

# 缙云山川鄂连蕊茶在不同群落类型中的 RAPD 分析

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**摘要:**采用随机扩增多态性 DNA (RAPD) 对常绿阔叶林灌木层广布种川鄂连蕊茶 (*Camellia rosthorniana*) 在缙云山 3 个群落类型 (毛竹林、针阔混交林和常绿阔叶林) 中的遗传结构和 DNA 多样性进行了研究。10 个引物共扩增出 138 个产物, 其中 115 个是多态性的, 多态位点比率为 83.33%。Shannon 指数估算的 3 个种群的遗传多样性为常绿阔叶林 0.3345, 毛竹林 0.3148, 针阔混交林 0.3085, 遗传分化为 8.12%; Nei 指数计算的 3 个种群的基因多样性为常绿阔叶林 0.2095, 毛竹林 0.1981, 针阔混交林 0.1934, 遗传分化为 7.51%。种群间遗传距离在 0.0177~0.0393 之间。川鄂连蕊茶在 3 个群落中的种群大小和生长状况不同, 各种群遗传多样性的高低可能是对所处群落生境的适应。

**关键词:** RAPD; 川鄂连蕊茶; 群落类型; DNA 多样性; 遗传分化

## RAPD analysis of *Camellia rosthorniana* populations in different communities in Jinyun Mountain

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**Abstract:** With special geographic location and climatic conditions, the subtropical areas in China has developed the largest and typical evergreen broadleaved forest in the world, evergreen shrubs is one important constituent of the forest. Unfortunately, little information is available with regard to the genetic variation of evergreen shrubs in evergreen broadleaved forest.

*Camellia rosthorniana* (Theaceae) is a long-lived evergreen shrub species, which distributes widely in subtropical areas of China and occurs in many types of communities in Jinyun mountain (29°50'N, 106°26'E). In the present study, RAPD (Random amplified polymorphic DNA) was used to detect the genetic structure and DNA diversity of three spatially isolated populations of *Camellia rosthorniana* in different communities, *i. e.*, giant bamboo forest, mixed coniferous broadleaved forest and evergreen broadleaved forest in Mt Jinyun. The distances between giant bamboo forest and evergreen broadleaved forest, between giant bamboo forest and mixed coniferous broadleaved forest, between mixed coniferous

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broadleaved forest and evergreen broadleaved forest were 100m, 1000m, and 1100m, respectively.

18 samples were collected from each population. To reduce the chance of consanguinity, trees sampled from the same population were selected at least 10m apart .

A total of 40 random primers were initially screened using 2 representatives from each of the three populations. 10 primers that gave distinct, repeatable fragment patterns were selected for final analysis. All 54 *Camellia rosthorniana* individuals sampled were genetically different from each other. The number of loci and that of polymorphic loci were 130,126,133 and 105,103,109 for giant bamboo forest、mixed coniferous broadleaved forest and evergreen broadleaved forest, respectively. On average, the percentage of polymorphic loci was 83.3%, indicating high polymorphism in *Camellia rosthorniana* population.

Based on the band frequency in terms of population and species, estimation of Nei's gene diversity ( $H$ ) and Shannon's index of diversity ( $I$ ) were performed using Popgene 1.31. Evergreen broadleaved community had the highest genetic diversity ( $H = 0.2095$ ,  $I = 0.3345$ ), followed by giant bamboo community ( $H = 0.1981$ ,  $I = 0.3148$ ) and mixed coniferous broadleaved community ( $H = 0.1934$ ,  $I = 0.3085$ ). Genetic differentiation  $(H_{SP} - H_{POP})/H_{SP}$  estimated with Shannon's index and coefficient of gene differentiation ( $G_{ST}$ ) were 0.0812 and 0.0751, respectively. Nei's (1972) genetic distances between populations were also caculated, which ranged from 0.0177 to 0.0393 and did not correspond to geographic distances.

Field survey showed that the three populations differed in both population size (largest in evergreen broadleaved community and smallest in giant bamboo community) and individual growth (basal diameters of the largest individual of *Camellia rosthorniana* in giant bamboo community, mixed coniferous broadleaved community and evergreen broadleaved community were 3.4cm, 5.3cm, and 6.4cm, respectively), thus, genetic diversity of each population may be the result of adaptation to its own microenvironments.

**Key words:** RAPD; *Camellia rosthorniana*; community types; DNA diversity; genetic differentiation  
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近年来,伴随着分子标记技术的迅速发展,有关热带森林、亚热带森林、温带森林乔木层优势种群的分子生态学研究已陆续展开<sup>[1~5]</sup>。由于独特的地理位置和气候条件,我国中亚热带地区分布着全球面积最大、发育最为典型的常绿阔叶林,常绿灌木是其重要的组成之一,但迄今为止,有关其分子生态学研究还未见报道。

川鄂连蕊茶(*Camellia rosthorniana*)属山茶科山茶属,常绿灌木,是我国亚热带地区常绿阔叶林灌木层广布种之一<sup>[6]</sup>,在缙云山也有较广泛的分布。本文拟运用 RAPD 技术,对其遗传多样性和在不同群落中的遗传分化进行研究。

1 材料与方法

1.1 研究地区概况与样品采集

缙云山 (29°50'N,106°26'E) 位于重庆市北碚区,最高海拔 900m,平均海拔 850~895m 之间,相对高差 640 左右,面积约 1400hm<sup>2</sup>。土壤主要为三叠纪砂岩为母质发育的酸性黄壤,其次是中性或酸性紫色土。缙云山具有亚热带季风湿润气候,热量充沛,雨量丰富。其地带性植被为中亚热带湿润常绿阔叶林,并存在次生暖性针叶林、竹林、灌丛和灌草丛,具有森林植被次生演替的不同阶段<sup>[7]</sup>。据野外调查,发现川鄂连蕊茶在常绿阔叶林、针阔混交林和毛竹林内均有分布。

样品于 2000 年 5 月采自贺龙园、派出所和青龙寨 3 个相对孤立的川鄂连蕊茶种群,它们分别代表了各种种群所处的群落类型。各群落的基本情况见表 1。群落组成为常绿阔叶林:川灰木(*Symplocos sedhunensis*) + 四川大头茶(*Gordonia acuminata*) + 大果杜英(*Elaeocarpus decipiens*) + 光叶山矾(*S. lancifolia*);针阔混

交林:川灰木(*S. sedhunensis*) + 马尾松(*Pinus massoniana*) + 薯豆(*E. japonicus*) + 杉木(*Cunninghamia lanceolata*);毛竹林乔木层以毛竹占绝对优势。其中常绿阔叶林和毛竹林相距 100m,毛竹林与针阔混交林均相距 1000m,常绿阔叶林与针阔混交林相距 1100m。各群落均采样 18 株,样品为当年生嫩叶,为尽量避免同一母树后代,样品株间相距至少 10 m。

1.2 DNA 的提取和扩增

采集的样品,按照陈亮等<sup>[8]</sup>的方法提取 DNA。DNA 样品经分光光度计和琼脂糖凝胶电泳检测纯度和浓度后,稀释到  $10\text{ng} \cdot \mu\text{l}^{-1}$  用于 RAPD 分析。用随意选取的 2 个样品 DNA 模板筛选 Sangon 系列引物 40 个( $S_1 \sim S_{40}$ ),经 3 次筛选,确定扩增效果良好,可重复的引物 10 个,用于全部样品的扩增。扩增在 PTC-100™ Programmable Thermal Controller 扩增仪上进行,反应体积为  $25\mu\text{l}$ ,含 20ng 模板 DNA,  $0.2\mu\text{mol} \cdot \text{L}^{-1}$  引物,  $1 \times \text{Taq}$  聚合酶反应缓冲液,  $1.0\mu\text{Taq}$  DNA 聚合酶,  $2\text{mmol} \cdot \text{L}^{-1}\text{MgCl}_2$ , 各  $0.2\text{mmol} \cdot \text{L}^{-1}\text{dNTPs}$ 。扩增程序为  $94\text{ }^\circ\text{C}$  预变性 180s,  $94\text{ }^\circ\text{C}$  变性 60s,  $38\text{ }^\circ\text{C}$  退火 90 s,  $72\text{ }^\circ\text{C}$  延伸 120 s, 共 45 个循环,最后  $72\text{ }^\circ\text{C}$  延伸 300 s。PCR 产物用 1.5% 的琼脂糖凝胶在  $1 \times \text{TAE}$  缓冲液电泳,EB 染色后在 BIO-RAD System 观察并保存结果。

1.3 数据分析

1.3.1 多态位点比率 在某一特定定位点上,若扩增片段出现的频率小于 0.99,则此位点称为多态位点。多态位点比率为在所有检到的位点中多态位点所占比例。

1.3.2 遗传参数 运用 POPGENE1.31 软件<sup>[9]</sup> 计算 Nei 指数和 Shannon 指数,再分别求各种群的平均值;计算相互间的遗传距离和遗传分化系数  $G_{ST}$ 。

2 结果和分析

2.1 多态位点比率

川鄂连蕊茶 3 个种群的多态位点数、总位点数及多态位点比例见表 2。3 个种群的多态位点比率非常接近,毛竹林略低,总的多态位点比率为 83.33%。

表 1 川鄂连蕊茶 3 个种群的基本特征

| 种群<br>Population | 群落类型<br>Community type | 地点<br>Site          | 海拔<br>Elevation(m) |
|------------------|------------------------|---------------------|--------------------|
| P1               | 毛竹林 <sup>①</sup>       | 贺龙园<br>Helongyun    | 840                |
| P2               | 针阔混交林 <sup>②</sup>     | 派出所<br>Paichushuo   | 780                |
| P3               | 常绿阔叶林 <sup>③</sup>     | 青龙寨<br>Qinglongzhai | 850                |

① Giant bamboo forest ② Mixed coniferous-evergreen broad-leaved forest ③ Evergreen broad-leaved forest

表 2 川鄂连蕊茶种内及种群内多态位点比率

| Table 2 Percentage of polymorphic loci within species and populations of <i>Camellia rosthorniana</i> |                         |                      |                                    |                                          |
|-------------------------------------------------------------------------------------------------------|-------------------------|----------------------|------------------------------------|------------------------------------------|
| 种群<br>Population                                                                                      | 样品数<br>Numb. of Samples | 位点数<br>Numb. of loci | 多态位点数<br>Numb. of polymorphic loci | 多态位点比率<br>Percentage of polymorphic loci |
| P1                                                                                                    | 18                      | 130                  | 105                                | 0.8077                                   |
| P2                                                                                                    | 18                      | 126                  | 103                                | 0.8175                                   |
| P3                                                                                                    | 18                      | 133                  | 109                                | 0.8195                                   |
| 合计 Total                                                                                              | 54                      | 138                  | 115                                | 0.8333                                   |

表 3 由 Shannon 指数估计的川鄂连蕊茶种群的遗传多样性

Table 3 Genetic diversity of *Camellia rosthorniana* estimated by Shannon index

| 引物 Primer       | P1     | P2     | P3     | $H_{POP}$ | $H_{SP}$ | $H_{POP}/H_{SP}$ | $(H_{SP}-H_{POP})/H_{SP}$ |
|-----------------|--------|--------|--------|-----------|----------|------------------|---------------------------|
| S <sub>11</sub> | 0.2348 | 0.1655 | 0.2610 | 0.2204    | 0.2662   | 0.8279           | 0.1721                    |
| S <sub>17</sub> | 0.3301 | 0.2988 | 0.3659 | 0.3316    | 0.3473   | 0.9548           | 0.0452                    |
| S <sub>18</sub> | 0.3044 | 0.2540 | 0.3064 | 0.2883    | 0.3241   | 0.8895           | 0.1105                    |
| S <sub>21</sub> | 0.2668 | 0.3461 | 0.2293 | 0.2807    | 0.3266   | 0.8595           | 0.1405                    |
| S <sub>22</sub> | 0.2919 | 0.1583 | 0.3097 | 0.2533    | 0.2775   | 0.9128           | 0.0872                    |
| S <sub>27</sub> | 0.3181 | 0.2739 | 0.3924 | 0.3281    | 0.3474   | 0.9444           | 0.0556                    |
| S <sub>31</sub> | 0.3896 | 0.4870 | 0.4133 | 0.4300    | 0.4470   | 0.9620           | 0.0380                    |
| S <sub>33</sub> | 0.2941 | 0.3393 | 0.2874 | 0.3069    | 0.3236   | 0.9484           | 0.0516                    |
| S <sub>38</sub> | 0.4541 | 0.4158 | 0.4008 | 0.4236    | 0.4514   | 0.9384           | 0.0616                    |
| S <sub>39</sub> | 0.1410 | 0.1548 | 0.2665 | 0.1874    | 0.2155   | 0.8696           | 0.1304                    |
| 平均 Mean         | 0.3148 | 0.3085 | 0.3345 | 0.3193    | 0.3475   | 0.9188           | 0.0812                    |

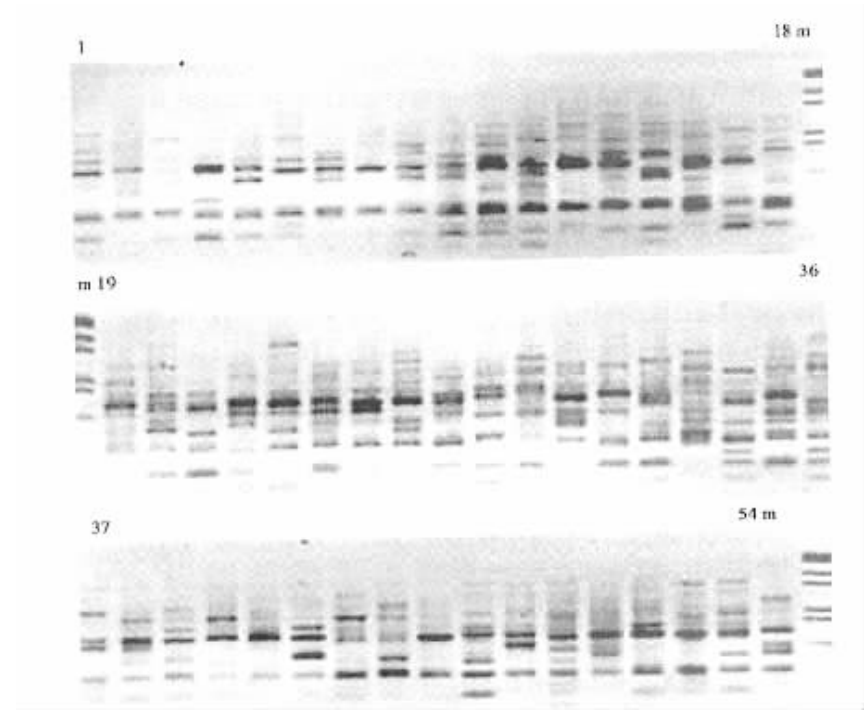


图 1 引物 S<sub>31</sub> 在全部 54 个分析样品中所产生的 RAPD 带型

Fig. 1 The RAPD profiles of the 54 samples analyzed in the experiment produced by primer S<sub>31</sub>

各种群的样品分别是 P1:1~18,P2:19~36,P3:37~54 Lanes 1~18 belong to P1, Lanes 19~36 belong to P2, Lanes 37~54 belong to P3

2.2 Shannon 信息指数

Shannon 指数计算的川鄂连蕊茶 3 个种群种群内、种群间的遗传多样性,以及各自在总变异中所占的比例,结果见表 3。由表 3 可见,10 个引物检测的川鄂连蕊茶各种群平均多样性大小顺序为:常绿阔叶林>毛竹林>针阔混交林。常绿阔叶林种群内变异明显高于其他两个种群。3 个种群平均遗传多样性为 0.3193,总遗传多样性为 0.3475,在总遗传变异中,有 91.88%存在于种群内,8.12%存在于种群间,即 Shannon 信息指数估计的川鄂连蕊茶 3 个种群间的遗传分化为 8.12%。

表 4 由 Nei 指数估计的川鄂连蕊茶种群的基因多样性

| Table 4 Gene diversity of <i>Camellia rosthorniana</i> estimated by Nei's index |        |        |        |                |                |                 |                 |
|---------------------------------------------------------------------------------|--------|--------|--------|----------------|----------------|-----------------|-----------------|
| 引物 Primer                                                                       | P1     | P2     | P3     | H <sub>S</sub> | H <sub>T</sub> | D <sub>ST</sub> | G <sub>ST</sub> |
| S <sub>11</sub>                                                                 | 0.1572 | 0.1106 | 0.1730 | 0.1469         | 0.1808         | 0.0339          | 0.1873          |
| S <sub>17</sub>                                                                 | 0.1922 | 0.1940 | 0.2160 | 0.2007         | 0.2061         | 0.0054          | 0.0260          |
| S <sub>18</sub>                                                                 | 0.2005 | 0.1661 | 0.1952 | 0.1873         | 0.2090         | 0.0217          | 0.1040          |
| S <sub>21</sub>                                                                 | 0.1712 | 0.2408 | 0.1511 | 0.1877         | 0.2140         | 0.0263          | 0.1229          |
| S <sub>22</sub>                                                                 | 0.1867 | 0.0909 | 0.1896 | 0.1557         | 0.1676         | 0.0119          | 0.0708          |
| S <sub>27</sub>                                                                 | 0.1868 | 0.1503 | 0.2388 | 0.1920         | 0.1995         | 0.0075          | 0.0378          |
| S <sub>31</sub>                                                                 | 0.2529 | 0.3280 | 0.2675 | 0.2828         | 0.2921         | 0.0093          | 0.0318          |
| S <sub>33</sub>                                                                 | 0.1783 | 0.2110 | 0.1760 | 0.1884         | 0.1973         | 0.0089          | 0.0449          |
| S <sub>38</sub>                                                                 | 0.3038 | 0.2780 | 0.2549 | 0.2789         | 0.3115         | 0.0326          | 0.1047          |
| S <sub>39</sub>                                                                 | 0.0733 | 0.0809 | 0.1586 | 0.1043         | 0.1139         | 0.0096          | 0.0846          |
| 平均 Mean                                                                         | 0.1981 | 0.1934 | 0.2095 | 0.2003         | 0.2166         | 0.0163          | 0.0751          |

2.3 Nei 指数

Nei 遗传分化指数是衡量种群遗传分化最常用的指标<sup>[10]</sup>。本项研究中,Nei 指数估算的川鄂连蕊茶 3 个种群种群内、种群间的基因多样性和遗传分化见表 4。由表 4 可见,川鄂连蕊茶 3 个种群平均基因多样性大小顺序与 Shannon 信息指数估计的结果一致。川鄂连蕊茶 3 个种群平均基因多样性为 0.2003,总基因多样性为 0.2166,种群间基因多样性为 0.0163,种群间遗传分化为 0.0751。就遗传分化程度而言,Nei 指数的估计值略低于 Shannon 指数的估计值。

2.4 Nei 遗传距离

为了进一步说明 3 个种群间的分化程度,计算了各种群间的遗传距离(表 5)。各种群间的遗传距离在 0.0177~0.0393 之间,也表明 3 个种群间的遗传分化较小。

3 讨论

山茶属(*Camellia*)是亚热带常绿阔叶林的主要组成植物之一。有关其天然种群遗传变异的研究主要集中在于山茶(*Camellia japonica*)<sup>[11~13]</sup>,但由于采样范围和采用的分子标记不同,研究结果差异较大。Wendel 等<sup>[18]</sup>在日本对山茶 60 个种群检测了 12 种等位酶的多态性,多态位点比率为 66%,期望杂合度为 0.265。

他认为日本山茶之所以有较高的遗传多样性,除了其具有长寿、异交、高生殖力和风媒等特征外,也与其在日本分布广泛、种群较大有关。Chung 等<sup>[11]</sup>比较了韩国山茶 6 个种群和日本山茶 10 个种群 13 种等位酶的遗传多样性,韩国山茶多态位点比率和期望杂合度分别为 66.44%和 0.209,日本山茶分别为 52.67%和 0.145,和 Wendel 的研究结果有很大差异。李力等<sup>[14]</sup>对我国山东青岛耐东山茶(*Camellia naidong*)进行了 7 种等位酶分析,多态位点比率为 83%。茶树(*Camellia sinensis*)由于其重要的经济价值,近年来 RAPD 分析开展较多<sup>[15~18]</sup>,如 Wachira 等<sup>[17]</sup>利用 RAPD 检测了几个产茶主要国家茶树栽培品种和野生茶树的遗传多样性,茶树和野生茶树平均基因多样性分别为 0.19、0.27,Shannon 指数分别为 0.33 和 0.404。与上述研究相比,川鄂连蕊茶的多态位点比率较高,基因多样性略低,Shannon 指数则处于中等水平。

在对其他长寿、木本、异交植物的 RAPD 分析中,以 Shannon 指数估计的遗传多样性分别为:墨西哥丁香(*Gliricidia sepium*)0.295<sup>[19]</sup>,红松(*Pinus koraiensis*)0.3654<sup>[20]</sup>,观光木(*Tsoongiodendron odorum*)0.3565<sup>[21]</sup>,白云杉(*Picea glauca*)0.404<sup>[22]</sup>,栓皮栎(*Quercus variabilis*)0.366<sup>[23]</sup>。与上述植物相比,川鄂连蕊茶的遗传变异也处于中等水平。

以 Shannon 指数和 Nei 指数估计的川鄂连蕊茶 3 个种群的遗传多样性均以常绿阔叶林最高。野外调查发现,川鄂连蕊茶在 3 个种群落内数量和生长很不一致。在常绿阔叶林内,川鄂连蕊茶分布最广,数量最多,长势良好,最大植株地径为 6.4cm,已成为灌木层的优势种群;在针阔混交林内,川鄂连蕊茶的分布、数量和长势为中等,最大植株地径为 5.3cm;在毛竹林内,川鄂连蕊茶分布面积小,数量较少,长势较差,最大植株地径仅有 3.4cm。因此,3 个种群遗传多样性的高低可能是各自对不同群落生境的适应。

以往的研究表明,群落生境差异对植物种群遗传分化的影响随物种差异很大,锥栗(*Castanopsis chinensis*)为 24.6%<sup>[3]</sup>,荷木(*Schima superba*)为 4.0%<sup>[13]</sup>,黄果厚壳桂(*Cryptocarya concinna*)为 0.95%<sup>[5]</sup>,大叶相思树(*Acacia auriculiformis*)为 9.27%<sup>[24]</sup>。本项研究中,以 Shannon 指数和 Nei 指数估算的川鄂连蕊茶种群间遗传分化分别为 8.12%和 7.51%,与上述植物相比,川鄂连蕊茶在不同群落中的遗传分化处于中等水平,也反映群落生境在一定条件下对种群结构的影响。

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| 表 5 川鄂连蕊茶 3 个种群的 Nei 遗传距离                                                                  |        |        |    |  |
|--------------------------------------------------------------------------------------------|--------|--------|----|--|
| Table 5 The matrix of genetic distance between populations of <i>Camellia rosthorniana</i> |        |        |    |  |
| 种群 Population                                                                              | P1     | P2     | P3 |  |
| P1                                                                                         |        |        |    |  |
| P2                                                                                         | 0.0177 |        |    |  |
| P3                                                                                         | 0.0340 | 0.0393 |    |  |

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