

Biology

**CONSERVATION OF GENETIC DIVERSITY OF
THREATENED CYCADS (CYCADACEAE) IN VIETNAM**

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Abstract. Cycads occur in lowland forests and are threatened by their degradation, fragmentation and over-exploitation. Our results indicate that cycad population sizes were very small (less than 100 individuals) with high propagules. These increased mating between closely related individuals growing in clumps. The allozyme data show although all studied cycads maintained high levels of genetic diversity, an average of 0.556 (0.491 in *C. hoabinhensis* to 0.598 in *C. chevalieri*), the degree of inbreeding was high. Factors that contribute to the high levels of genetic variability include dioecy, perennial and complex habitat structure. The priorities for cycad conservation is discussed as well.

1. INTRODUCTION

Cycad habitats are threatened in two major ways, by the clearing of land for cultivation and settlements and by over-exploitation. Their habitats have suffered severe reduction and degradation. Many cycads are known to occur as fragmentary populations. The remaining fragments are usually small in size; hence a small number of individuals comprised each fragment. Due to fluctuations in the number of individuals through random demographic and environmental forces, such small populations face an increased probability of extinction. Recolonization of fragments in which the species has become extinct may occur if migration between fragments is still possible. However, when the distances between the remaining fragments are great, subpopulations may become isolated from each other with a change of gene flow between subpopulations and the probability of the species being greatly reduced. Moreover, because of their attractive leaves; most species are collected for cultivation in collector's gardens or to sell in local markets as ornamental plants.

In order to conserve threatened cycads, effective conservation not only maintains their habitats to manage their population dynamics, but also conserve the genetic diversity that provide the raw material for adaptive responses to environmental change. The goals of this study are to use electrophoresis and field measurements to determine the level and structure of genetic variation, population characteristics related to heterozygosity and genetic consequences under disturbed habitats. This study also aims to determine the ecological factors and set up priorities for genetic conservation of cycads in Vietnam.

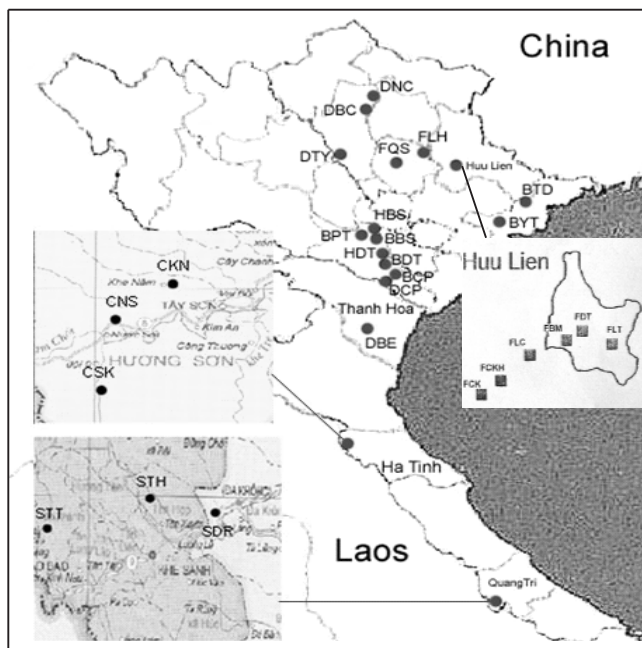


Fig. 1. Map showing the studying sites: DNC: Nam Chang, DBC: Ban Chu, DBE: Ben En, DCP: Cuc Phuong, DTY: Thuy Uy, FDT: Deo Tra, FBM: Ba Min, FLT: Lan Ti, FGK: Goc Khe, FCK: Cai Kinh, FLH: Lang Hang, FQS: Quang Son, HDT and BDT: Dong Tam, HBS and BBS: Ba Sao, BPT: Phu Thanh, BCP: Yen Quang, BTD: Tan Dan, BYT: Yen Tu, SDR: Dackrong, STH: Tan Hop, STT: Tan Thanh, CSK: Son Kim, CNS: Nuoc Sot, and CKN: Khe Nam.

2. MATERIALS AND METHODS

518 leaf samples of 6 threatened cycads, *Cycas dolichophylla*, *C. ferruginea*, *C. hoabinhensis*, *C. balansae*, *C. chevalieri* and *C. simplicipinna* had been collected from November 2001 to May 2003 in 26 studied sites of 10 provinces: in Ninh Binh: Cuc Phuong (20°21N-105°36E, 492m alt.), Thanh Hoa: Ben En (19°33N-105°29E, 110m alt.), Tuyen Quang: Nam Chang (22°22N-105°27E, 380m alt.), Ban Chu (22°21N-105°27E, 570m alt.) and Thuy Uy (21°44N- 105°21E, 664 m alt.) for *C. dolichophylla*; Lang Son: Deo Tra (21°36N-106°20E, 373m alt.), Ba Min (21°42N-106°23E, 476m alt.), Lan Ti (21°37N-106°24E, 311m alt.), Lang Chua (21°43 N-106°22E, 306m alt.), Goc Khe (21°45N-106°22E, 460m alt.), Cai Kinh (21°34N-106°27E, 420m alt.). Thai Nguyen: Lang Hang (21°45 N - 106°01E, 333m alt.), Quang Son (21°44N - 105°54 E, 331m alt.) for *C. ferruginea*; Hoa Binh: Dong Tam (20°14N-105°50E, 360m alt.), Ha Nam: Ba Sao (20°30N-105°56E, 334m alt.) for *C. hoabinhensis*; Hoa Binh: Phu Thanh (20°16N-105°51E, 158m alt.) and Dong Tam (236m alt.), Ha Nam: Ba Sao (246m alt.), Ninh Binh: Yen Quang (20°21N – 105°36E, 365m alt.), Quang Ninh: Tan Dan (21°08 N-106°52 E, 50m alt.) for *C. balansae*; Ha Tinh: Son Kim (18°26N-105°15 E, 212m alt.), Nuoc Sot (18°28'12N-105°18'23 E, 358m alt.) and Khe Nam (18°32'54N-105°21'09E, 547m alt.) for *C. chevalieri* ; and Quang

Tri: Dackrong (16°40'00N-106°52'14E, 387m alt.), Tan Thanh (16°36'15N-106°38'23E, 281m alt.) and Tan Hop (16°39'21N-106°44'32E, 481m alt.) for *C. simplicipinna* (Fig. 1). Samples were immediately kept in the icebox. They were transferred to the Lab. Molecular Biology, Institute of Ecology and Biological Resources and subsequently stored at -80°C until used enzyme electrophoresis. Parameters of population size, habitats and interviews were determined and implemented in the field.

Polyacrylamide gel electrophoresis was used to obtain allozyme data. Leaf tissue was ground in 1 ml extraction buffer of Triest (1989). The electrode buffer was Tris-Cl and Glycine. The gels contained two parts, stacking gel with 0.5M Tris-Cl, pH 6.8 and 4.5% acrylamide, and running gel with 1.5M Tris-Cl, pH 8.8 and 10% acrylamide. Gels were run for 3 hours at 70 mA and 4 ° C and then stained at room temperature for 12 enzymes: Shikimate dehydrogenase (SKDH), Aspartate aminotransferase (GOT), 6-P-Gluconate dehydrogenase (6PGDH), Isocitrate dehydrogenase (IDH), Leucine aminopeptidase (LAP), β -esterase (β -EST), Esterase (EST), Alcohol dehydrogenase (ADH), Malate dehydrogenase (MDH), Xanthine dehydrogenase (XPH), Acid phosphate (APH) and Malic enzyme (ME). Six enzymes resulted clearly consist of GOT, LAP, SKDH, 6PGDH, IDH and β -EST and were used to assess genetic variability, using the FSTAT and TFPGA programs (Goudet, 1995; Miller, 1997)

3. RESULTS

Hill et al. (2004) identified areas for six species, of these, four *Cycas dolichophylla*, *C. ferruginea*, *C. hoabinhensis* and *C. balansae* in North Vietnam and two remaining species, *C. simplicipinna* and *C. chevalieri* in central Vietnam. *C. dolichophylla* occurs in shade of lowland rainforests, on loamy soils over limestone, schist or granites. This species was recorded in the little disturbed forest of Nam Chang, Ben En and Cuc Phuong. Habitat structure is complex and includes three distinct strata. The canopy of tall trees, up to 35-40m in height, with wet and warm summer; and dry and cool winter. The understorey is made up of young trees of the canopy and shrubs. Ban Chu and Thuy Uy were heavily suffered by commercial logging and destroyed for cultivation. *C. ferruginea* and *C. hoabinhensis* occur in shrub forests, on bare limestone faces on steep bluffs. They were found in completely destroyed forests on steep or ridge of limestone hills at Lang Chua, Cay Khe, Lang Hang, Cai Kinh, Quang Son or Dong Tam and Ba Sao or disturbed forests at Deo Tra and Lan Ti. *C. balansae* was found in shade in pine plantations at Tan Dan and valleys, on sandy loamy soils over schist or granites at Ba Sao, Phu Thanh and Dong Tam. Due to the distribution at low and medium elevation, its habitats had been greatly fragmented by human activities. At present, over-exploitation of fuel wood is a major cause for degraded habitats and creates growth of a light-demanding species. *C. chevalieri* was found in shade in illegal logged lowland forests at Khe Nam and regenerating secondary forests along Rao An river at Son Kim. Three species, *C. dolichophylla*, *C. balansae* and *C. chevalieri* prefer high humidity, with low temperature in winter and high temperature in summer. *C. simplicipinna* occurs on sandy loams over schists in boulder beds along river bank of Rao Quan, in greatly disturbed forests. This was also found in

shrubs along river bank of Dackrong and arable land at Tan Thanh. The geographical distribution of the cycads is greatly influenced by human activities. Their distribution is highly fragmented. All studied populations are known to be very small for their survival in nature. The population size varied from 3 individuals at Ban Chu, the buffer zone of the Na Hang Nature Reserve to 37 at Cuc Phuong, an average of 25 individuals per *C. dolichophylla* population. Similarly for *C. ferruginea* populations varied from 28 at Lan Ti to 75 at Cai Kinh, averaged 47 individuals. Population size averaged 31.5 for *C. hoabinhensis*, 58.8 (25 at Ba Sao-87 at Yen Quang) for *C. balansae*, 28.7 (23 at Tan Thanh-41 at Tan Hop) for *C. simplicipinna* and 26.7 (23 at Son Kim-33 at Khe Nam) for *C. chevalieri*. Cycads in each population grow scatterly or the form of small clumps on determined faces of the hills. Most cycads of *C. ferruginea* and *C. hoabinhensis* grow on flanks of limestone hills in East-south. For this reason, these flanks receive light abundant favorable environment, while cycads in remaining species grown in shade of overstorey and understorey.

Demographic data from all studied populations showed the number of propagules produced by females in a population varied. This depended on environmental conditions and their habitats. Low proportion of propagules was determined at Lang Hang (34.21%) for *C. ferruginea*, Ba Sao (35%) for *C. balansae*, Tan Hop (27.5%) for *C. simplicipinna* and Khe Nam (21.9%) for *C. chevalieri*. Higher values were found for remaining populations. The highest proportions were recorded at Tan Thanh (95.8%), Dong Tam (80%) and Cuc Phuong (70.27%) for *C. simplicipinna*, *C. hoabinhensis* and *C. dolichophylla*, respectively. These differences may be relative to age structure within populations. It was difficult to discriminate male and female for young individuals, even mature ones. A number of males and females observed for each population were very small. Three females of *C. balansae* produced seeds were recorded at Yen Quang and Dong Tam. However, sex ratio (female/male) was also determined at some populations of *C. simplicipinna*. This value was 12.5% at Tan Hop. Due to human impact on their habitats and over-exploitation, these influenced greatly population structure. Only one male was observed at Tan Thanh, Dackrong and Son Kim. Asexual propagules produced from cut parent individuals were high, an average of 6.7 per parent at Tan Hop. Three propagules were produced from one parent individuals at Dackrong. Asexual propagules were observed at Cuc Phuong, Na Hang and Nuoc Sot. Clearly, this will affect greatly on mating system within populations.

Six enzyme systems provided 10 interpretable loci for 88 individuals from 4 populations of *Cycas dolichophylla*, 80 from 3 populations of *C. chevalieri* and 29 from 2 populations of *C. hoabinhensis*, 12 loci for 76 from 7 populations of *C. ferruginea*, 11 loci for 152 from 6 populations of *C. balansae* and 9 loci for 86 individuals from 3 populations of *C. simplicipinna*. A number of alleles was 36 for *C. dolichophylla* and *C. chevalieri*; 43, 38, 32 and 31 for *C. balansae*, *C. ferruginea*, *C. simplicipinna* and *C. hoabinhensis*. A mean number of alleles per locus were recorded at population and species levels. There were differences on the number of alleles between populations. The highest value was found at BCP population (4.45) and lowest was found at FGK population (2.4). Three alleles were found for each locus at species levels for *C. dolichophylla*, *C. balansae*, *C.*

simplicipinna and *C. chevalieri*. Lower values were found for two remaining species, *C. ferruginea* and *C. hoabinhensis*. High proportions of polymorphic loci were determined at population and species levels. This proportion at 95% criterion varied from 95% for *C. ferruginea* to 100% for 3 species *C. dolichophylla*, *C. hoabinhensis* and *C. chevalieri*. Observed heterozygosity averaged 0.326, ranging from 0.273 at DBE to 0.433 at DBC for *C. dolichophylla*; 0.321 ranging from 0.284 at FGK to 0.383 at FLT for *C. ferruginea*. Similarly, these values were 0.318 (2.7 at HBS - 3.1 at HDT), 0.347 (0.283 at BDT - 0.406 at BBS), 0.393 (0.349 at STH - 0.457 at SDR) and 0.403 (0.368 at CNS - 0.426 at CKN) for *C. hoabinhensis*, *C. balansae*, *C. simplicipinna* and *C. chevalieri*, respectively.

The monomorphic loci were found only in 2 populations of FBM and FQS for *C. ferruginea*. There were no monomorphic loci to be found in all remaining studied populations for all six species. Three loci, namely, Got-1 in BDT and BBS, Got-2 in BBS and β -est-2 in BPT and BBS showed low levels of polymorphism, with a frequency of the most common alleles of over 0.9 and lowest heterozygosities under HW equilibrium for these loci, an average of 0.152, ranging from 0.08 at β -est-2 in BPT to 0.199 at Got-1 and 2 in the same population BBS. Higher levels of genetic variation were found for a population at Got-2 in the frequencies ranging from 0.83 in BYT to 0.63 in BCP with the heterozygosities ranging from 0.333 to 0.476, respectively. Similarly, only one locus Got-2 in STT and one Idh-2 in FQS showed low level of polymorphism, with a frequency of the most common allele of over 0.9 and lowest heterozygosity of 0.093 and 0.089 under HW equilibrium, respectively. Higher variation was found for all remaining loci in all studied populations.

Data on the distribution of genotypes showed high levels of homozygotes for most common alleles in all studied populations. These values ranged from 11.1% at Lap-1 and 2 to 74.2% at Got-2, average of 32.6% for *C. chevalieri*; from 8.3% at β -est-1 to 85.1% at Got-2, an average of 35.9% for *C. simplicipinna*, from 6.1% at β -est-1 to 75.6% at β -est-2, average of 36.4% for *C. balansae*, from 20.3% at Lap-1 to 75.7% at Idh-2, average of 41.7% for *C. ferruginea*, from 5.7% at Skdh-1 to 65.4% at Got-2, an average of 41.8% for *C. dolichophylla*, from 13.7% at Lap-1 to 82.7% at Got-1, average of 45.7% for *C. hoabinhensis*. At population level, these values ranged from 35.2% at BCP to 42% at BYT and BDT (average of 39.3%) for *C. balansae*; from 33.5% at CKN to 38.7% at CSK, average of 35.7% for *C. chevalieri*; from 32.4% at SDR to 39.4% at STH, average of 35.8% for *C. simplicipinna*, from 33.3% at DBC to 50.6% at DBE, average of 43.5% for *C. dolichophylla*, from 38% at FDT to 52% at FBM, average of 46.2% for *C. ferruginea*, and from 46.1% at HDT to 50.3% at HBS, average of 48.2% for *C. hoabinhensis*.

The mean fixation values were also found at each locus and indicated a great deficit of heterozygotes compared to HW equilibrium. The coefficient of excesses of homozygotes or heterozygotes compared with panmictic expectations within populations for all populations of 0.343 (0.218 at SDR to 0.404 at STH), 0.306 (0.281 at CKN to 0.401 at CNS), 0.377 (0.357 at HBS to 0.397 at HDT), 0.386 (0.244 at BBS to 0.470 at BYT), 0.453 (0.388 at DBC to 0.49 at DBE) and 0.396 (0.253 at FLT to 0.49 at FCK) for *C. simplicipinna*,

C. chevalieri, *C. hoabinhensis*, *C. balansae*, *C. dolichophylla* and *C. ferruginea*, respectively showed a pronounced effect of inbreeding among individuals within populations. Eight loci, seven loci, six loci, five loci and four loci for *C. dolichophylla*, *C. ferruginea*, *C. hoabinhensis*, *C. simplicipinna* and *C. chevalieri*, respectively had high positive values (> 0.3) and were suggested a significant decrease in heterozygosities among individuals for each species. Eight loci for *C. balansae* also had high positive values. The coefficient within the entire populations indices showed individuals decreased 35.9% and 30.8% in heterozygosity under HW equilibrium for *C. simplicipinna* and *C. chevalieri*, respectively. Similarly, these indices were 52.2%, 44.5%, 40.9% and 43% for *C. dolichophylla*, *C. ferruginea*, *C. hoabinhensis* and *C. balansae*, respectively. However, there were differences in a number of loci having high positive values within populations. For example, three loci were found within SDR. Five and six loci were found for STT and STH, respectively. Similarly, our data found four loci within CSK and five within CNS and CKN. The mean values of the coefficient of population differentiation (0.024 and 0.003 for *C. simplicipinna* and *C. chevalieri*, respectively) were very low compared with *C. balansae*, *C. dolichophylla* and *C. ferruginea*.

The mean genetic diversity calculating from differences among individuals within populations of *C. simplicipinna* (Hs) was found at 0.589, ranging from 0.226 for Got-2 to 0.698 for β -est-1. Similarly, mean total genetic diversity (Ht) was 0.697, ranging from 0.232 for Got-2 to 0.719 for β -est-1. The value of both Hs and Ht was equal (0.604) for *C. chevalieri*. Hs averaged 0.587, ranging from 0.484 at Got-1 to 0.712 at Idh-1, 0.536 ranging from 0.263 at 6pgdh to 0.729 at β -est-1, 0.579 ranging from 0.371 at β -est-2 to 0.711 at Skdh-1, and 0.513 ranging from 0.332 at Got-1 to 0.682 at Lap-1 for *C. dolichophylla*, *C. ferruginea*, *C. balansae* and *C. hoabinhensis*, respectively. Ht averaged 0.621 (from 0.490 at Got-1 to 0.732 at Idh-1 and β -est-1), 0.569 (from 0.336 at Idh-2 to 0.750 at β -est-1), 0.522 (from 0.324 at Got-1 to 0.684 at 6pgdh) and 0.619 (from 0.394 at β -est-2 to 0.743 for β -est-3) for *C. dolichophylla*, *C. ferruginea*, *C. hoabinhensis* and *C. balansae*, respectively. Mean values obtained for genetic diversity among populations (Dst) and genetic differentiation (Gst) were found for each species. Dst values were 0.034, 0.032, 0.01, 0.039 and 0.009 for *C. dolichophylla*, *C. ferruginea*, *C. hoabinhensis*, *C. balansae* and *C. simplicipinna*, respectively. Values of Gst were 0.055, 0.057, 0.018, 0.064 and 0.015 for *C. dolichophylla*, *C. ferruginea*, *C. hoabinhensis*, *C. balansae* and *C. simplicipinna*, respectively. These values were zero for *C. chevalieri*.

4. DISCUSSION

The amount of genetic variation within populations and species, determined by enzyme loci is high in six studied species, *Cycas dolichophylla*, *C. ferruginea*, *C. balansae*, *C. hoabinhensis*, *C. chevalieri* and *C. simplicipinna*. Based on priori predictions, they are related to long-lived perennial habit and outcrossing breeding behavior. Cycads are dioecious and their reproduction is by seeds on open carpophylls. Males have pollen cones and females have fertile zone with long megasporophylls. They are insect-pollinated or wind-pollinated. Many individuals can live more 50 years old (our data). Cycads

occur in the complex habitat structure. Moreover, due to their distribution in lowlands, cycads have usually been influenced by environmental conditions and human interventions for many decades. When comparing groups with the same life history trait, mode of reproduction and breeding system (Hamrick *et al.*, 1979; Hamrick and Godt, 1989), the cycads maintained higher levels of genetic diversity. The values of genetic diversity in six studied cycads also were higher than found in coniferous species with similar outcrossed mating systems as *Picea abies* (Lundkvist, 1979), *Pinus longoeva* (Hiebert and Hamrick, 1983), *P. ponderosa*, *Pseudotsuga menziesii* (Linhart *et al.*, 1981) and for other plants as *Lupinus texensis* (Babbell and Selander, 1974), *Eucalyptus obliqua* (Brown *et al.*, 1975). These may reflect the long evolutionary origin of cycads.

There were high differences between observed heterozygosities and expected heterozygosities in relation to high inbreeding degree (> 0.2) and a result of the population structure caused by related individuals growing in clumps within the range of outcrossing perennials. Two cycads, *C. simplicipinna* and *C. chevalieri* were found in shrubs along Dakrong and Rao An rivers, near the villages, in arable land at Tan Thanh, in shade in secondary forests at Tan Hop and Khe Nam. *C. balansae* was found in shade, in secondary forests in Phu Thanh, Dong Tam, Ba Sao and Yen Quang on limestone hills with little soil; and on sandy loamy soil over graites in forest plantations of pines in Tan Dan or cultivated in gardens in Yen Tu. Two cycads *C. ferruginea* and *C. hoabinhensis* were found in shrubs or secondary forests, on limestone hills in Lan Ti, Deo Tra and Ba Min, Cai Kinh, Goc Khe, Lang Hang, Dong Tam and Ba Sao. Due to distributing on lowland (below 500 m elevation), cycads were greatly affected by human activities. Consequently, their habitats were suffered from destruction, e.g. by commercial logging, firewood collection and clearance for agriculture in 1970s. Soil fertility was heavily decreased to create favorable conditions for development of shrubs and grass. Clearly, the original vegetation has been greatly modified and fragmented. In addition, cycads have been collected for purposes as ornamental plants. The sizes of all the studied populations were very small (< 100 individuals). Therefore, the effects of sib-mating may happen at high intensity within populations. Moreover, a number of asexual individuals produced through fragmentation of cut stems were high. During our surveys, 26 males observed were generated from one father, or 6 females found from one mother. Due to a seed with high weight and large size (our data restricted and limited dispersed seeds). No squirrel was observed. These could lead to the integrated mosaic of genotypes among individuals within clumps. High proportion of homozygous loci per individual was found within populations and averaged 60.5% for each population.

Observed data indicated that individuals within STT population were reconstructed from survival seeds by 2-3 years ago after secondary vegetation (shrubs and grass) were cleared and burned for planting banana. Proportion of propagules was very high (95.8%). Although, the secondary vegetation at SDR site did not burned every year, it exists near the village and along Rao Quan River. Soils usually are relatively infertile and fertile soils rapidly lose its nutrients. Cycads are often influenced by human disturbance and domestic animals (Pigs, dogs and buffalos) growth rate of them are very slow and often subjected

exploitation by local people when they are 4-5 years old. Therefore, its population size is very small (about 20 individuals) and proportion of propagules within the population was high (52.6%). Only one cone was observed during a period of March-April. For levels of disturbed habitats of *C. chevalieri* differed in comparison with mentioned vegetation of *C. simplicipinna*. Secondary vegetation at CSK, near Rao An river was heavily suffered by flood in September. Many individuals were died. Cycads at CKN are recruited after their leaves were cut together with shrubs and lianas in 2000. No cone was observed at three sites CSK, CNS and CKN. These disturbances may contribute to genetic diversity within these populations. Cone not occurring the same time could increase levels of inbreeding within populations. Cultivated population from Yen Tu showed considerable differences in alleles and genotype frequencies. The data seem to indicate that individuals can originate vegetatively from other locations in comparison with individuals within remaining populations BDT, BTD, BBS, BPT and BCP. They did not originate directly from each other. Habitats in which populations including from Phu Thanh, Dong Tam, Ba Sao, Yen Quang, Cai Kinh, Lang Hang, and Goc Khe were sampled are similar, dominant shrubs on limestone hills with similar covering and consequently a similar amount of high light intensity, together with a high temperature are considered as factor unfavorable to growth and seed production. Cycads from Tan Dan were established after ecological conditions for more suitable of their survival were reconstructed. Pines were planted in 1970s after disturbance in the form of clearance for agriculture. The canopy of this type consists of only pine species *Pinus merkusii*, up to 35 m in height. However, dominant shrubs in families Myrsinaceae, Araceae and Rubiaceae along streams were found in the ground layer and understorey. Proportion of propagules was very high (83.3%) in the population. Hypothetically explanation may be proposed that the small populations have originated from a few founder plants, subsequently an increased opportunity for inbreeding. Over-exploitation of mature cycads was altered age of structure and influenced productivity activity within populations, although some ecological conditions may be maintained in some population in protected areas.

5. CONCLUSION AND RECOMMENDATION

All studied cycad species maintained high levels of genetic diversity and low levels of genetic population differentiation in relation to nature history characteristics in studied regions. Several factors contribute to the high levels of genetic diversity including dioecy, long-lived time and existence in complex habitat structure. However, our results also human disturbances increased matings between close relatives in small populations.

In order to cycad conservation, requirements of both genetic and ecological factors would be considered. The following priorities for threatened cycads to ensure long-term sustainability might be suggested (1) Cycad habitat conservation is a first priority. As a result showed that genetic diversity of six studied cycads was mostly partitioned among populations. This is related to their habitat fragmentation. (2) Ex-situ conservation should be implemented soon. These activities will be the source material to re-establish

as necessary in order to maintain cycads for long term sustainability. It will prevent potential genetic erosion of wild cycad populations. And (3) Future research might continue to ensure that the conservation value of cycads is maintained for long-term sustainability. A program should be established to monitor ecological changes, identify threats to conservation, and assess the effectiveness of cycad management.

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