations from Hardy–Weinberg equilibrium with an excess of homozygotes. Although this could indicate that there are significant levels of inbreeding in the populations studied it could equally be due to the presence of null alleles at these loci. Secondly, there was no assessment of the genetic diversity present in planted restoration plots in the previous study. Similarly, the previous study made no attempt to examine gene flow in natural and planted populations. Therefore in this study, a new set of seven microsatellites were used to examine diversity in naturally occurring trees in Doi Suthep-Pui National Park and gene flow in intact forest and restoration plots to see whether the trees in the restoration plots are genetically isolated, or whether they receive pollen from outside the plots.

Materials and methods

Sample collection

Plant material was sampled from two areas within Doi Suthep-Pui National Park, Chiang Mai Province, Thailand (18°43′–19°08′ N; 98°48′–98°58′ E). These consisted of three FORRU forest restoration plots located in Mae Sa Mai, and an area of primary forest, approximately 2 km from the restoration plots. The area of primary forest, which was sampled consisted of 16 trees. These natural trees population was surrounded by large numbers of *P. cerasoides* trees, which had been planted along roadside by the Thai Royal Forest Department. The seed used to establish the restoration plots (A, B, C located within 1,500 m of each other) by FORRU in 1997, 1998 and 1999 were collected from fewer than 10 seed trees (exact number unknown) located within Doi Suthep-Pui National Park (Fig. 1).

Young leaves were also collected from all 96 flowering trees in FORRU's restoration plots; 23, 28 and 45 trees from restoration plots A, B and C, respectively. Leaf material was stored at −80°C until use. For paternity analysis, seeds were collected from five mother trees in Doi Suthep natural forest and all fruiting trees including three mother trees in restoration plot A (trees 1, 7, 9) and eight mother trees in plot restoration plot C (53, 54, 55, 64, 65, 83, 86, 87) (Fig. 1). Trees on restoration plot B was not set fruit. Seeds from each mother tree were collected and sown in modular germination trays. The first true leaves of each germinated seeds from each seed tree were harvested, stored at −80°C and used at a later date for DNA extraction. The available number of seed from each mother tree varied from 6 to 20. All DNA extractions were carried out in the laboratories of East Malling Research, England prior to their analysis.

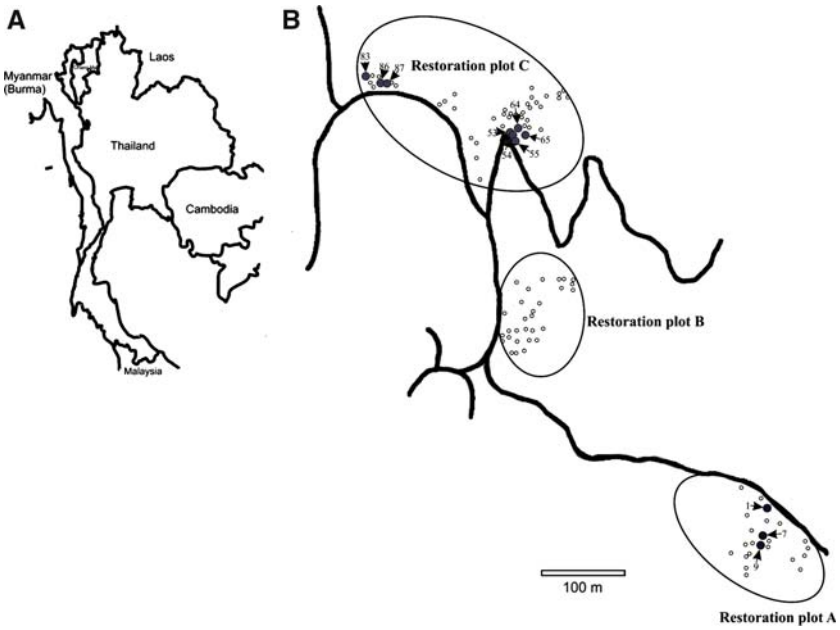

Fig. 1 (**A**) Map of Thailand and (**B**) FORRU restoration plot A, B and C, located in Chiang Mai Province, Thailand

Microsatellite analysis

Total genomic DNA was extracted from a young leaf of a seed tree and seedlings of individual trees using the hexadecyltrimethyl ammonium bromide (CTAB) method described by Murray and Thompson (1980). PCR reactions were performed in a final volume of 12.5 µl containing 2.5 ng genomic DNA, 1× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 µM each primer and 0.25 U *Taq* polymerase (Invitrogen). The following touchdown PCR conditions were used: 94°C for 90 s followed by 10 cycles of 94°C for 30 s, 60°C for 45 s (−0.5°C per cycle), 72°C for 1 min and then 25 cycles of 94°C for 30 s, 55°C for 45 s, 72°C for 1 min with an elongation step of 72°C for 5 min. Primer pairs which were developed in *Prunus avium* by Clarke and Tobutt (2003), were tested for amplification in *Prunus cerasoides*. The forward primers were re-ordered fluorescently tagged with 6-FAM, VIC, NED or PET (Applied Biosystems). The screening of the populations was undertaken using a 3100 ABI prism Sequencer (Applied Biosystems). Genescan 500-250LIZ was used as the internal size standard and data were analysed using GENESCAN and GENOTYPER software (Applied Biosystems).

The mean observed heterozygosity (H_O, direct count estimate), the Hardy–Weinberg expected heterozygosity (H_E) and the inbreeding coefficients (F_{IS}, F_{IT} & F_{ST}) (Wright 1965) were computed using GENEPOP computer program (version 3.3, Raymond and Rousset 1995) for each locus and averaged over all loci. A bilateral test for departure from Hardy-Weinberg equilibrium was also performed using GENEPOP. Paternity analysis was computed using CERVUS 2.0 (Marshall et al. 1998) by comparing genotypes of candidate fathers and seedlings after subtraction of the maternal contribution to each seed. This system was based on the assumption that in the cases where no adult tree from those genotyped within the plot of origin of the mother tree matched the paternal contribution to the seed, then the father of that seed was not one of the genotyped trees within the plot.

Results

Total genetic diversity in all samples

All 10-primer pairs from *Prunus avium* amplified polymorphic loci in *P. cerasoides*, although three, (EMPA013, EMPA015 and EMPA016) were not included in the study due to complex banding patterns. No more than two clearly discernible alleles were amplified for each tree/primer pair combination. The seven loci were found to be highly polymorphic, with 7–14 alleles detected (Table 1). A total of 75 alleles were identified among the 401 individuals. The average number of alleles from the seven populations (four adult and three seed populations) was greatest for locus EMPA005 and least for locus EMPA010. The observed heterozygosity (H_O) for each locus ranged from 0.327 (EMPA010) to 0.842 (EMPA001) (Table 1) and deviated significantly from Hardy–Weinberg expectations for locus EMPA001, EMPA005, EMPA014, EMPA018 and EMPAJ54 (heterozygote deficiency, $P < 0.001$).

Genetic diversity of the parental populations

Both the observed and expected heterozygosity were greatest in the trees in the Doi Suthep natural population. The total number of alleles detected was least in the natural population

Table 1 Characteristics of the seven microsatellite markers (Clarke and Tobutt 2003)

Locus	No. of Alleles	Size range of produces (bp)	Mean effective number of alleles	PIC	H_E	H_O
EMPA001	13	129–179	6.72	0.834	0.852	0.842***
EMPA005	14	230–266	3.75	0.693	0.734	0.701***
EMPA009	8	213–227	2.34	0.531	0.573	0.559
EMPA010	7	100–138	1.56	0.339	0.358	0.327
EMPA014	12	230–258	4.35	0.742	0.771	0.776***
EMPA018	11	77–116	6.67	0.833	0.851	0.786***
EMPAJ54	10	187–213	2.56	0.577	0.610	0.537***

Significant departure from Hardy–Weinberg equilibrium (***$P < 0.001$); PIC, Polymorphic information content; H_E, Expected heterozygosity; H_O, observed heterozygosity

of 16 trees and greatest in restoration plot C. The total number of alleles in the adult population was 64 of which, 40, 43, 45 and 52 occurred in the natural population and restoration plots A, B and C, respectively (Table 2). The effective number of alleles was greatest in the restoration plot C and least in restoration plot B. Within the four adult populations there were 3, 3, 4 and 6 unique alleles in the natural population and restoration plots A, B and C, respectively. The F_{ST} value of 0.0575 demonstrates that there is some degree of differentiation between the four adult populations. With the exception of restoration plot C the majority of F_{IS} values at each locus for the other adult populations were largely negative indicating an excess of heterozygotes. This suggests that null alleles at these loci are rare.

Genetic diversity of the offspring populations

Diversity parameters for seven microsatellite loci in offspring populations are reported in Table 2. Observed and expected heterozygosities in the seed from the Doi Suthep natural

Table 2 The number of individuals genotyped, observed and expected heterozygosity, the F_{IS} value, total number of alleles, the number and effective number of alleles and the number of unique alleles for the seven loci in the adult and seed populations

Population		N	H_O	H_E	F_{IS}	Total number of alleles	Number of alleles per locus	Effective number of alleles	Number of unique alleles[a]
Doi Suthep	Adult	16	0.77	0.71	−0.17	40	5.71	3.66	0
	Seed	100	0.61	0.62	−0.04	58	8.29	3.75	8
Restoration plot A	Adult	23	0.75	0.69	−0.11	43	6.14	3.53	2
	Seed	53	0.62	0.61	−0.03	35	5.00	2.98	0
Restoration plot B	Adult	28	0.64	0.60	−0.09	45	6.43	3.11	1
Restoration plot C	Adult	45	0.57	0.66	0.14	52	7.57	3.94	2
	Seed	136	0.60	0.61	0.01	49	7.00	3.40	2
Total		401	0.65	0.68	−0.04	75	10.71	3.99	15

[a] Defined as unique within the seven population (four adult and three seed populations)

population were similar to those in restoration plot A and C. The total number of alleles was 58, 35 and 49 in the seed gathered from the natural population and the trees in restoration plots A and C, respectively. The seeds from the Doi Suthep trees had 16 alleles, which were absent from the other two seed populations. In contrast, the seed from restoration plot A had no unique alleles whereas restoration plot C had six alleles, which were absent from the other two seed populations. There was an excess of heterozygotes at the majority of loci in the three seed populations. A comparison of the genetic diversity between seed populations collected in the natural population and restoration plots showed that the seed analysed from natural population had a greater number of alleles, and more effective and unique alleles than restoration plot A and C. Seed were collected from mother tree in plot A and C was three and eight mother trees, respectively mother, whereas only five mother trees contributed to the seed analysed from natural population.

Comparison of genetic diversity of the adult and seed populations

A comparison of diversity in the adult and seed populations showed that, in most cases, observed and expected heterozygosities were greater in the adult than the seed populations (Table 2). There were more alleles in the seed than the adult natural population at Doi Suthep. In contrast the seed population in restoration plot A had a total of 35 alleles compared to 43 in the equivalent adult population (Table 2). Similarly, the seed population from restoration plot C had 49 alleles compared with a total of 52 in the adult population. The effective number of alleles in adult population from restoration plot A and C was also higher than in seed population, but effective number of alleles in adult natural population was lower than in seed population.

In Doi Suthep subpopulation, there are more alleles in the 100 seeds than in the 16 adult trees that were sampled. This shows that there are more alleles in the pollen contribution to the seed than is represented in the 16 sampled adult trees. In contrast, in restoration plots (A and C) there are fewer alleles in the seed than in the flowering adult trees in the plot. This is despite the fact that many more seeds than adult trees were sampled. The seed population of the natural Doi Suthep population contained eight unique alleles which were absent from the other six populations whereas the seed population from restoration plot A had no such alleles and plot C had only two allele which was unique.

Paternity assignment

Results of paternity assignment for progenies of 16 mother trees are recorded in Table 3. Between 6 and 20 seeds were analysed per mother tree to provide a grand total of 289 seeds. For the Doi Suthep natural population: 100 seedlings were analysed from five mother trees. Seventeen seeds (17%) had a paternal contribution which matched at least one of the 16 naturally occurring trees sampled in Doi Suthep and 83 seeds (83%) had a paternal contribution which did not match any of the 16 naturally occurring mother trees. The number of pollen donors which were not in the sampled trees was relatively high in the Doi Suthep natural population when compared with seeds from restoration plots A and C. Of the 53 seeds from three trees in restoration plot A, 23 seeds (43%) had a pollinator genotype, which matched at least one of the trees in restoration plot A. A further 13 seeds did not match a tree in plot A but did match a tree in the other restoration plots. The remaining 17 seeds had a pollinator profile which did not match any of the trees in

Table 3 Summary of paternity assignment for 16 mother trees

Subpopulation	Unidentified father from ungenotyped trees	Identified father from trees inside the plot	Identified father from genotyped trees in another restoration plot	Total
Doi Suthep	83 (83%)	17 (17%)	0	100
Restoration plot A	17 (32%)	23 (43%)	13 (25%)	53
Restoration plot C	34 (25%)	77 (57%)	25 (18%)	136

restoration plots A, B or C. A total of 136 seeds from eight seed trees from restoration plot C were analysed. Of these, 77 (57%) had a pollinator profile which matched at least one tree within restoration plot C, and 25 seeds (18%) did matched a tree in the other restoration plot. The remaining 34 seeds had a paternal profile, which did not match any of the trees in any of the three restoration plots.

Discussion

Microsatellites in *Prunus cerasoides*

This study has demonstrated that 10 of the primer pairs developed for *Prunus avium* amplify in *Prunus cerasoides* and seven of these produce no more than two bands per diploid individual. Our work also demonstrates the utility of heterologous amplification using primers from closely related species, which can be for genetic analysis to support a practical forest restoration programme. The transfer of published primers from one species into another avoids the time and costs associated with generating and sequencing a new microsatellite library (Brondani et al. 1998; Cipriani et al. 1999; Dayannandan et al. 1997; Khadari et al. 2001; Pakkad et al. 2004; Roa et al. 2000; White and Powell 1997).

The ability to amplify these additional loci extends the number of primers available for genetic studies in this species beyond the five that were previously transferred from peach, wild cherry and sour cherry by Pakkad et al. (2004). The diversity in the microsatellites studied in the wild population of *Prunus cerasoides* was much greater than when the same loci were used by Clarke and Tobutt (2003) in 14 cultivars of sweet cherry used in the breeding programme and mapping studies at East Malling. The average number of alleles in the six loci used both in this study and that of Clarke and Tobutt (2003) was 9.8 and 4, respectively.

The seven microsatellite loci employed detected a total of 75 alleles (n = 401). Polymorphic information content (PIC) varied from 0.34 to 0.83. The average number of alleles in seven microsatellite loci in this study is higher than the average number of 8.2 detected by Pakkad et al. (2004) using the five microsatellites transferred from peach, wild cherry and sour cherry.

In the previous study of *Prunus cerasoides*, Pakkad et al. (2004) obtained an average number of 3.6 alleles in the 16 naturally occurring trees at Doi Suthep National Park. With the new set of seven microsatellites, the same sixteen trees tested in Doi Suthep National Park produced more alleles per locus (average 5.7), once again evidence that the new set of microsatellite loci are more variable. In the previous study the F_{IS} values for each locus were positive, indicating an excess of homozygotes, which may reflect the incidence of

null alleles in these loci. In contrast, the current study, using the different set of microsatellites, showed little evidence of homozygote excess in the 16 trees at Doi Suthep and only one of the seven loci produced a positive F_{IS} value. As null alleles are a problem in paternity analysis these results suggest that the current set of microsatellites are likely to be better suited for paternity analysis than the original set of five. Pakkad et al. (2004) do not provide the F_{IS} values for the 16 naturally occurring trees at Doi Suthep alone.

Forest restoration

Knowledge of the genetic variability between and within natural and plantation *Prunus cerasoides* populations is essential for the development of efficient strategies for seed collection for the restoration programme which is being actively pursued by FORRU in the seasonally dry forests in Thailand. There is a need to know how many trees are needed to form the basis of seed collections for the establishment of restoration plots and whether the new restoration plots are in genetic contact with other populations. Previous work on the species has shown moderate differentiation between populations of *Prunus cerasoides* with an F_{ST} for three populations of 0.115 (Pakkad et al. 2004).

Within the adult population there was only moderate genetic differentiation with an F_{ST} value of 0.0575, which suggests that the restoration plots had a similar genetic composition to that of the natural population. The adult tree population in restoration plots were based on seed collected from no more than ten seed bearing mother trees and it is important to know whether this is sufficient to capture the genetic diversity in the natural population. The results are slightly ambiguous in relation to this question. The restoration plots tended to have slightly lower observed and expected heterozygosities but contained more alleles than the 16 trees in the natural population at Doi Suthep. A comparison of the adult trees in the restoration plots, based on seed collected from Doi Suthep showed there is a need to analyse a greater number of naturally occurring trees in Doi Suthep to get a better estimate of the diversity within the natural population. This would clarify whether the restoration plots based on seed collected from about ten trees capture the majority of the natural diversity present in the forest. A further suggestion that the number of trees, which are used as seed suppliers is important is seen in the results of the seed analysis. Seed was collected from only three seed trees in plot A and eight trees for plot C. The expected heterozygosities and the number of alleles were lower in the seed than in the restoration plot from which they were collected. The reduction in diversity between the adult and seed population was greater in plot A where seed was collected from only three mother trees. Once again this suggests that the number of trees on which the seed collections are based is important and should be from as many mother trees as possible (Barnes and Burley 1990; Blakesley et al. 2004; Kitzmiller 1990; Konnert and Ruetz 2003; Schmidt 2000). The results also suggest that it is probably best to collect seed for the establishment of further restoration plots from the natural population than from seed in restoration plots.

The results of the gene flow studies should be considered preliminary as they are based on the analysis of relatively few seeds. They do, however, the gene flow assessment provides some interesting insights into the genetic diversity of *P. cerasoides*. In the 16 naturally occurring trees over 80% of the genotyped seed were fathered by unidentified trees whereas in restoration plot A only about 48% of the pollen came from an unidentified father. This proportion was even less in plot C where only 31% of the pollen parents were unidentified. The naturally occurring trees within Doi Suthep were surrounded by planted trees, which were clearly contributing to the paternity of the seed crop. This result

demonstrates that ''fill in'' planting should consist of locally sourced material if it is considered important to conserve the genetic integrity of the local populations. Although we have demonstrated that there is less gene flow from unidentified trees in the restoration plots, there is, nevertheless, significant pollen flowing into these planted plots. In terms of their genetic connectivity with surrounding populations such gene flow is certainly a good thing. It means that, even in situations where the material that has been used to establish the restoration plots has a narrow genetic base, the fact that the population is not isolated genetically means that it has the opportunity to increase its diversity in succeeding populations.

Acknowledgements The authors thank Rungtiwa Punyayod for helping with seed collection and the germination of seedlings in the nursery. We are deeply grateful to Jake Clarke of East Malling Research (EMR) for technical advice and support, Joan Cottrell and Tom Connolly for helping with data analysis. We are also grateful for institutional support of this work provided by Chiang Mai University and the Herbarium especially Dr. Vilaiwan Anusarnsunthorn and the Forest Restoration Research Unit (FORRU) attached to the Biology Department, Faculty of Science. Financial support came from International Plant Genetic resources (IPGRI-APO) and East Malling Research (EMR, UK).

References

Blakesley D, Pakkad, G (2004) Conserving Thailand's forest genetic resources. fgr research highlights, pp 10–11

Blakesley D, Anusarnsunthorn V, Kerby J, Navakitbumrung P, Kuarak K, Zangkum S, Hardwick K, 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, Anusarnsunthorn V (eds) Forest restoration for wildlife conservation. ITTO and Chiang Mai University, pp 207–222

Brondani RPV, Brondani C, Tarchini R, Grattapaglia D (1998) Development, characterisation and mapping of microsatellite markers in *Eucalyptus grandis* and *E. urophylla*. Theor Appl Genet 97:816–827

Barnes RD, Burley J (1990) Tropical forest genetics at the Oxford Forestry Institute: exploration, evaluation, utilization and conservation of genetic resources. Forest Ecol Manag 35:159–170

Bussell JD, Hood P, Alacs EA, Dixon KW, Hobbs RJ, Krauss SL (2006) Rapid genetic delineation of local provenance seed-collection zones for effective rehabilitation of an urban bushland remnant. Austral Ecol 31:164–175

Chase M, Kesseli R, Bawa KS (1996) Microsatellite markers for population and conservation genetics of tropical trees. Am J Bot 83:51–57

Cipriani G, Lot G, Huang WG, Marrazzo MT, Peterlunger E, 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 Genet 99:65–72

Clarke JB, Tobutt KR (2003) Development and characterization of polymorphic microsatellites from *Prunus avium* 'Napoleon'. Mol Ecol Notes 3:578–580

Dakin EE, Avise JC (2004) Microsatellite null alleles in parentage analysis. Heredity 93:504–509

Dayanandan S, Bawa K, Kesseli R (1997) Conservation of microsatellites among tropical trees (Legumninosae). Am J Bot 84:1658–1663

Dow BD, Ashley MV, Howe HF (1995) Characterization of highly variably $(GA/CT)_n$ microsatellites in the bur oak, *Quercus macrocarpa*. Theor Appl Genet 91:137–141

Downey SL, Iezzoni AF (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, Anusarnsunthorn V, Garwood N, Blakeyley D (1995) Research needs for restoring the forest of Thailand. Nat Hist Bull Siam Soc 43:179–184

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

Food and Agriculture Organization (1997) State of the world's forests

Food and Agriculture Organization (2001) State of the world's forests

Forest Restoration Research Unit (1998) Forests for the future: growing and planting native trees for restoring forest ecosystems. In: Elliott S, Anusarnsunthorn V, Blakesley D (eds) Biology Department, Science Faculty, Chiang Mai University, Thailand

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

Goosem SP, Tucker NIJ (1995) Repairing the rainforest – theory and practice of rainforest re-establishment in North Queensland's wet tropics. Wet Tropics Management Authority, Cairns

Khadari B, Hochu T, Santoni IS, Kjellberg F (2001) Identification and characterization of microsatellite loci in the common fig (*Ficus cariaca* L.) and reprenstative species of the genus *Ficus*. Mol Ecol Notes 1(3):191–193

Kitzmiller JH (1990) Managing genetic diversity in a tree improvement program. Forest Ecol Manag 35:131–149

Konnert M, Ruetz W (2003) Influence of nursery practices on the genetic structure of beech (*Fagus sylvatica* L.) seedling populations. Forest Ecol Manag 184:193–200

Lamb D, Parrotta J, Keenan R, Tucker N (1997) Rejoining habitat fragments: restoring degraded rainforest lands. In: Laurance WF, Bierregaard RO (eds) Tropical forest remnants: ecology, management and conservation of fragmented communities. The University of Chicago Press, Chicago, USA, pp 366–385

Leungaramsri P, Rajesh N (1992) The future of people and forests in Thailand after the logging ban. Project for Ecological Recovery, Bangkok, Thailand

Marshall TC, State J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. Mol Ecol 7:639–655

Muluvi GM, Sprent JI, Soranzo N., Provan J, Odee D, Folkard G, Mcnicol JW, Powell W (1999) Amplified fragment length polymorphism (AFLP) analysis of genetic variation in *Moringa oleifera* Lam. Mol Ecol 8:463–470

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acid Res 8:4321–4325

Pakkad G, James C, Torre F, Elliott S, Blakesley D (2004) Genetic variation of *Prunus cerasoides* D. Don, a framework tree species in northern Thailand. New Forests 27:189–200

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

Roa AC, Chavarriaga-Aguirre P, Duque MC, Maya MM, Bonierbale MW, Iglesias C, Thome J (2000) Cross-species amplification of cassava (*Manihot esculenta*) (Euphorbiaceae) microsatellites: allelic polymorphism and degree of relationship. Am J Bot 87:1647–1655

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

Sosinki B, Gannavarapu M, Hager LD, Beck LE, King GJ, Ryder D, Rajapakse S, Baird WV, Ballard RE, Abbott AG (2000) Characterization of microsatellite markers in peach [*Prunus persica* (L.) Batsch]. Theor Appl Genet 101:421–428

Streiff R, Labbe T, Bacilieri R (1998) Within-population genetic structure in *Quercus robur* L. and *Quercus petraea* (Matt.) Liebl. assessed with isozymes and microsatellites. Mol Ecol 7:317–328

Tucker NIJ, Murphy TM (1997) The effects of ecological rehabilitation on vegetation recruitment: some observations from the Wet Tropics of North Queensland. Forest Ecol Manag 99:133–152

Ueno S, Yoshimaru H, Kawahara T, Yamamoto S (2000) Isolation of microsatellite markers in *Castanopsis cuspidata* var. *sieboldii* Nakai from an enriched library. Mol Ecol 9:1171–1193

White G, Powell W (1997) Isolation and characterization of microsatellite loci in *Swietenia humilis* (Meliaceae); an endangered tropical hardwood species. Mol Ecol 6:851–860

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