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Allozyme variations in *Michelia formosana* (Kanehira) Masamune (Magnoliaceae), and the inference of a glacial refugium in Taiwan

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Abstract *Michelia formosana* is distributed islandwide in Taiwan and also occurs in the Ryukyu Archipelago, Japan. Allozyme genetic variability in *M. formosana* (Magnoliaceae) was investigated using five polymorphic loci (*Pgm-1*, *Idh-1*, *Mr-1*, *Skdh-1*, and *Ppo-3*) from five enzyme systems. The average value of expected heterozygosity (H_e) describing within-population variation was 0.241. The overall F_{is} (0.0736) indicates a significant deficiency of heterozygotes at the population level. This positive value of F_{is} is mainly contributed from populations found at Pinglin, Chienshi, Wufeng, and the Ryukyus, all located at northern latitudes, and is also caused by locus *Ppo-3*. Among-population variation, F_{st}, accounted for 10.6% of the total heterozygosity and deviates significantly from zero. This populational differentiation agrees well with that of general tropical woody species outcrossed by animals. The population on Lanyu (Orchid Island) has some morphological differences from the plants native to Taiwan, but this was not reflected in the cluster analysis using the unweighted pair group method. *Pgm-1d*, however, is the diagnostic characteristic for the plants growing on Lanyu as it is entirely absent from all other populations of *M. formosana*. About 18% of the alleles of this study show clinal geographical variation and were found to be significantly related to latitudinal gradients throughout the species range. This, however, is not a linear relation but a curve with a peak form which is exactly the same as the relationship between expected heterozygosities of populations and latitude. This observation suggests that Nantou County, in central Taiwan could be a glacial refugium for genetic diversity of *M. formosana*. This inference is further supported by analysis of published studies on seven widely spread plant species in Taiwan. Location of this glacial refugium is probably related to the first emergence of the Central Mountain Ridge millions of years ago that naturally becamed the major window to receive genetic diversity from the Asian mainland.

Keywords Allozyme variation · *Michelia formosana* · Refugium

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Introduction

Michelia formosana (or *M. compressa* a term still used in the flora of Taiwan; see Keng 1996) is a widely distributed species of the Magnoliaceae and is dominant in subtropical forests in Taiwan and Lanyu. It can be found at elevations from sea level up to 2000 m. Recently people have tended to use *M. formosan* for the plants grown in Taiwan and *M. compressa* for the plants native to southern Japan (Shimabuku 1997). *M. formosana* is also distributed throughout the southern Ryukyus (Irimote and Ishigati Islets). That the distribution of *M. formosana* is confined to only Taiwan and the Ryukyus is probably related to paleoclimatic changes. The genus *Michelia* is native to tropical, subtropical, eastern Asia, and the Malay Archipelago (Hutchinson 1973). Palynological evidence dated the the earliest fossil record of *Michelia* in Taiwan back to the Holocene, in the Quaternary (Huang 1992). To date, no conclusion has been drawn with respect to the evolution of *M. formosana* – whether it migrated from the Malay Archipelago, southern China or was newly evolved in Taiwan.

M. formosana is insect-pollinated, mostly by honey bee (*Apies* sp.) and less by Syrphidae and the bumble bee (Bombidae) (Y.P. Feng, personal communication). It produces elongate cone-like fruit with coalescent follicles. When mature, the follicles split open only on one side to expose four to six fleshy scarlet-pink seeds. The removable outer portion of the outer seed coat is fleshy, oily, and soft. The fleshy seed is usually suspended from the open follicle for some time by a slender, elastic thread.

Table 1 Elevation, latitude, longitude, average observed heterozygosities (H_o), average expected heterozygosities (H_e), the average number of alleles per polymorphic locus (A), and fixation indices (F_{is}) in nine populations of *Michelia formosana*

Locality	Population Code (*n*)[a]	Elevation (m)	Latitude	Longitude	P[b]	A	H_o	H_e	F_{is}
Pinglin	4 (34)	250–430	24.93	121.68	80	1.80	0.139	0.172	0.196*
Sanshia	3 (31)	390–500	24.93	121.35	80	2.20	0.249	0.228	–0.096
Chienshi	2 (36)	370–1430	24.70	121.18	80	2.20	0.216	0.272	–0.209**
Wufeng	1 (37)	600-1430	24.65	121.12	80	2.00	0.212	0.260	–0.187*
Lienhuachih	7 (30)	600–750	23.88	120.87	80	2.20	0.303	0.306	–0.004
Liukuei	8 (27)	730–980	22.98	120.62	80	2.00	0.160	0.160	–0.003
Taimali	9 (31)	520–1270	22.60	120.98	80	2.00	0.227	0.271	0.166
Lanyu	6 (20)	200–400	22.08	121.48	80	2.00	0.323	0.321	–0.005
Ryukyu	5 (8)	100–400	–	–	40	1.60	0.125	0.182	0.327**

* $P<0.05$, ** $P<0.01$

[a] Number of samples per population is indicated in parenthesis

[b] Percentage of polymorphic loci per individual

Fruits are eaten and dispersed by birds or sometimes monkeys. The flattened seeds, 7–8 mm in length, exhibit orthodox storage behavior and usually have a germination percentage of between 50% and 80% (Lin and Wu 1995; Lin 1996).

The plants growing on Lanyu show morphological differentiation from those in Taiwan. For example, the plants of Lanyu have larger and broader (elliptic) leaves, and in artificial plantations they begin flowering at about 3 years old. Thus, the plants growing on Lanyu have recently, been renamed as *M. formosana* (Kaneh.) Masam. var. *kotoensis* (Lu et al. 1998). To obtain a general picture of genetic variation of *M. formosana* throughout its range of distribution, and with the expectation of finding significant differentiation between the plants growing in Taiwan and on Lanyu, we used allozyme analysis to investigate the genetic diversity and the differentiation of nine selected populations.

In the study reported here, Nantou County (in the central part) and the south-eastern part of Taiwan were found to have the highest expected heterozygosity for *M. formosana.* A thorough review of published papers on the genetic diversity of widely spread plant species in Taiwan and our present results led to the discovery, for the first time, of a major refugium for plant genetic diversity. The discovery and the formation of this refugium are discussed in this paper.

Material and methods

Sampling

In total, 225 samples of *Michelia formosana* were collected from seven natural populations, i.e. Pinglin (34), Sanshia (31), Chienshi (36), Wufeng (37), Lienhuachih (30), Liukuei (27), and Taimali (31) (Table 1). Samples from Ryukyu (8) were collected from the Taipei Botanical Garden where the plants were grown from seeds collected from unknown mother trees in Ryukyu, and samples from Lanyu (20) were from two artificial plantations. The elevation, latitude, and longitude for each population are listed in Table 1. *M. formosana* native to Lanyu has a slightly different morphology, with broader and thicker leaves that may have been an adaptation to the tropical climate there. *M. formosana* of Taiwan produces valuable wood and is considered to be one of the most valuable broadleaf trees locally.

Young leaves and flower buds covered with hairy winter scales were collected from August to the beginning of September 1999. They were carried back to the laboratory in sealed polyethylene bags and frozen at –70°C, or stored overnight at room temperature (24°C), before enzyme extraction. Samples were ground with extraction buffer according to procedures described in Feret (1971) and absorbed onto Whatman 3 mm filters (4×12 mm). Paper strips were arranged in a plastic petri dish and stored at –70°C until use. It was found that flower buds generated much less browning than young leaves during grinding.

Electrophoresis

Electrophoresis and staining methods followed the procedures described in Cheliak and Pitel (1984). Buffer system H was used to resolve isocitrate dehydrogenase (IDH, EC 1.1.1.42), phosphoglucomutase (PGM, EC 5.4.2.2), polyphenol oxidase (PPO, E.C.1.14.18.1), and shikimate 5-dehydrogenase (SKDH, EC 1.1.1.25). Buffer system B was used to resolve meandione reductase (MR, EC 1.6.99.2). Putative loci were designated sequentially, with the most anodally isozyme designated as 1, the next 2, and so on. Likewise, alleles were designated sequentially with the most anodally migrating alleles designated a, the next b, and so on.

Data analysis

Allele frequencies in each population of the species were calculated for the five polymorphic loci encoding the five enzyme systems. BIOSYS-2 (from William C. Black IV, Department of Microbiology, Colorado State University), a modified version of the BIOSYS-1 program (Swofford and Selander 1981), was used to estimate the following genetic parameters: mean number of alleles per locus (A), percentage polymorphic loci (P) (99% criterion), and observed (H_o) and expected heterozygosities (H_e). The significance of an F_{st} value per locus different from zero was checked by FSTAT v 1.2 (from Jerome Goudet, University of Lausanne, Switzerland). Conformance of the investigated populations to the Hardy-Weinberg (HW) equilibrium of F_{IS} was estimated using boostrapping over loci (FSTAT). Linear regressions and peak regression were performed to study the relationships between the expected heterozygosities (H_e) and the latitudinal range of eight populations except those of the Ryukyus, using the general linear models procedure (GLM) of the SAS system (SAS Institute, Cary, N.C.).

Table 2 Allele frequencies in nine populations of *M formosana*

Population[a] / Locus	1	2	3	4	5	6	7	8	9
Pgm									
(*n*)[b]	36	33	31	34	8	18	30	27	31
a	0.000	0.000	0.032	0.000	0.000	0.000	0.000	0.000	0.000
b	0.861	0.742	0.742	0.779	1.000	0.333	0.700	0.889	0.790
c	0.139	0.258	0.226	0.221	0.000	0.500	0.300	0.111	0.210
d	0.000	0.000	0.000	0.000	0.000	0.167	0.000	0.000	0.000
H_o	0.222	0.273	0.516	0.324	0.000	0.556	0.333	0.222	0.290
H_e	0.243	0.388	0.404	0.349	0.000	0.629	0.427	0.201	0.337
Idh									
(*n*)	37	36	31	34	8	20	30	27	30
a	0.000	0.014	0.000	0.000	0.000	0.000	0.017	0.000	0.000
b	1.000	0.986	1.000	1.000	1.000	1.000	0.983	1.000	0.000
H_o	0.000	0.028	0.000	0.000	0.000	0.000	0.033	0.000	0.000
H_e	0.000	0.028	0.000	0.000	0.000	0.000	0.033	0.000	0.000
Mr									
(*n*)	37	34	31	32	8	20	30	27	30
a	0.919	0.897	0.903	0.875	1.000	0.800	0.900	0.926	0.700
b	0.081	0.103	0.097	0.125	0.000	0.200	0.100	0.074	0.300
H_o	0.108	0.147	0.129	0.125	0.000	0.400	0.200	0.148	0.467
H_e	0.151	0.187	0.178	0.222	0.000	0.328	0.183	0.140	0.427
Skdh									
(*n*)	34	36	30	33	8	19	30	26	30
a	0.191	0.111	0.033	0.091	0.188	0.079	0.083	0.019	0.033
b	0.735	0.847	0.933	0.909	0.688	0.921	0.767	0.885	0.900
c	0.074	0.042	0.033	0.000	0.125	0.000	0.150	0.096	0.067
H_o	0.324	0.250	0.133	0.182	0.625	0.158	0.467	0.231	0.200
H_e	0.424	0.272	0.129	0.168	0.508	0.149	0.389	0.212	0.188
Ppo									
(*n*)	32	34	30	31	8	18	27	25	17
a	0.391	0.603	0.300	0.065	0.750	0.417	0.426	0.140	0.735
b	0.609	0.397	0.700	0.935	0.250	0.583	0.574	0.860	0.265
H_o	0.406	0.382	0.467	0.065	0.000	0.500	0.481	0.200	0.176
H_e	0.484	0.484	0.427	0.123	0.400	0.500	0.498	0.246	0.401

[a] See Table 1 for numerical code for each population
[b] *n*, Number of samples
*, ** Significant at the 5% and 1% levels, respectively

The genetic distance between the *j*th population and the remaining populations was calculated by population differentiation, *Dj*, of Gregorius and Roberts (1986):

$$Dj = \frac{1}{2}\sum_i \left| pi(j) - \overline{pi}(j) \right|,$$

where *pi* (*j*) and *p* (*j*) are the frequencies of the *i*th allele in the *j*th population and in the remaining populations, respectively. This measure indicates the proportion of alleles by which one population differs from all other populations. If the *j*th population is identical to, or completely different from the remaining populations, the values of *Dj* are 1 or 0, respectively.

Results

Seven enzyme systems tested could be resolved clearly enough for genetic diversity analysis: Pgm, Idh, Mdh, Skdh, Mr, Sod, and Ppo. Among these loci, *Pgm-2*, *Mr-2*, and *Sod* are monomorphic in all populations. Mdh has multiple bands, but the locus could not be defined. *Pgm-1*, *Skdh-1*, *Mr-1*, and *Ppo-3* are polymorphic. *Idh-1* is also polymorphic, but allele *Idh-1a* has very low frequency and was found only in the Lienhuachih and Chienshi populations. The observed allelic frequencies at each locus for each population are listed in Table 2. Among these alleles, *Pgm-1a* was only found in the population Sanshia, and *Pgm-1d* was only found on Lanyu. The distribution of allelic frequency of PGM in population Lanyu is quite different from those of the other populations. The Ryukyu population has fixed allele frequencies for PGM, IDH, and MR, and no heterozygote of PPO was observed. This probably is because the sample size is too small, but SKDH has quite uniform frequencies of alleles, thus giving high heterozygosities.

Pattern of allele distribution

Despite most of the frequencies fluctuating greatly along the geographical transect, 2, *Pgm-1d* and *Skdh1-c* (18%), of the 11 alleles were found to be significantly related to latitudinal gradients throughout the species range (Table 4). However *Skdh-1c* was observed to have not a linear but a quadratic relationship with a peak was found in population Lienhuachih. The regression model for allele

Table 3 Fixation indices (F_{is}) at polymorphic loci in nine populations of *M. formosana*

Locus	F_{IS}	F_{ST}	F_{IT}	*P*[a]
Pgm	0.0646	0.0847	0.1438	0.108
Idh	–0.0155	0.0114	–0.0040	0.999
Mr	0.0634	0.0493	0.1096	0.198
Skdh	–0.0307	0.0416	0.0122	0.665
Ppo	0.1574	0.1855	0.3137	0.012*
Mean	0.0736	0.1056**	0.1714	0.005**

*,** Significant at the 5% and 1% levels, respectively
[a] *P* Testing by Hardy-Weinberg within samples for F_{IS}

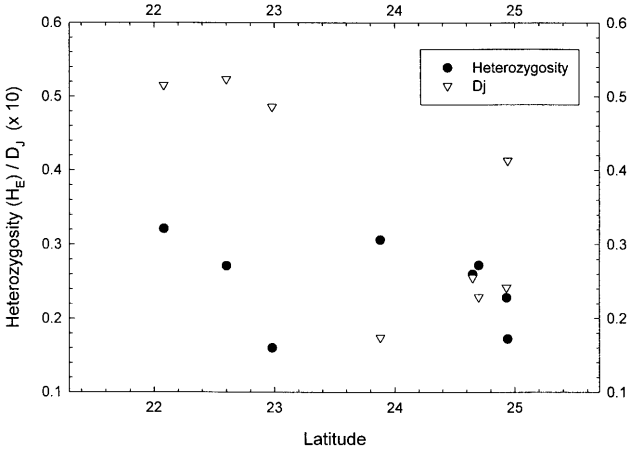

Fig. 1 Relationships of expected heterozygosities (*H_e*; *black circles*) and the proportions of alleles by which 1 population differs from all other populations (*D_j*; *open triangles*) against latitude for eight populations of *Michelia formosana* native to Taiwan

frequencies against the effect of latitude can be written as follows:

Skdh-1c frequency=–33.860+2.883 lat.–0.061 lat.^{2}.

Genetic diversity within populations

Measures of genetic diversity are presented in Table 1. Since there were only 5 polymorphic loci, the genetic diversity expression was entirely based on these loci. At the population level, the average number of alleles per polymorphic locus (A) was 1.95, the percentage of polymorphic loci per individual (P) was 75.5%, observed heterozygosity (H_O) varied from 0.125 to 0.323, with an average of 0.217, and the expected heterozygosity (H_e) varied from 0.160 to 0.321, with an average of 0.241. The species showed a considerable deficiency of heterozygotes because values of H_o were lower than H_e in 4 of the 9 populations (see F_{IS} of Table 1), thus most of the populations obey the Hardy-Weinberg equilibrium. In conclusion, populations at Pinglin, Ryukyu, Wufeng, and Chienshi show lower genetic diversity (H_o) than do the other populations, and the populations of Lienhuachih, Lanyu, and Sanshia have higher levels of genetic diversity than do the others.

The Ryukyu population has a low genetic diversity (H_e=0.182) that is similar to that of population Pinglin but much lower than those of most of the populations of Taiwan. Similar observation have been made on another tree species *Trochodendron aralioides* (Wu et al. 2000).

Figure 1 shows that the H_e (black dots) of populations tends to decrease with increasing latitude above 24°, and decreases with decreasing latitude below 24°. However, populations of Lanyu and Taimali have H_e values comparable to that of Lienhuachih.

Genetic differentiation

Wright's F-statistics showed that at the population level, mean F_{is}=0.0736, and at the species level, mean F_{it}=0.1714 (Table 4). While testing for HW equilibrium in loci within a population employing the FSTAT program, we found that 0.0736 significantly deviates from zero. This is caused by the locus *Ppo-3*. Again, F_{st} is the measure of absolute gene differentiation, and values range from 0.0114 for *Idh-1* to 0.1855 for *Ppo-3*, with an average of 0.1056, which is significantly different from zero (*P*=0.05) as tested using Weir and Cockerham (1984) estimators of F-statistics (θ) (FSTAT program). Thus, about 89% of the total variation resides within populations.

Figure 1 shows that the *Dj* (open triangle) of populations tends to decrease with increasing latitude (Student *t*-test, *t*=57.59369, *P* <0.00000, *df*=14). Averaging values of *Dj* across all populations, excluding Ryukyu, showed that the proportion of alleles by which a population differs from all the other populations is 0.0355.

Discussion

Level of genetic variation within populations and extent of population differentiation

M. formosana has white medium-sized (about 2 cm in diameter), and insect-pollinated flowers, and thus should favor outcrossing The fixation indices (F_{is}=0.0736), however, indicate a slightly deficiency of heterozygotes. This is because populations at Pinglin, Chienshih, and Wufeng at higher latitudes contribute to the positive fixation index, and populations in central and southern areas generally agree with HW equilibrium. This is probably related to dramatic differences in climatic conditions. Taiwan is geographically situated well within the Pacific monsoon zone; the amounts of rainfall in different parts of the island are strongly influenced by the prevailing winds. In winter months, rains are mainly due to northeast monsoon winds, which bring much rain to northern, northeastern, and eastern Taiwan, but have little effect on the climate of the central and southern parts. In early February during the flowering season for *M. formosana*, there is also a lot of rain which could prevent the mechanism of outcrossing by pollinators (honey bees). A deficiency of heterozygotes in northern populations has also been observed in other plant species (Cheng et al. 2000).

Table 4 Significance tests for regression coefficients of allele frequencies against latitudinal effect for nine populations of *Michelia formosana*

Locus	Allele	Range of frequency	Latitude model			
			R^2	Effect	F	*P*
Pgm-1	*a*	0.000–0.032	0.2377			0.5073
				Linear	0.97	0.3706
				Quadratic	0.59	0.4762
	b	0.333–0.889	0.3553			0.3337
				Linear	1.66	0.2543
				Quadratic	1.10	0.3427
	c	0.111–0.500	0.1874			0.5952
				Linear	0.88	0.3922
				Quadratic	0.28	0.6212
	d	0.000–0.167	0.7679			0.0259*
				Linear	8.37	0.0340*
				Quadratic	8.17	0.0355*
Mr-1	*a*	0.700–0.926	0.4764			0.1984
				Linear	3.82	0.1080
				Quadratic	0.73	0.4322
	b	0.074–0.300	0.4764			0.1984
				Linear	3.82	0.1080
				Quadratic	0.73	0.4322
Skdh-1	*a*	0.019–0.191	0.1910			0.5887
				Linear	0.96	0.3711
				Quadratic	0.22	0.6621
	b	0.707–0.933	0.4857			0.1897
				Linear	0.56	0.4864
				Quadratic	4.16	0.0970
	c	0.000–0.138	0.9209			0.0018**
				Linear	0.36	0.5724
				Quadratic	57.36	0.0006**
Ppo-3	*b*	0.065–0.621	0.0894			0.7912
				Linear	0.45	0.5328
				Quadratic	0.04	0.8443
	b	0.265–0.935	0.0894			0.7912
				Linear	0.45	0.5328
				Quadratic	0.04	0.8443

*, ** Significant at the 5% and 1% levels, respectively

As a general impression, the four northern populations have higher densities than the populations at Lienhuachih and the southern ones. This is supported by observations made by Dr. Chang-Fu Hseih, Department of Botany, Taiwan University. The density and number of plants with a stem diameter greater than 1 cm per hectare of *M. formosana* in plots at Lanjenchi (330 m altitude, 120°49', 22°01'), Mt. Nanjen (250 m latitude, 120°50', 22°05'), Lanyu (150 m altitude, 121°29', 22°05'), Mt. Peitungyen (2050 m altitude, 121°07', 24°04'), and Mt. Lopei (1150 m altitude, 121°27', 24°48') was 24, 20, 12, 101, and 228, respectively (provided by Dr. Hseih). Mt. Peitungyen in central Taiwan and Mt. Lopei in northern Taiwan definitely have a much higher density of *M. formosana* than Lanjenchi (south end of Taiwan), Mt. Nanjen (south end) and Lanyu (south-eastern). Thus, the effect of a large population size is independent of the event of inbreeding phenomenon.

In *M. formosana*, 10.6% of the total genetic diversity measured over the polymorphic loci is attributable to differentiation among populations. This value is slightly less than the 13.5% (G_{st}) of tropical woody species based on 124 taxa (Hamrick 1994). Geographic range has been considered as one major characteristic influencing genetic diversity (Hamrick et al. 1992). Few widely spread broadleaf tree species in Taiwan have been studied for variations in allozymes. Two examples, *Cinnamomum kanehirae* (H_e=0.214) and *Myrica rubra* (H_e=0.163), showed rather high genetic variation. The same should occur in *M. formosana* as much variation was noted in other enzyme systems which were not justified to be used for our calculations. Breeding system and seed dispersal are other factors influencing genetic diversity; outcrossing by animals and ingestion for seed dispersal result in the highest H_e. These factors all contribute to a presumed high genetic diversity in *M. formosana*.

Geographical pattern of genetic diversity

Variations in populations tend to be lower both north and south of latitude 23°50' than in the population at latitude 23°50', as indicated by the H_e values. Allele frequencies in 2 loci (*Shdh-1b* and *Skdh-1c*) also showed significant clinal variation with H_e values across the entire range of latitude. Clinal variation of allelic frequencies along latitudinal and/or longitudinal gradients has been reported for some tree species (Leonardi and Menozzi 1995; Zanetto and Kremer 1995, Tomaru et al. 1997). They proposed that patterns of geographical variation in allelic frequencies resulted from postglacial migration and the

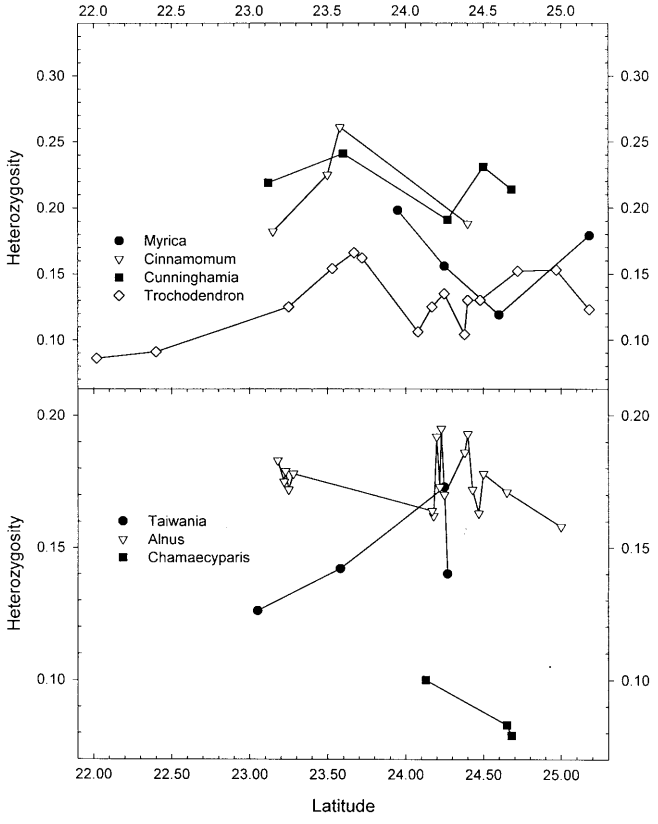

Fig. 2 Relationships of expected heterozygosities (H_e) for seven widely spread plant species with latitude. **Upper figure** ● *Myrica rubra* (Cheng et al. 2000), ▽ *Cinnamomum kanehirae* (Lin et al. 1997), ■ *Cunninghamia konishii* (Lin et al. 1998), ◇ *Trochodendron aralioides* (Wu et al. unpublished observation). Lower figure ● *Taiwania cryptomerioides* (Lin et al. 1993), ▽ *Alnus formosana* (Su and Huang 2000); ■ *Chamaecyparis taiwanensis* (Lin et al. 1994)

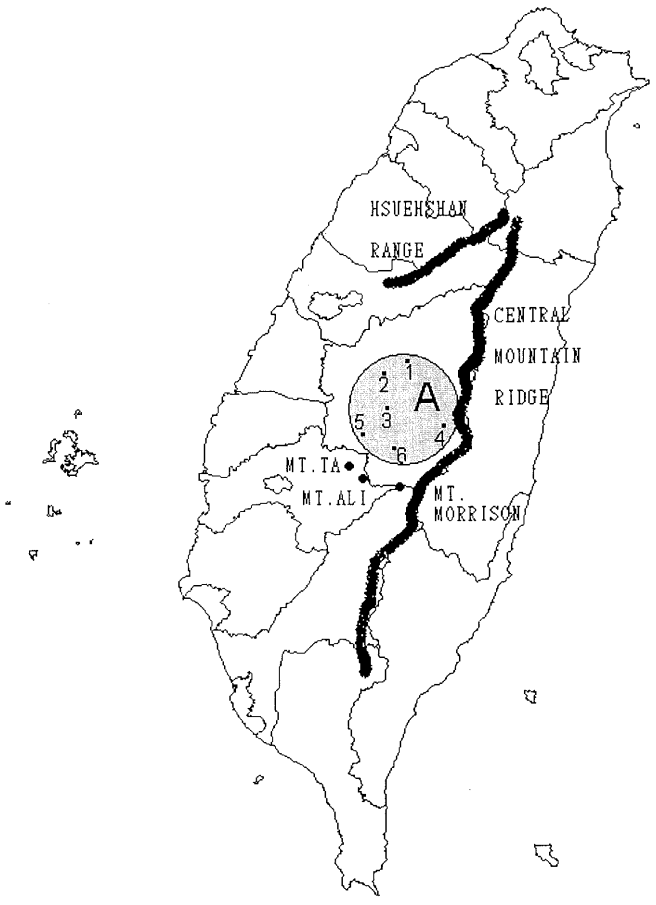

Fig. 3 Map of Taiwan showing the location of the major refugium where the populations for five species with highest expected heterozygosities (H_e) are included. *1* Puli for *Myrica rubra*, *2* Lienhuachih for *Michelia formosana*, *3* Jenlun for *Trochodendron aralioides*, *4* Denta for *Cunninghamia konishii*, *5* Chitou for *Trochodendron aralioides*, *6* Wangshiang for *Cinnamomum kanehirae*. The Central Mountain Ridge and Hsuehshan Range are also shown

founding effect. Clinal variation in *M. formosana* suggests migration in two directions.

Population differentiation of *M. formosana* appears to have proceeded less in northern populations, as indicated by *Dj* values. This indicates the population differentiation of the southwestern part of Taiwan is unique. Cluster analysis using the unweighted pair group method also showed that populations at Taimali and Lanyu have higher genetic distances from the other populations (data not shown). This also agrees with the isolated situations of these two populations. The population at Lienhuachih has a high heterozygosity but a low D_j values. This is because, in general, the allelic frequencies for each locus in population Lienhuachih are close to the average and the allelic frequency distributions are more uniform.

Inference of a possible major refugium in Taiwan

We found a peak of highest expected heterozygosities throughout the entire latitudinal range in Taiwan (Fig. 1) for this species. This indicates that the expected heterozygosity is related to the ecological amplitude of the population; this characteristic is also probably related to the migration history of *M. formosana*. Thus we searched for all published studies on widely spread plant species in Taiwan. A total of seven widely spread species, not including *M. formosana*, grow in various altitudinal ranges and different environmental conditions. Figure 2 represents the relationships between latitude and expected heterozygosities of these seven species: *Myrica rubra* (Lour.) Sieb. & Zucc. (Cheng et al. 2000), *Cinnamomum kanehirae* Hay. (Lin et al. 1997), *Cunninghamia konishii* Hay. (Lin et al. 1998), *Trochodendron aralioides* Sieb& Zucc. (Wu et al. unpublished observation), *Taiwania cryptomerioides* Hay. (Lin et al. 1993), *Alnus formosana* (Burkill.) Mak. (Sue et al. 2000), and *Chamaecyparis taiwanensis* Masam. & Suzuki (Lin et al. 1994). Most of these seven species have their highest expected heterozygosities between latitudes 23.6° and 23.9°. The situations with both *Alnus formosana* and *Taiwania cryptomerioides* are not clear since no populations in the above-mentioned latitudes have been sampled. That means that 75% of the species examined have a common center for their genetic diversity. In addition,

we found that another subordinate or secondary refugium may exist between 24.2° and 24.8°, because four species showed another slight peak in this region.

The major refugium found is located in Nantou County, in central Taiwan (Fig. 3). Why is the refugium located in the center of Taiwan? To answer this question, we need to look back at the creation of Formosa, probably 5–6 million years ago (Ma), when the Central Mountain Ridge was the first landmass to emerge above sea level (Teng 1990). The area corresponding to present-day Nantou County was probably the first subordinate landmass to emerge at this time. Geotectonic evidence reveals that the Eastern Coastal Range Island had been colliding with the Gutaiwan Block, which is rifted Eurasian continental margin to the incipient Taiwan Terrain. At about 3 Ma, this narrow Eastern Coastal Range Island began to indent into the Gutaiwan Block, virtually completing the basic geotectonic process of the current island of Taiwan (Lu and Hsu 1992). Strong climatic oscillations that occurred up to 700000 years ago, with a dominant series of cold and dry glacial periods being interrupted by intervals of warmer and moister interglacial climates, have been documented for the Quaternary (Webb and Bartlein 1992; Bond et al. 1993). During glacial stages, sea levels decreased so that Taiwan probably would have been connected with the Asian landmass by a land bridge (Liu 1988), and plants could have migrated towards the warmer south without a barrier. Thus, it is reasonable to presume that the central area of the Central Mountain Ridge (mostly in Nantou County) should have been the first place to accommodate various genetic diversity and was the location from which dispersal of organisms originated in various directions.

In view of its geographical location, Nantou County has another advantage as the major refugium. On the eastern side of the north-south extending Central Mountain Ridge there are numerous peaks over 3000 m in elevation which prevent cold weather from reaching the eastern side. To the north, the Hsuehshan Range also has many peaks over 3000 m high, thereby forming a barrier to prevent winter cold fronts coming from the north. To the south, a smaller range is formed by Mt. Morrison or Jade Mt. (3952 m), Mt. Ali (2489 m), and Mt. Ta (2489 m). Only the western opening happens to be the window facing the Taiwan Strait to receive genetic diversity.

Special attention should be paid to the southeastern populations of *M. formosana* where high expected genetic diversity (H_e) and high population differentiation (*Dj*) coexist. Good adaptation to the humid and warm climate is probably the major reason for the species growing and flourishing here. This should be considered as a second refugium center for *M. formosana*.

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