Annals of Botany **98**: 601–608, 2006
doi:10.1093/aob/mcl135, available online at www.aob.oxfordjournals.org

Allozyme Variation of Populations of *Castanopsis carlesii* (Fagaceae) Revealing the Diversity Centres and Areas of the Greatest Divergence in Taiwan

YU-PIN CHENG[1,3], SHIH-YING HWANG[2], WEN-LIANG CHIOU[3] and TSAN-PIAO LIN[1,*]

[1]*Institute of Plant Biology, National Taiwan University, Taipei 106, Taiwan,* [2]*Graduate Institute of Biotechnology, Chinese Culture University, Yangmingshan, Taipei 111, Taiwan and* [3]*Division of Forest Biology, Taiwan Forestry Research Institute, Taipei 100, Taiwan*

Received: 22 March 2006 Returned for revision: 10 April 2006 Accepted: 18 May 2006 Published electronically: 27 June 2006

• *Background and Aims* The genetic variation and divergence estimated by allozyme analysis were used to reveal the evolutionary history of *Castanopsis carlesii* in Taiwan. Two major questions were discussed concerning evolutionary issues: where are the diversity centres, and where are the most genetically divergent sites in Taiwan?
• *Methods* Twenty-two populations of *C. carlesii* were sampled throughout Taiwan. Starch gel electrophoresis was used to assay allozyme variation. Genetic parameters and mean F_{ST} values of each population were analysed using the BIOSYS-2 program. Mean F_{ST} values of each population against the remaining populations, considered as genetic divergence, were estimated using the *FSTAT* program.
• *Key Results* Average values of genetic parameters describing the within-population variation, the average number of alleles per locus ($A = 2{\cdot}5$), the effective number of alleles per locus ($A_e = 1{\cdot}38$), the allelic richness ($A_r = 2{\cdot}38$), the percentage of polymorphic loci ($P = 69\,\%$), and the expected heterozygosity ($H_e = 0{\cdot}270$) were estimated. High levels of genetic diversity were found for *C. carlesii* compared with other local plant species. Genetic differentiation between populations was generally low.
• *Conclusions* From the data of expected heterozygosity, one major diversity centre was situated in central Taiwan corroborating previous reports for other plant species. According to the mean F_{ST} value of each population, the most divergent populations were situated in two places. One includes populations located in north central Taiwan between 24·80°N and 24·20°N. The other is located in south-eastern Taiwan between 22·40°N and 23·10°N. These two regions are approximately convergent with the most divergent locations determined for several other plant species using chloroplast DNA markers published previously. An important finding obtained from this study is that unordered markers like allozymes can be used to infer past population histories as well as chloroplast DNA markers do.

Key words: Diversity centre, allozymes, *Castanopsis carlesii*, Fagaceae, genetic divergence.

INTRODUCTION

The floristic composition of Taiwan as an island exhibits high levels of endemism and species diversity (Hsieh *et al*., 1994). Most plants are thought to have originated from the Asian mainland during cycles of temperature oscillations. Geological data indicate that ice ages have occurred at regular intervals of approx. 100 000 years followed by 20 000 years of warmer periods (the Milankovitch cycle) (Bennett, 1990). Although only the highest elevations in Taiwan show evidence of glaciation, the large temperature and climate changes undoubtedly influenced plant distributions and evolution throughout the island. During glacial expansions, most subtropical species would have retreated into more-southerly or warmer lowland areas, i.e. refugia. At the same time, temperate species would have expanded their ranges of distribution to lower elevations, and lowland forests would have been dominated by conifers (Tsukada, 1967). When temperatures rose again, a reverse course of events occurred with subtropical species recolonizing from south to north, and from lower to higher elevations. The last glacial age was undoubtedly an important factor in shaping the current genetic structure of plant species in Taiwan. No recent geological changes, e.g. in the height of the Central Mountain Range (CMR), are known to have occurred since the last glacial period ended.

Castanopsis carlesii Hayata (Fagaceae) is widely distributed in East Asia, including Japan, mainland China and Taiwan. It is one of the most important tree species of the *Machilus–Castanopsis* forest and evergreen oak forests in Taiwan (Hsieh *et al*., 1994). It generally occurs in forests at medium to high elevations in central Taiwan (1000–2300 m), but occurs at low elevations on both the northern and southern parts of the island. *Castanopsis carlesii* is monoecious (Liao, 1996), but staminate and pistillate flowers are separated on different inflorescences. The small flowers and unisexual catkins facilitate pollination by wind and promote an outcrossing mating system that is likely to increase levels of gene flow. The acorns of *C. carlesii* are principally dispersed by gravity in the field.

Genetic variation in a species indicates its evolutionary potential and the ability to adapt to various environments (Hamrick *et al*., 1992). Therefore, the level of genetic diversity in natural populations and the factors influencing genetic variability have been a primary concern of evolutionary geneticists. The diversity centres of tree species according to expected heterozygosity in Taiwan have been suggested by many studies using allozymes (see references in Lin, 2001). Central Taiwan, especially Nantou

* For correspondence. E-mail tpl@ntu.edu.tw

TABLE 1. *Genetic diversity for each studied population of* Castanopsis carlesii *using allozyme analysis*

Population	Elevation (m)	Geographical area†	*n*	*A*	A_r	A_e	*P*	H_o	H_e	Mean F_{ST}	*Z* value
1. Fushan (FS)	600–800	W	30	2·3	2·06	1·27	54·5	0·145	0·210	0·102	1·836
2. Tungyanshan (TY)	800–1000	W	32	2·3	2·14	1·36	45·5	0·131	0·265	0·057	–0·491
3. Kungliao (KL)	50–100	W	24	2·5	2·50	1·56	72·7	0·227	0·361	0·080	0·698
4. Tungao (TA)	500–700	E	20	2·5	2·42	1·39	72·7	0·164	0·283	0·089	1·164
5. Chenghsipao (CH)	1500–1800	W	31	2·8	2·53	1·39	72·7	0·158	0·276	0·056	–0·543
6. Kuanwu (KW)	2000–2200	W	24	2·3	2·16	1·34	63·6	0·136	0·251	0·067	0·026
7. Chialishan (CL)	1500–1700	W	30	2·9	2·65	1·40	81·8	0·152	0·288	0·043	–1·215
8. Anmashan (AM)	2000–2200	W	31	2·6	2·34	1·30	72·7	0·117	0·233	0·086	1·008
9. Lishan (LS)	2100–2200	W	19	2·3	2·25	1·35	72·7	0·144	0·259	0·056	–0·543
10. Meifeng (MF)	1800–2000	W	26	2·6	2·45	1·34	90·9	0·129	0·251	0·066	–0·026
11.Hsinkangshan (HK)	800–1000	E	17	2·0	2·00	1·28	54·5	0·118	0·216	0·108	2·146*
12. Nanheng (NH)	2000–2200	W	24	2·5	2·42	1·37	72·7	0·148	0·270	0·041	–1·319
13. Lichia (LC)	800–1200	E	28	2·4	2·22	1·30	81·8	0·140	0·229	0·060	–0·336
14. Liyushan (LY)	400–500	E	27	2·5	2·36	1·39	81·8	0·182	0·278	0·054	–0·646
15. Tengchih (TC)	1200–1400	W	31	2·5	2·36	1·33	63·6	0·109	0·247	0·052	–0·750
16. Alishan (AL)	1700–2100	W	22	3·0	2·84	1·46	81·8	0·190	0·313	0·045	–1·112
17. Souchia (SC)	500–600	E	21	2·2	2·12	1·35	63·6	0·104	0·258	0·057	–0·491
18. Tawu (TW)	400–500	E	30	2·2	2·04	1·36	63·6	0·152	0·262	0·093	1·371
19. Nanjenshan (NJ)	400–500	E	30	2·5	2·31	1·37	72·7	0·164	0·272	0·066	–0·026
20. Lienhuachih (LH)	600–700	W	28	2·9	2·77	1·66	90·9	0·179	0·398	0·064	–0·129
21. Tahanshan (TH)	1100–1200	E	26	2·5	2·44	1·38	72·7	0·108	0·275	0·044	–1·164
22. Tona (TN)	1000–1200	W	30	2·8	2·72	1·32	81·8	0·142	0·241	0·077	0·543
Mean (s.d.)			26·4 (4·44)	2·5 (0·25)	2·38 (0·28)	1·38 (0·09)	69·0 (11·5)	0·147 (0·029)	0·270 (0·043)	0·067 (0·019)	

n, Number of individuals; *A*, mean number of alleles per locus; A_r, allelic richness; A_e, mean number of effective alleles per locus; *P*, average of polymorphic loci (95 % criterion); H_o, observed heterozygosity; H_e, expected heterozygosity; mean F_{ST}, mean genetic differentiation of each population against the remaining populations.

† The division of geographical areas was based on the location of populations along the Central Mountain Range.

* For the 95 % confidence interval, the difference was statistically significant.

County harbours the highest expected heterozygosities for many trees species (Lin, 2001). A second diversity centre is located in the northern part of central Taiwan. Also climatic influences on tree species in Taiwan during the last glacial age have been proposed by several studies (Tsukada, 1966; Huang *et al*., 2002, 2004; Hwang *et al*., 2003; Cheng *et al*., 2005). Such studies are important for showing how historical events have influenced the distribution and genetic structure of plant species in Taiwan. Predicted refuge sites were reported to exist in two major locations: a south-eastern refugium (Huang *et al*., 2002; Cheng *et al*., 2005) and a central-northern refugium (Hwang *et al*., 2003; Huang *et al*., 2004; Cheng *et al*., 2005). Those studies predicted the refuge sites according to the genetic divergence (differentiation) among haplotypes of the populations studied. It was found that for many tree species in Europe, the genetic divergence (mean F_{ST}) of southern populations was significantly more differentiated from the pooled remaining populations of central or northern populations (Grivet and Petit, 2002; Petit *et al.,* 2003; Heuertz *et al.,* 2004). Thus, the most genetically divergent populations coincided with refuge sites in the last glacial age in Europe.

However, previous genetic variation studies using allozyme markers in Taiwan used only limited populations and did not even include populations on the eastern side of the CMR which runs north to south and is a major barrier to gene exchange between plants growing on both sides. It is important to use a species with an island-wide distribution and which is representative as a dominant subtropical tree species in order to thoroughly survey the genetic diversity centre(s) by sampling populations covering the entire island of Taiwan. Moreover, genetic analysis using allozymes also estimates parameters such as genetic differentiation (F_{ST}), which can be used for comparison with sites having the highest genetic divergence as determined using chloroplast DNA markers in several previous studies, including *C. carlesii* (Cheng *et al*., 2005). Allozyme markers are often used to study the population genetic structure of species, but few have been used to discuss the influence of geological changes or phylogeographical issues (Tomaru *et al*., 1997; Hiramatsu *et al*., 2001; Widmer and Lexer, 2001). In this study, an attempt was made to reveal the genetic variation of *C. carlesii* using allozyme analysis and to study its evolutionary history. The following two questions were addressed: (1) Where are the diversity centres exhibiting the highest expected heterozygosities? (2) Where are the most genetically divergent sites in Taiwan?

MATERIALS AND METHODS

Plant material

Twenty-two natural populations, with 17–32 individuals each, were sampled throughout the entire range of *C. carlesii* in Taiwan (Table 1 and Fig. 1). Young leaves of large trees were collected from the field and immediately stored on ice, then transported to the laboratory at

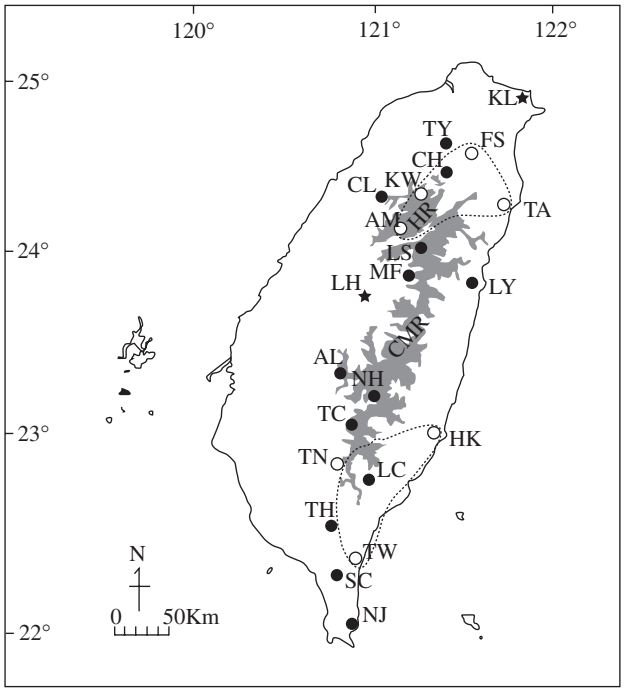

FIG. 1. Map of Taiwan and sampling sites of *Castanopsis carlesii* in this study. Stars indicate areas with higher genetic diversity (LH, 0·398; KL, 0·361). Dotted lines indicate the regions with high genetic divergence. Open circles indicate populations with higher values of genetic divergence (HK, 0·108; TW, 0·093; TN, 0·077; FS, 0·102; TA, 0·089; AM, 0·086; KW, 0·067), closed circles indicate the remaining populations. CMR, Central Mountain Range; HR, Hsuehshan Range.

the Taiwan Forestry Research Institute, Taipei. Fresh leaves were cleaned and stored at −80 °C prior to protein extraction. Fresh foliage was ground in mortars using liquid nitrogen, and enzymes were extracted with a buffer, following the method of Feret (1971). Enzyme extracts were absorbed onto Whatman 3-mm filters. Paper strips were arranged in a plastic Petri dish and stored at −80 °C until being analysed.

Enzyme electrophoresis

Allozymic variation was assayed by horizontal electrophoresis and resolved on 12 % starch gels. Electrophoresis and staining followed the procedures described by Cheliak and Pitel (1984). Two buffer systems were used for electrophoresis to resolve the putative loci. Nine enzyme systems involving 11 putative loci were reliably scorable. Buffer system H was used to resolve PGM (phosphoglucomutase, EC 5.4.2.2), SkDH (shikimate 5-dehydrogenase, EC 1.1.1.25), MDH (malate dehydrogenase, EC 1.1.1.37) and IDH (isocitrate dehydrogenase, EC 1.1.1.42); and buffer system B was used to resolve MR (meadione reductase, EC 1.6.5.2), LAP (leucine aminopeptidase, EC 3.4.11.1), PGI (phosphoglucose isomerase, EC 5.3.1.9), EST (esterase, EC 3.1.1.1) and GPD (glucose-6-phosphate dehydrogenase, EC 1.1.1.49). The genotypes of the allozyme patterns were inferred from the known subunit compositions and number of isozymes commonly observed in plant species (Soltis and Soltis, 1989; Murphy *et al*., 1996). To ensure the accuracy of allozyme identification, every gel was run using the same individual as a control. Sometimes protein samples of different individuals were mixed together and pooled samples were run on the same lane in order to ascertain whether protein samples from the different plants involved were actually identical.

Data analysis

The allelic frequencies at each of the allozyme loci were estimated using the BIOSYS-2 program (from William C. Black IV, Department of Microbiology, Colorado State University, CO, USA), which is a modified version of the BIOSYS-1 program by Swofford and Selander (1981). The following genetic parameter estimates were calculated based on the allelic frequencies: the mean number of alleles per locus (A), the effective number of alleles per locus (A_e) (Crow and Kimura, 1970), the percentage of polymorphic loci (P) (using the 95 % criterion), and the observed (H_o) and expected heterozygosities (H_e) (Nei, 1978). The allelic richness (A_r) and genetic differentiation (F_{ST}) between populations were analysed using the *FSTAT* program (Goudet, 1995). Computation of allelic richness for specified sample sizes was based on the rarefaction method developed by Hurlbert (1971). Allelic richness corresponding to the number of different alleles found in the specified sample size at a locus was measured as described by El Mousadik and Petit (1996). The BIOSYS-2 program was used to conduct cluster analysis based on genetic distances (Nei, 1978) via the unweighted pairwise group method using arithmetic averages (UPGMA) (Sneath and Sokal, 1973) to present relationships among populations. Principal component analysis (PCA) based on the correlation matrix of population allelic frequency was performed using PCAGEN version 1·2 software (Goudet, 1999). A Mantel test (Mantel, 1967) was performed between the two matrices of genetic differentiation and geographic distance to test for a pattern of isolation by distance. The pairwise genetic differentiations between populations were expressed as $F_{ST}/(1 - F_{ST})$. The conventional F_{ST} according to the allelic frequency of the population was estimated using the Arlequin program (Schneider *et al*., 2000), while the level of divergence for each population from the remaining populations was calculated as a mean value of pairwise F_{ST} values for each population against the remaining populations (Petit *et al*., 2003).

In order to infer recent population bottlenecks from within-population allozyme allelic frequencies, the Wilcoxon signed-ranks test (with 1000 simulation iterations) was performed under the Infinite Allele Model (IAM), using BOTTLENECK, which tests for excessive population heterozygosity (Cornuet and Luikart, 1996; Piry *et al*., 1999).

RESULTS

Genetic diversity

Castanopsis carlesii is predicted to be diploid according to the banding patterns of isozymes even though no

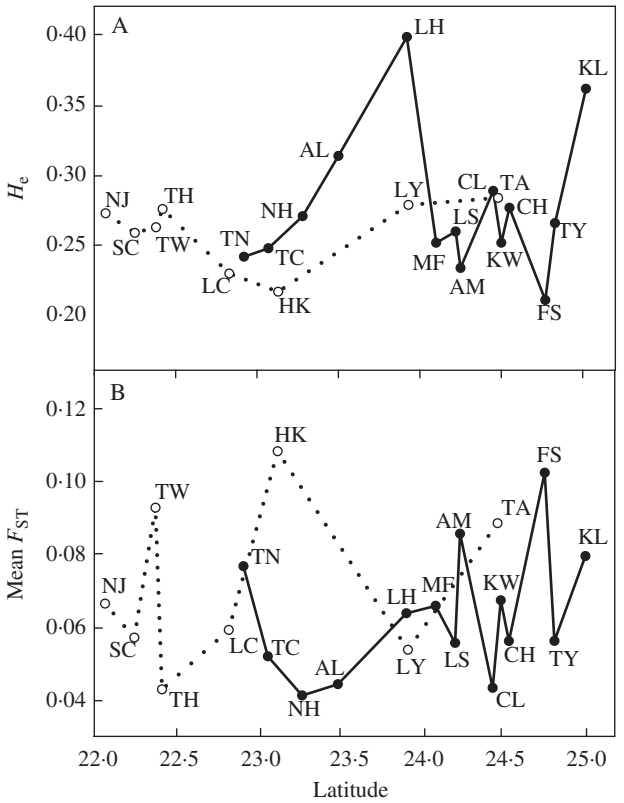

FIG. 2. Relationships of expected heterozygosity (H_e) and mean F_{ST} with latitude: (A) H_e of each population in this study; (B) mean F_{ST} of each population. Closed circles indicate western populations, and open circles indicate eastern populations.

chromosome number counts are available in the literature. In total, 11 putative loci were scored in this study: PGM-2, SkDH-1, MDH-2, MR-2, LAP-1, PGI-1, PGI-2, EST-1, EST-2, IDH-1 and GPD-1. In total, 40 alleles were scored for all loci, and the number of alleles detected at each locus ranged from two at PGI-2 and GPD-1 to six at MDH-2 and PGI-1. No significant correlations were found between allelic frequencies in relation to longitudinal or latitudinal gradients. Occurrences of the common alleles lacked geographic structure, except for the rare allele, PGM-2d, which was only recorded in the two western populations of Lishan (LS) and Nanheng (NH).

Measures of genetic diversity are presented in Table 1. The average number of alleles per locus (A) was 2·5, and the effective number of alleles per loci (A_e) was 1·38. Allelic richness (A_r) ranged from 2 to 2·84, with an average of 2·38, and the average percentage of polymorphic loci (P) was 69 %. The expected heterozygosity ranged from 0·210 to 0·398, with an average of 0·270. Among these populations, Lienhuachih (LH) in central Taiwan exhibited the highest diversity, followed by the population at Kungliao (KL), northern Taiwan; Fushan (FS) also of northern Taiwan had the lowest diversity (Fig. 2A). Because the CMR is a major barrier to gene flow of plants in Taiwan (Huang *et al*., 2002), these populations were separated into two groups according to their geographical location, i.e. located on the eastern or western side of the CMR. Estimates of genetic parameters of the populations were regressed with latitude, and no significant correlations were found between the parameters and latitude of each population, regardless of whether all populations or the two geographical groups were analysed individually (data not shown).

Test of isolation by distance and detection of recent bottlenecks

Genetic differentiation between populations was generally low as revealed by the allozyme markers, and F_{ST} varied from 0·001 to 0·192 (data not shown). A Mantel test for evaluating patterns of isolation by distance also showed no significant correlation between population differentiations measured as $F_{ST}/(1 - F_{ST})$ and the natural logarithm of the geographical distance between populations (r = 0·134318; p = 0·078 with 1000 permutations; NB 'p' is used here for probability to distinguish from 'P' for percentage of polymorphic loci). Even when examined individually, there were no significant correlations between the differentiation and geographical distances for each side of the CMR (western side, r = –0·025059, p = 0·465553; eastern side, r = 0·000765, p = 0·495833).

There were no significant excesses of heterozygosity ($H_e > H_{eq}$) ($p > 0·05$) shown in any of the populations tested indicating no evidence of recent bottlenecks.

Pair-wise F_{ST} *and genetic divergence*

The mean F_{ST} values of each population against the remaining populations ranged from 0·041 to 0·108. The Hsinkangshan (HK) population had the highest value (Table 1 and Fig. 2B). On the western side of the CMR, major peaks were found in the populations of Kungliao (KL), Fushan (FS), Anmashan (AM) and Tona (TN). On the eastern side of the CMR, major peaks were found in the three populations of Tungao (TA), Hsinkangshan (HK) and Tawu (TW). The z test was used to evaluate the significance of each population according to its z value: $z = (m - \mu)/\text{s.d.}$; where m is the average of mean F_{ST} of all sampled populations, μ is the value of mean F_{ST} to be tested, and s.d. is the standard deviation. For a 95 % confidence interval ($p < 0·05$), the z value is either greater than 1·96 or less than −1·96. Only Hsinkangshan (HK) was significant at the 5 % level, and Fushan (FS) had a high but non-significant z value.

Genetic relationships among populations

The Nei's genetic identity (Nei, 1978) which means the extent of genetic similarities between two populations as measured by a function of allele frequencies for all population pairs ranged from 0·896 to 0·997, with an average of 0·967 (data not shown), which is well within the range of values expected for conspecific populations (Crawford, 1989). The UPGMA dendrogram produced from Nei's genetic distance (Nei, 1978) among all population pairs clustered into two major groups (not shown). These groupings provided few insights into correlations with the geographical range of the populations. The PCA

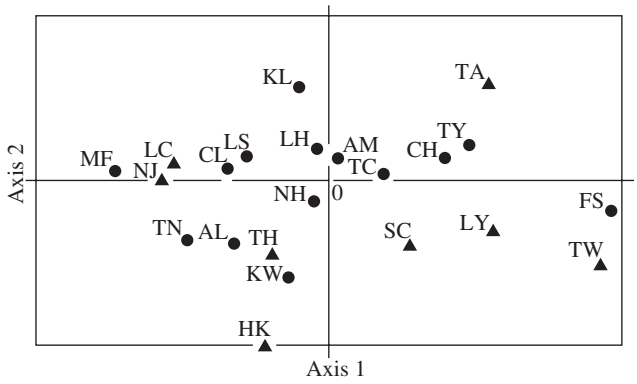

FIG. 3. Two-dimensional representation of the principal component analysis (PCA) of the genetic relationships among 22 populations (indicated as a two-letter code) of *Castanopsis carlesii*, inferred from the frequencies of 40 alleles. The symbols of filled circles and triangles indicated populations on the western and eastern sides of the CMR, respectively. The percentages of the total variability explained by the two first components were 28·5 % for axis 1 and 19·1 % for axis 2.

(Fig. 3) also indicated no clustering of populations related to the geographic structure. The results of the UPGMA dendrogram and PCA indicated that the CMR is not a barrier to gene flow among populations of both sides.

Comparison of mean F_{ST} *obtained from cpDNA and allozyme data*

Correlations between the two matrices of mean F_{ST} derived from the allozyme and noncoding cpDNA sequence (Cheng *et al*., 2005) datasets against latitude were evaluated using the partial Mantel test in the zt software (Bonnet and Van de Peer, 2002). Pairwise comparisons showed that mean F_{ST} values for each population on the eastern side of the CMR were significantly correlated, and this positive correlation was not due to chance (r = 0·430730, p = 0·005994). However, correlations between mean F_{ST} values for each population on the western side of the CMR, as analysed by the simple Mantel test, showed no correlations (r = 0·188902, p = 0·119880). This discrepancy was due to populations having high values for the allozyme data which contrasted with low values for the cpDNA markers. However, populations having high values of mean F_{ST} on the western side of the CMR for both markers were all located in the northern part of central Taiwan, between latitudes of 24·20°N and 24·80°N. Thus the region covering several populations (FS, AM and TA) in the northern part of central Taiwan has the greatest genetic divergence on the western side of the CMR.

DISCUSSION

Comparison of genetic diversity between C. carlesii *and other local species*

The results of this study revealed that *C. carlesii* is highly diversified. The mean expected heterozygosity (H_e) within populations and other genetic parameters were all well above the average values of species with widespread geographical ranges with an outcrossing, wind-pollinated mating system (A = 2·56, A_e = 1·33, P = 74·3 % and H_e = 0·228; Hamrick *et al*., 1992) and of a long-lived tree species (Hamrick and Godt, 1989). *Castanopsis carlesii* had a mean H_e of 0·270, which is higher than those of other widespread tree species that have been reported in Taiwan, such as *Alnus formosana* (H_e = 0·175; Sue *et al*., 2000), *Cunninghamia konishii* (H_e = 0·219; Lin *et al*., 1998), *Michelia formosana* (H_e = 0·241; Lin, 2001), *Myrica rubra* (H_e = 0·191; Cheng *et al*., 2000) and *Trochodendron aralioides* (H_e = 0·133; Wu *et al*., 2001).

The centre for genetic diversity

As one of the most dominant and widespread tree species in Taiwan, *C. carlesii* shares habitats with other evergreen broadleaf species, and this contributes to the diversity of broadleaf forests in various types of habitat (Hsieh *et al*., 1994). According to the fossil pollen record in central Taiwan for the last glacial maximum, the vegetation was predicted to have descended by about 1500 m (Liew *et al*., 2005). Judging from its present elevational distribution (up to 2300 m a.s.l.), *C. carlesii* would have been able to survive at low elevations in northern and central Taiwan during the last glacial maximum. Also from the highly diversified genetic variations maintained at present, it is thought that a bottleneck effect did not occur to *C. carlesii* in the last glacial period. The test of recent effective population size reductions from the allelic frequency data showed no significant excesses of heterozygosity, which supports the conclusion presented above.

The geographical region in central Taiwan has been proposed as being a major diversity centre for several tree species based on allozyme variation (Lin, 2001). Those species include *Cinnamomum kanehirae*, *Cunninghamia konishii*, *Michelia formosana, Myrica rubra* and *Trochodendron aralioides*. Moreover, island-wide samplings of *T. aralioides* revealed a secondary diversity centre between latitudes 24·7°N and 25·0°N in northern Taiwan (Wu *et al*., 2001). The distribution profile of expected heterozygosity with latitude (Fig. 2A) of *C. carlesii*, in fact, is very similar to that reported by Wu *et al*. (2001). The Lienhuachih (LH) population (of central Taiwan) exhibited the highest heterozygosity, followed by Kungliao (KL) (northern Taiwan). In addition, Lienhuachih (LH) had the highest values for the other genetic parameters of A_e, A_r and P. The central Taiwan population of Alishan (AL) also exhibited higher values of allelic richness than did other populations (Table 1). This major area in central Taiwan agrees well with the results for other tree species (Lin, 2001; Wu *et al*., 2001). In the results of the cpDNA study, the Lienhuachih (LH) population (π = 0·00136) also had the highest nucleotide diversity, followed by Fushan (FS) (π = 0·00116) and Lichia (LC) (π = 0·00091) (Cheng *et al*., 2005). The Lienhuachih (LH) population appears to be the common site for a diversity centre for *C. carlesii*.

Why is an area which exhibits high levels of genetic diversity interesting to evolutionary biologists? It is suggested that the high diversity in the Lienhuachih (LH) population and nearby areas resulted from an admixture of different colonization routes from north and south after the glacial period. The region of Kungliao (KL) may represent another centre of mixture of diversity. Comparisons between the allozyme and cpDNA data point to populations in central Taiwan for conservation priority.

No correlation between population groupings and geographical ranges

The UPGMA dendrogram and PCA method generated population groupings that do not conform to groupings according to geographic locations. The results indicated that the CMR is not a barrier to gene flow among populations of both sides. The intermixture of different nuclear loci or introgression between species (Martinsen *et al*., 2001), and selection (Hamrick, 1982) possibly being another factors causing the erosion of the spatial genetic structure has so far not been elucidated in Taiwan. These effects might have caused the absence of a relationship between genetic distance and geographic distance. Alternatively, the absence of correlation could be due to the fact that populations of *C. carlesii* have not reached mutation-drift equilibrium yet. Many European studies of mixed species populations of oaks and their close relatives have found abundant evidence of polymorphisms shared among sympatric species, while allopatric conspecifics possess different suites of genotypes, either through introgression or incomplete lineage sorting of ancestral polymorphisms (Belahbib *et al*., 2001; Petit *et al*., 2002). In Taiwan, *C. carlesii* is found intermixed with other oak species, e.g. *Pasania hancei*, *P. harlandii*, *Castanopsis kawakamii*, *C. fargesii* and *Limlia uraiana* at low elevations, and with *Quercus longinux*, *Q. morii*, *Q. sessilifolia*, *Q. stenophylloides*, *P. hancei*, *P. kawakamii* and *Lithocarpus lepidocarpus* at medium to high elevations. However, hybridization among sympatric species has never been studied. Further studies are needed to investigate possible interspecific hybridization and introgression of all of the potential participants in these associations.

No allelic frequencies observed at any loci were significantly related to the elevational or latitudinal gradients in *C. carlesii*. This might be associated with the complicated range expansion process. Temperate tree species colonized from south to north after the glaciers retreated (Hewitt, 1996). However, it is thought that the recolonization process in Taiwan was probably more complicated than that in temperate areas. The colonization routes in Taiwan might have been more than one direction because of the CMR and whether there was more than one refuge site. The multidirectional colonization effect would have promoted intermixing among populations of a given species.

The most genetically divergent locations

The value of the mean F_{ST} of each population in comparison with the remaining populations can be used to examine the consequences of historical and contemporary geographical population subdivisions on evolutionary processes (Johnson *et al*., 2000). Petit *et al*. (2003) tested the hypothesis that glacial refuge areas harbour a larger fraction of intraspecific diversity. They revealed a latitudinal pattern, with populations in the south being significantly more differentiated from the pooled remaining populations than were the central or northern populations, and concluded that plant populations in refuge areas have higher genetic divergence rather than a higher number of haplotypes.

A very interesting discovery in this study is the conformance of the most divergent region between species. In terms of the mean values of pairwise F_{ST} for each population against the remaining populations, the most divergent populations were situated in two places: in the region of the Anmashan (AM), Fushan (FS) and Kungliao (KL) populations on the western side of the CMR, and Tungao (TA) at a comparable latitude on the eastern side of the CMR (Fig. 2B). This region is located in the northern part of central Taiwan between 24·80°N and 24·20°N. This is in proximity to the potential refugia determined for *Trochodendron aralioides* (Huang *et al*., 2004) and *Cunninghamia konishii* (Chung *et al*., 2004). Kungliao (KL), the only population in close proximity to the seashore, has the lowest elevation level and was probably colonized from the Fushan (FS) population. However, the moderately high F_{ST} value may imply the uniqueness of this population. A second peak of F_{ST} was found for the populations of Hsinkangshan (HK), Tona (TN) and Tawu (TW), a site similar to the south-eastern Taiwan location found for *Q. glauca* (Huang *et al*., 2002). This region is located between 22·40°N and 23·10°N. It seems reasonable that south-eastern Taiwan would harbour high genetic diversity in an ice age because of a warmer temperature. However, it is more difficult to imagine why north central Taiwan could be a potential refugium. One of the possibilities was the location where the two mountain ranges (Hsuehshan Range and Central Mountain Range) crossed here (Fig. 1) and allowed moisture to be absorbed from the ocean.

The most divergent populations identified in this study were not among those highlighted by analyses of cpDNA data. Such contradictions between genetic structures based on these two types of markers have also been reported in several situations, as discussed by Avise (1994). The discrepancy in mean F_{ST} values between the allozyme and cpDNA data was probably because of different evolutionary dynamics of nuclear DNA and chloroplast DNA. For instance, consider a specific population which exhibits high mean genetic differentiation in cpDNA markers, but receives pollen from various sources; then a new population generated from seeds of this specific population might have a much lower genetic differentiation from other populations at the allozyme level than for the cpDNA markers.

Phylogeographical studies usually used ordered sequence data because DNA can be organized into hierarchically ordered networks of descent and can provide historical information (Schaal *et al*., 1998). Allozyme

markers are usually used to reveal population genetics, and have seldom been used to conduct phylogeographical studies. Ordered cpDNA markers may reflect the long-term historical structure and the unordered allozyme markers may hint at the contemporary structure, characterizing different evolutionary dynamics (Avise, 1994). The present study suggests that genetic information using allozyme markers may be used to reveal the questions addressed and corroborate with the results of previous studies reported in Taiwan.

ACKNOWLEDGEMENTS

This investigation was funded by grants from the Taiwan Forestry Research Institute (contribution no. 367) and the National Science Council (NSC92-2313-B-002-117), Executive Yuan, Taiwan.

LITERATURE CITED

Avise JC. 1994. *Molecular markers, natural history and evolution*. New York, NY: Chapman and Hall.

Belahbib N, Pemonge MH, Ouassou A, Sbay H, Kremer A, Petit RJ. 2001. Frequent cytoplasmic exchanges between oak species that are not closely related: *Quercus suber* and *Q. ilex* in Morocco. *Molecular Ecology* **10**: 2003–2012.

Bennett KD. 1990. Milankovitch cycles and their effects on species in ecological and evolutionary time. *Paleobiology* **16**: 11–21.

Bonnet E, Van de Peer Y. 2002. zt: a software tool for simple and partial Mantel tests. *Journal of Statistical Software* **7**: 1–12.

Cheliak WM, Pitel JA. 1984. *Techniques for starch gel electrophoresis of enzymes from forest tree species*. Information report PI-X-42. Chalk River, Ontario: Petawawa National Forestry Institute, 19–45.

Cheng YP, Chien CT, Lin TP. 2000. Population genetics of geographically restricted and widespread species of *Myrica* (Myricaceae). *Journal of Heredity* **91**: 61–66.

Cheng YP, Hwang SY, Lin TP. 2005. Potential refugia in Taiwan revealed by the phylogeographical study of *Castanopsis carlesii* Hayata (Fagaceae). *Molecular Ecology* **14**: 2075–2085.

Chung JD, Lin TP, Tan YC, Lin MY, Hwang SY. 2004. Genetic diversity and biogeography of *Cunninghamia konishii* (Cupressaceae), an island species in Taiwan: a comparison with *Cunninghamia lanceolata*, a mainland species in China. *Molecular Phylogenetics and Evolution* **33**: 791–801.

Cornuet JM, Luikart G. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* **144**: 2001–2014.

Crawford DJ. 1989. Enzyme electrophoresis and plant systematics. In: Soltis DE, Soltis PS, eds. *Isozymes in plant biology*. Portland, OR: Dioscorides Press, 146–164.

Crow JF, Kimura M. 1970. *An introduction to population genetics theory*. New York, NY: Harper and Row.

El Mousadik A, Petit RJ. 1996. High level of genetic differentiation for allelic richness among populations of the argan tree (*Argania spinosa* (L.) Skeels) endemic to Morocco. *Theoretical and Applied Genetics* **92**: 832–839.

Feret PP. 1971. Isozyme variation in *Picea glauca* (Moench) Voss seedlings. *Silvae Genetica* **20**: 46–50.

Goudet J. 1995. FSTAT, vers. 1.2: a computer program to calculate F-statistics. *Journal of Heredity* **86**: 485–486.

Goudet J. 1999. PCA-GEN, vers. 1.2. http://www2.unil.ch/izea/softwares/pcagen.htm.

Grivet D, Petit RJ. 2002. Phylogeography of the common ivy (*Hedera* sp.) in Europe: genetic differentiation through space and time. *Molecular Ecology* **11**: 1351–1362.

Hamrick JL. 1982. Plant population genetics and evolution. *American Journal of Botany* **69**: 1685–1693.

Hamrick JL, Godt MJW. 1989. Allozyme diversity in plant species. In: Brown AHD, Clegg MT, Kahler AL, Weir BS, eds. *Plant population genetics, breeding, and genetic resources*. Sunderland, MA: Sinauer Associates, 43–63.

Hamrick JL, Godt MJW, Sherman-Broyles SL. 1992. Factors influencing levels of genetic diversity in woody plant species. *New Forester* **6**: 95–124.

Heuertz M, Hausman JF, Hardy OJ, Vendramin GG, Frascaria-Lacoste N, Vekemans X. 2004. Nuclear microsatellites reveal contrasting patterns of genetic structure between western and southern European populations of the common ash (*Fraxinus excelsior* L.). *Evolution* **58**: 976–988.

Hewitt GM. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society* **58**: 247–276.

Hiramatsu M, Ii K, Okubo H, Huang KL, Huang CW. 2001. Biogeography and origin of *Lilium longiflorum* and *L. formosanum* (Liliaceae) endemic to the Ryukyu Archipelago and Taiwan as determined by allozyme diversity. *American Journal of Botany* **88**: 1230–1239.

Hsieh CF, Shen CF, Yang KC. 1994. Introduction to the flora of Taiwan. 3. Floristic, phytogeography, and vegetation. In: *Flora of Taiwan*. Vol. 1, 2nd edn. Taipei, Taiwan: Editorial Committee of the Flora of Taiwan, 7–18.

Huang SSF, Hwang SY, Lin TP. 2002. Spatial pattern of chloroplast DNA variation of *Cyclobalanopsis glauca* in Taiwan and East Asia. *Molecular Ecology* **11**: 2349–2358.

Huang SF, Hwang SY, Wang JC, Lin TP. 2004. Phylogeography of *Trochodendron aralioides* (Trochodendraceae) in Taiwan and its adjacent areas. *Journal of Biogeography* **31**: 1251–1259.

Hurlbert SH. 1971. The nonconcept of species diversity: a critique and alternative parameters. *Ecology* **52**: 577–586.

Hwang SY, Lin TP, Ma CS, Lin CL, Chung JD, Yang JC. 2003. Postglacial population growth of *Cunninghamia konishii* (Cupressaceae) inferred from phylogeographical and mismatch analysis of chloroplast DNA variation. *Molecular Ecology* **12**: 2689–2695.

Johnson KP, Adler FR, Cherry JL. 2000. Genetic and phylogenetic consequences of island biogeography. *Evolution* **54**: 387–396.

Liao JC. 1996. Fagaceae. In: *Flora of Taiwan*. Vol. 2, 2nd edn. Taipei, Taiwan: Editorial Committee of the Flora of Taiwan, 51–123.

Liew PM, Huang SY, Kuo CM. 2005. Pollen stratigraphy, vegetation and environment of the last glacial and Holocene—a record from Toushe basin, central Taiwan. *Quaternary International* **147**: 16–33.

Lin TP. 2001. Allozyme variations in *Michelia formosana* (Kanehira) Masamune (Magnoliaceae), and the inference of glacial refugium in Taiwan. *Theoretical and Applied Genetics* **102**: 450–457.

Lin TP, Wang CT, Yang JC. 1998. Comparison of genetic diversity between *Cunninghamia konishii* and *C. lanceolata*. *Journal of Heredity* **89**: 370–373.

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

Martinsen GD, Whitman TG, Turek RJ, Keim P. 2001. Hybrid populations selectively filter gene introgression between species. *Evolution* **55**: 1325–1335.

Murphy RW, Sites JW, Buth DG, Haufler CH. 1996. Protein: isozyme electrophoresis. In: Hillis DM, Moritz C, Mable BK, eds. *Molecular systematics*. Sunderland, MA: Sinauer Associates, 51–120.

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

Petit RJ, Latouche-Halle C, Pemonge MH, Kremer A. 2002. Chloroplastic DNA variation of oaks in France and the influence of forest fragmentation on genetic diversity. *Forest Ecology and Management* **156**: 115–129.

Petit RJ, Aguinagalde I, De Beaulieu JL, Bittkau C, Brewer S, Cheddadi R, *et al.* 2003. Glacial refugia: hotspots but not melting pots of genetic diversity. *Science* **300**: 1563–1565.

Piry S, Luikart G, Cornuet JM. 1999. BOTTLENECK: a computer program for detecting recent reductions in the effective size using allele frequency data. *Journal of Heredity* **90**: 502–503.

Schaal BA, Hayworth DA, Olsen KK, Rauscher JT, Smith WA. 1998. Phylogeographic studies in plants: problems and prospects. *Molecular Ecology* **7**: 465–474.

Schneider S, Roessli D, Excoffier L. 2000. *Arlequin vers. 2.0: a Software for Population Genetic Data Analysis*. Geneva: Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Sneath PH, Sokal RR. 1973. *Numerical taxonomy: the principles and practice of numerical classification*. San Francisco, CA: Freeman.

Soltis DE, Soltis PS. 1989. *Isozymes in plant biology*. Portland, OR: Discorides Press.

Sue CY, Fang K, Huang S. 2000. Patterns of genetic variation of *Alnus formosana* in Taiwan. *Taiwania* **45**: 95–106.

Swofford DL, Selander RB. 1981. BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *Journal of Heredity* **72**: 281–283.

Tomaru N, Mitsutsuji T, Takahahi M, Tsumura Y, Uchida K, Ohba K. 1997. Genetic diversity in *Fagus crenata* (Japanese beech): influence of the distributional shift during the late-Quaternary. *Heredity* **78**: 241–251.

Tsukada M. 1966. Late Pleistocene vegetation and climate in Taiwan (Formosa). *Proceedings of the National Academy of Sciences of the USA* **55**: 543–548.

Tsukada M. 1967. Vegetation in subtropical Formosa during the Pleistocene glaciations and the Holocene. *Palaeogeography Palaeoclimaology Palaeoecology* **3**: 49–64.

Widmer A, Lexer C. 2001. Glacial refugia: sanctuaries for allelic richness, but not for gene diversity. *Trends in Ecology and Evolution* **16**: 267–269.

Wu JE, Huang S, Wang JC, Tong WF. 2001. Allozyme variation and the genetic structure of populations of *Trochodendron aralioides*, a monotypic and narrow geographic genus. *Journal of Plant Research* **114**: 45–57.