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锥栗种群在鼎湖山三个群落中的遗传分化研究

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摘要：采用扩增片段长度多态性（AFLP，Amplified fragment length polymorphism）方法对锥栗（*Castanopsis chinensis*）在 3 个不同群落亚种群的遗传分化进行了研究。研究地点位于广东省鼎湖山，3 个群落分别是：针叶林群落，针阔叶混交林群落，常绿阔叶林群落。采集的叶样进行 DNA 提取、酶切、连接、PCR 扩增后进行变性聚丙烯酰胺的凝胶电泳，银染后把所得带进行 1/0 标记。结果表明，四组引物对分别扩增出 27，20，33，39 条带，其中分别有 15，15，18，26 条是多态性带，说明其多样性较高。用 AMOVA（Analysis of molecular variance）分析表明 75.36 %的遗传变异表现在种群内，24.64 %的遗传变异表现在种群间，极显著（$P<0.001$）。使用 Nei 遗传距离进行了算术平均数的非加权成组配对法分析（UPGMA），其聚类结果表明常绿阔叶林群落亚种群和针阔叶混交林群落亚种群的种群聚合在一起。上述结果说明锥栗不同亚种群有不同的遗传多样性，种群间遗传分化较大。这是种群本身遗传特性的反映，并与群落演替密切相关，即不同群落微环境对种群遗传的影响。

关键词：锥栗（*Castanopsis chinensis*）；AFLP；遗传多样性；遗传分化；亚种群

Genetic differentiation of *Castanopsis chinensis* population in three communities in Dinghu Mountain, China

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Abstract: The extent of genetic differentiation among populations has become a topic of increasing interest in the related spheres of ecology. However, relatively few studies were reported concerning population genetic differentiation following with community succession.

Dinghu Mountain (112°30′39″—112°33′41″E, 23°09′21″～23°11′30″N) is situated in Guangdong Province China, and belonging to the lower subtropics. According to community physiognomy, species composition and successional stage, three typical vegetation types are found: pioneer coniferous community, midsuccessional mixed coniferous evergreen broadleaved community and climax evergreen broadleaved forest. They coexist in Dinghu Mountain and make it ideal place for inversigating population genetic differentiation related to community succession.

Then, this paper focused on species *Castanopsis chinensis* to study its genetic differentiation. For it no genetic study was reported before.

C. chinensis is one of the heliphyte species in lower subtropics, and dominates at the altitude between 50～800m in Dinghu Mountain, China. As mid-successional species, it can be found in coniferous community, mixed coniferous evergreen broadleaved community and evergreen broadleaved community.

In May 1999, samples were collected from three communities above. The sample number for coniferous community was 10, for mixed coniferous evergreen broadleaved community 18, and for evergreen broadleaved community 20. Here, the individuals belonging to the same community composed a subpopula-

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tion.

The distance between coniferous community and mixed coniferous evergreen broadleaved community was 600m,coniferous community and evergreen broadleaved community 800m,and mixed coniferous evergreen broadleaved and evergreen broadleaved community 350m.

We confined our samples in their own communities,to reduce probability of confounding variable. A minimum distance separated the trees,>50m,from parent trees to avoid progeny of one-parent trees.

Recently,a proliferation of genome marker techniques is advanced,for example,RFLP(restriction fragment length polymorphism),RAPD(random amplified polymorphic DNA),DAF(DNA amplification fingerprinting),AP-PCR(arbitrarily primed PCR). The drawback for all of these techniques is sensitive to the reaction condition and DNA quality. As a simple,cheap,efficient and informative tool,AFLP(amplified fragments length polymorphism)is used in our study. The abundance of genetic markers of AFLP allows for a more accurate genetic study in *C. chinensis* population.

The PCR reaction products were analyzed in 5% denaturing polyacrylamide gels. After electrophoresis,silver staining was performed.

Then,AFLP markers were scored for presence(1)or absence(0). Only polymorphic markers that could score unequivocally in all genotypes were induced in the anlysis below.

The pairwise distances between genotypes were estimated using Euclidean distances,then were used in AMOVA(analysis of molecular variance,1.55)package to estimate the genetic differentiation among subpopulations. Pairwise similarities of 1/0 data from all markers were also calculated between subpopulations using Nei's genetic distance. The resultant similarity matrix was put into UPGMA(unweighted pair group with arithmetic average)cluster analysis with PHYLIP 3.5C.

The results showed that four AFLP primer combinations yielded 27,20,33 and 39 AFLP bands respectively,in which 15,15,18,26 bands were polymorphic.

AMOVA analysis revealed 75.36% of the variation occurred within subpopulations,24.64% of variation occurred among subpopulations with great significant p-value(<0.05). Phist($\phi_{\rm st}$)was 0.246 with significance whith indicated 24.6% genetic differentiation among subpopulations.

Nei's genetic distance was calculated and used in cluster analysis,and the results showed subpopulation of coniferous community was separated from the other two subpopultions.

With these genetic analysis,this paper obtained meaningful results in *C. chinensis* related to community succession.

Previous researches suggested that for $F_{\rm st}$,the range 0～0.05 indicated little differentiation,0.05～0.15 moderate,0.15～0.25 large differentiation and above 0.25 very large genetic differentiation. Φ_{st} in our study,analogue to F_{st},was 0.246,between 0.15～0.25,showing large differentiation.

We were not surprised about this result. As species of family Fagaceae,the seeds of *C. chinensis* were gravity-dispersed and confined themselves near their parents,which restricted gene flow and resulted in high genetic differentiation among subpopulations.

Moreover,in our study,three communities represented different microenvironment. Tree compositions in these communities could alter the local environment with respect to the natural of throughfall,soil moisture,soil nutrient availability and a myriad of other factors,which had been described in plenty of previous studies. We thought these microenvironment differences also influence the species distribution and resulted in high genetic differentiation.

Then,in pioneer successional community,new immigrants of *C. chinensis* possessed an edge occasion,surviving not well. However,there might be ample opportunity for its growth in mid-successional and climax communities because of suitable community environment. Consequently,the subpopulation of *C. chinensis* in pioneer successional community obviously diverged from the other two subpopulations.

To our knowledge, our analysis first provides a view of genetic differentiation in subtropical forest as influenced by different community composition and dynamics. These results provide a rational basis for decision concerning the conservation, management and restoration.

Key words: *Castanopsis chinensis*; AFLP; genetic diversity; genetic differentiation; subpopulation
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种群是生活在一定时间与一定空间上的所有同种个体的总和[1],这些个体由于自然或者人为的原因会发生种群隔离，使种群间产生遗传分化;或者种群间基因流虽没有割断，但是不同环境的自然选择作用也可造成种群间的遗传分化。因此种群间遗传分化引起了生态工作者的广泛注意[2,3]。引起种群遗传分化的原因有两种:一是内因,即种群本身的遗传特性所引起的[2],如花粉、种子的传播方式;另一种是外因,即由于环境变化、人为干扰所引起的隔离、遗传漂变等[4]。后者包括大的环境的变化和由于微环境的不同造成的种群遗传分化。本文拟就微环境不同所引起的种群间遗传分化进行初步研究。地点选择地处我国南亚热带上的鼎湖山。

我国的南亚热带地区具有优越的气候条件,水热资源充足,发育着世界上面积最大、最典型的南亚热带常绿阔叶林。鼎湖山是其中的典型地区,植物资源丰富,保存着比较完整的天然植物,是研究南亚热带森林种群和群落的理想地方。

锥栗是南亚热带常绿阔叶林的演替先锋种,阳生性植物,在鼎湖山分布很广,是优势树种。对它已有较多研究[5~8],但多集中在群落与种群的宏观探讨上,本文拟对它们在不同群落中的遗传分化进行探讨,以进一步了解种群特性及其环境对它们生长的影响。

1　材料与方法

1.1　采样地区自然地理概况和样品采集　鼎湖山位于东经 112°30′39″～112°33′41″,北纬 23°09′21″～23°11′30″,年降雨量 1956 mm ,年均温 20.9℃，最冷月和最热月的平均温度分别为 12 ℃ 和 28 ℃。土壤由泥盆纪厚层变质砂岩、砂页岩发育形成,主要土壤类型有赤红壤、红壤等[8]。叶样(自然更新种)采集于 1999 年 5 月,3 个采样点分别代表 3 个不同群落(表 1)。3 个群落的组成是,常绿阔叶林群落:厚壳桂(*Cryptocarya chinensis*)+黄果厚壳桂(*Cryptocarya concinna*)+锥栗(*Castanopsis chinensis*)+荷木(*Schima superba*);针阔叶混交林群落:马尾松(*Pinus massoniana*)+荷木;针叶林群落:马尾松-芒萁(*Dicranopteris dichotoma*)。常绿阔叶林采样点与针阔叶混交林采样点相距 600m，针阔叶混交林采样点与针叶林采样点相距 350m，常绿阔叶林采样点与针叶林采样点相距 800m。

1.2　样品处理　新鲜树叶 1 克采用 CTAB 法提取 DNA[9],用电泳法测其含量。AFLP 方法参照 Vos 等[10]。

使用 *Eco*R Ⅰ和 *Mse* Ⅰ(New England Co.)对 DNA 进行双酶切(37℃,2h),之后 70℃灭活;加入 *Eco*R 和 *Mse* 接头,T_4DNA 连接酶(New England Co.),16℃反应 16h;PCR 扩增反应的 2 个 *Eco*R 引物和 2 个 *Mse* 引物分别是:

*Eco*R1: 5′-GACTGCGTACCAATTC-*AGG*-3′
*Eco*R2: 5′-GACTGCGTACCAATTC-*ACT*-3′
*Mse*1: 5′-GATGAGTCCTGAGTAA-*CAG*-3′
*Mse*2: 5′-GATGAGTCCTGAGTAA-*CTC*-3′

PCR 反应:94℃变性 5min，之后 94℃变性 30s，65℃退火 30s,72℃延伸 60s。每次降低退火温度 1℃,反应 10 个循环,固定于退火温度 56℃反应 24 个循环。72℃延伸 5min。PCR 产物用 5%变性聚丙稀酰胺进行电泳,电泳结束后使用银染显色[11]。

1.3　数据处理　把变性聚丙稀酰胺电泳产物所得的各带进行 1/0 标记。首先计算衡量种群遗传变异的平均非偏差杂合度(Unbiased mean herterozygosis)和其无偏差方差(Unbiased variance)[12]。这一指标不依赖于人为定义的多样性,从基因频率的角度来度量种群遗传变异。

种群间遗传分化分析采用 AMOVA (Analysis of molecular variance,AMOVA 1.55)[13]分析软件进行。

Nei 的遗传距离计算如下:

$$D = -\ln \frac{\sum_{l}\sum_{i} P_{1/li}P_{2/li}}{\sqrt{\sum_{l}\sum_{i} P_{1/li}^{2}}\sqrt{\sum_{l}\sum_{i} P_{2/li}^{2}}}$$

$P_{1/li}$和 $P_{2/li}$分别是第 1 个亚种群和第 2 个亚种群的第 i 条带在 l 位点上出现的频率[12]。使用算术平均数的非加权成组配对法(UPGMA)对这一结果进行聚类分析,使用的软件是 PHYLIP 3.5C[14]。

表 1 样品采集群落

Table 1 Communities of collecting samples

群落类型 Community type	位置 Location	海拔 Elevation (m)	面积 Area (m^2)	采样数目 No. of sample
常绿阔叶林群落①	111°32′50″E 23°11′05″N	300	3000	20
针阔叶混交林群落②	111°32′55″E 23°10′35″N	150	3000	18
针叶林群落③	111°32′56″E 23°09′63″N	150	3000	10

①Evergreen broadleaved community ②Mixed coniferous- evergreen broadleaved community ③Coniferous community

2 结果与分析

4 对引物分别扩增出 27,20,33,和 39 条 AFLP 带(表 2)。从表 2 可以看出 4 个引物对所扩增的多态性带十分丰富,最多的是 *Eco*R2-*Mse*2 引物对和 *Eco*R2-*Mse*1 引物对所得的多态性百分率,66.7%;最低是 *Eco*R1-*Mse*2 引物对,54.5%。

从表 2 看出这些引物对扩增所得的平均非偏差杂合度也较高(表 2),平均非偏差杂合度代表了种群遗传多样性程度,因此锥栗 3 个亚种群的遗传多样性较高,但各亚种群结果不同。从 4 个引物对扩增出的所有多态性带进行计算得出,锥栗常绿阔叶林亚种群平均非偏差杂合度为 0.311(±0.058),针阔叶混交林亚种群平均非偏差杂合度为 0.297(±0.062),针叶林亚种群平均非偏差杂合度为 0.197(±0.067)。可以看出常绿阔叶林亚种群平均非偏差杂合度和针阔叶混交林亚种群平均非偏差杂合度相差不大,而针叶林亚种群平均非偏差杂合度明显小于这两者。

表 2 4 对引物扩增结果

Table 2 Amplification results with four primer combinations

引物对 AFLP primer combination	所有带 Total bands	单态带 Monomorphic bands	多态性带 Polymorphic bands	多态性百分率 Polymorphic bands(%)	亚种群 Subpopulation	平均非偏差杂合度 Unbiased mean heterogeneity(±SE)
*Eco*R1-*Mse*1	27	12	15	55.6%	常绿阔叶林亚种群①	0.326(±0.055)
					针阔叶混交林亚种群②	0.304(±0.069)
					针叶林亚种群③	0.293(±0.078)
					平均④	0.308(±0.067)
*Eco*R2-*Mse*1	20	5	15	66.7%	常绿阔叶林亚种群	0.163(±0.064)
					针阔叶混交林亚种群	0.190(±0.067)
					针叶林亚种群	0.067(±0.049)
					平均	0.140(±0.060)
*Eco*R1-*Mse*2	33	15	18	54.5%	常绿阔叶林亚种群	0.331(±0.062)
					针阔叶混交林亚种群	0.220(±0.063)
					针叶林亚种群	0.205(±0.057)
					平均	0.252(±0.061)
*Eco*R2-*Mse*2	39	13	26	66.7%	常绿阔叶林亚种群	0.373(±0.055)
					针阔叶混交林亚种群	0.409(±0.055)
					针叶林亚种群	0.212(±0.074)
					平均	0.331(±0.061)

①Evergreen broadleaved subpopulation ②Mixed coniferous- evergreen broadleaved subpopulation ③Coniferous subpopulation ④Average

从表 3 可以看出锥栗种群有 24.64 %的遗传变异存在于亚种群间，显著度检测为极显著；另外有 75.36%的遗传变异存在于亚种群内。

表 3 对锥栗 48 个样品的 AMOVA 分析

Table 3 AMOVA analysis for 48 individuals of *Castanopsis chinensis*

变异来源 Source of variation	自由度 *d. f.*	方差 *SSD*	均方差 *MSE*	变异成分 Variance component	变异百分率 % total variance	Phist Φ_{ST}	显著度检测 *p*-value
亚种群间①	2	51.512	25.756	3.548	24.64%	0.246	<0.001
亚种群内②	45	488.344	10.852	10.852	75.36 %		—

①Among subpopulation ②Within subpopulation 显著度检测：对 AFLP 扩增产物带进行 1000 次随机重复 Significant test：1000 random permutations for AFLP amplification results

表 4 锥栗 3 个亚种群的 Nei 遗传距离

Table 4 Estimates of Nei's genetic distance of *Castanopsis chinensis* in three subpopulations

亚种群 Subpopulation	常绿阔叶林亚种群 Evergreen broad-leaved subpopulation	针阔叶混交林亚种群 Mixed coniferous-evergreen broadleaved subpopulation	针叶林亚种群 Coniferous subpopulation
常绿阔叶林亚种群	0		
针阔叶混交林亚种群	0.1366	0	
针叶林亚种群	0.1529	0.1978	0

Φ_{ST} 相当于分化指数，Wright，引自 Buso[15]，认为分化指数介于 0～0.05 之间说明种群间分化很弱，0.05～0.15 之间表示中等分化，0.15～0.25 之间表示分化大，大于 0.25 表明分化极大，本研究结果为 0.246，大致说明种群之间有大的遗传分化。

Nei 遗传距离见表 4，常绿阔叶林亚种群和针阔叶混交林亚种群之间的遗传距离最小为 0.1558。使用 UPMGA 聚类法得出的树状图如图 1，显示出常绿阔叶林亚种群和针阔叶混交林亚种群聚合在一起。

3 结论与讨论

Liao & Hsiao[16] 研究发现植物 *Acorus gramineus* 在不同地点有 46.84%的遗传变异，不同种群间有 36.28%的遗传变异，他们认为这是由于自然选择或遗传漂变引起的，但是他们并没有发现不同种群个体形态上的差别，也没有发现不同的生态型。但他们取样是在整个台湾省，各种环境变化较大，造成了种群较大的遗传分化。本实验在较小的范围内采样，所不同的只是群落组成的不同，但却发现了锥栗 3 个亚种群有大的遗传分化。

锥栗是演替先锋种，是壳斗科、阳生性植物。种子成熟后落在母树旁，因此在一定程度上限制了基因交流，使得种群间遗传分化大。另外锥栗在演替早期群落（针叶林）、演替中期群落（针阔叶混交林），和演替顶极群落（常绿阔叶林）中都有分布，但是在演替早期群落（针叶林）中数量较少，在此群落中只采集到 10 株个体的叶样，并其中只有一株是大树，说明锥栗并不能很好的适应演替早期群落（针叶林），因此，这一阶段的自然选择作用较大，使得锥栗针叶林亚种群表现出不同于

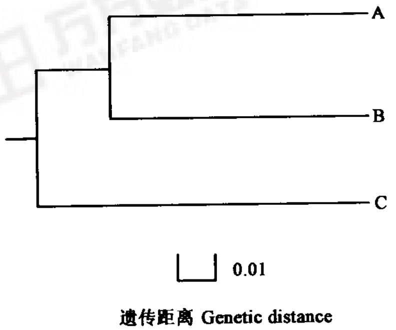

图 1 3 个亚种群 UPMGA 聚类法得出的树状图

Fig. 1 Dendrogram showing Nei's genetic distance between three subpopulations using UPGMA cluster analysis

A. 常绿阔叶林群落亚种群 Evergreen broadleaved community subpopulation

B. 针阔叶混交林群落亚种群 Mixed coniferous-evergreen broadleaved community subpopulation

C. 针叶林群落亚种群 Coniferous community subpopulation

另外两个亚种群的遗传特性,如针叶林亚种群平均非偏差杂合度的计算结果为 0.197,而另外两个亚种群分别为 0.311 和 0.297。从 Nei 遗传距离的 UPGMA 聚类结果也看出常绿阔叶林亚种群和针阔叶混交林亚种群聚合在一起的,同样表明虽然 3 个亚种群出现了极显著的遗传分化,并且两两亚种群间也表现出较大的遗传距离,但针叶林亚种群更不同与常绿阔叶林亚种群和针阔叶混交林亚种群。

以前有很多关于群落微环境的研究,表明不同的群落有着不同的土壤 pH 值、土壤微生物活性[18]、土壤水分、水势[19],土壤温度[20]、土壤物理结构[21]、光照、空气温度、空气湿度,蒸腾作用[22]。进一步是王伯荪等[23]进一步对群落小气候的研究,发现种群在不同群落中有不同的外表、生理生态分化。对照本文结果,进一步从分子角度证实了亚种群间遗传分化的存在,这种分化是与锥栗生物学特性与演替系列群落,即群落微环境有关的。

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