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Environmental Biology and Conservation Genetics Research in the Context of Artificial Intelligence

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Abstract: Due to the deterioration of the environment, many species are in an endangered state. Conservation genetics has emerged for the protection of endangered species. So far, it has a great application in the protection of animals and plants. Conservation genetics is mainly devoted to protecting the genetic diversity and evolutionary potential of populations and providing a theoretical basis for the formulation of conservation strategies. However, for some endangered plants lacking basic genetic information, it is difficult to formulate effective management measures. DNA sequence analysis can provide highly repetitive and informative data, which plays a huge role in population genetics, phylogenetics, environmental biology, and more. In this paper, artificial intelligence technologies such as microsatellite marker technology and DNA sequencing technology were used to explore golden camellia, to explore the genetic diversity of golden camellia, and to put forward suggestions to guide future protection strategies for the survival and evolution of golden camellia.

1. Introduction

The utilization and protection of biological genetic resources has always been a research hotspot in international environmental law. With the development of biological science technology and life science, the importance of biological genetic resources in medical, consumer goods and other fields has become prominent, making a large number of countries rich in biological genetic resources more and more aware of the use of intelligent technology to protect and utilize biological genetic resources. importance.

At present, many scholars have adopted artificial intelligence technology in the research of environmental biology and conservation genetics, and have achieved good research results. For example, a scholar used 10 microsatellite (SSR) loci to assess the genetic characteristics of three

adjacent ex situ wax palm species and correlated them with three wild populations in the mountains to infer that The grouping structure between the ex situ population and the wild population was collected, and the study showed that the expected heterozygosity of the wax palm ex situ population was lower than that of the wild population, and the private allele ratio of the wild population was higher than that of the ex situ collected population [1]. Some scholars used 8 pairs of SSR primers to study the genetic diversity and genetic structure of a total of 516 individuals in 22 natural populations of Phellodendron Phellodendri. It shows that there is a significant positive correlation between the genetic distance and geographic distance of Cork chinensis, and the results of UPGMA support that the 22 populations of Cork Phellodendri are divided into two genetic groups. The lack of and the reduction of genetic diversity may be caused by the northward migration of individual refuges after the glacial period, and finally scholars have proposed suitable conservation strategies for Phellodendron chinensis [2-3]. A scholar used microsatellite (SSR) to detect the level of genetic diversity and the distribution of genetic diversity in the Pussythia population. The results showed that there was a low level of genetic diversity in the population, and the analysis results of PCoA and STRUCTURE showed that there was a serious population decline. , and may even become extinct, which has attracted the attention of the local committee [4]. It can be seen that the application of artificial intelligence in biological heritage has been very deep, and the research progress of genetics is inseparable from artificial intelligence technology.

This paper first introduces the research fields of environmental biology and conservation genetics, and then analyzes the application of conservation genetics in genetic diversity. Genetic structure analysis, according to the research results, the genetic information of camellia camellia was found, which provided the basis for the protection of camellia camellia species.

2. Relevant Theories and Applications

2.1. Environmental Biology and Conservation Genetics

The seven core themes of the environmental biology section are: commonalities and differences between living things and between living things and non-living things, heredity, evolution, reproduction and development, homeostasis, living things and the environment, and humans and the environment [5].

Conservation genetics is an emerging discipline that focuses on biological diversity, especially genetic diversity and conservation, through genetic principles and research methods. 6].

2.2. Application of Conservation Genetics

Genetic diversity is an important part of biodiversity conservation, and accurate assessment of the genetic diversity and genetic structure of a species is the basis for proposing conservation strategies for that species. In particular, for the description of management strategies and conservation units, avoiding the potential risk of inbreeding decline, and clarifying the reduction of population size, understanding the genetic diversity of endangered species can protect the reproductive evolution of these species [7]. In recent years, many studies have shown that external factors such as climate change and human activities have an increasingly strong impact on endangered species, and many endangered species have experienced habitat fragmentation or population reduction. The ultimate goal of protecting endangered species is to protect the evolutionary potential and self-renewal capacity of the species as much as possible. The level of genetic diversity determines the potential of species evolution, so it is necessary to investigate the

genetic diversity, adaptation potential and long-term conservation status of endangered species, which will help to propose more effective management methods [8-9]. The methods for judging the genetic structure are:

$$G = (1 - F_{ST}) / 4F_{ST} \quad (1)$$

$$P = (N - F_{ST}) / N \quad (2)$$

G represents gene flow, N represents the number of substitutions, and F_{ST} represents the coefficient of genetic differentiation.

Molecular markers commonly used in conservation genetics are microsatellite markers and DNA sequence markers [10]. cpDNA (chloroplast) sequence analysis is the most widely used molecular marker technology in the study of plant protection genetics. Therefore, the non-coding region is a hot research object in conservation genetics [11]. The chloroplast genes inherited from one parent do not undergo genetic recombination, and their structure is simple, the molecular weight is relatively small, and they are rarely subjected to selection pressure. However, chloroplast inheritance as a single parent can only explain the evolution of a single gene. In conservation genetics research, it is often necessary to analyze the nuclear genes inherited from both parents. SSR can perform cluster analysis on all samples of this species according to the frequency of genes, which just complements the insufficiency of cpDNA. Therefore, the combination of cpDNA and SSR is widely used in conservation genetics research [12]. The development and application of SSR (simple repeat sequence) molecular markers have further promoted the development of conservation genetics, which consists of multiple repeated 1-6bp nucleotides in tandem to form a sequence of tens to hundreds of bp in length, which is abundant in all Among the coding regions and non-coding regions of higher organisms, the occurrence probability of non-coding regions is greater than that of coding regions [13].

3. Research Experiments

3.1. Experimental Subjects

In recent years, conservation strategies for endangered species have focused on maximizing the preservation of genetic diversity and heterozygosity within populations. The changes of biological genetic diversity are complex, not only affected by natural factors (environment and disasters), but also by human factors (habitat destruction, introduction and pollution, etc.) [14]. Generally, Camellia sinensis grows in limestone mountains, with a high degree of habitat heterogeneity, and the population has been fragmented. In addition, due to the serious poaching and digging, the populations in the protected areas are temporarily well protected, while the size of the populations not in the protected areas is decreasing day by day; the development of tourism in some places, the development of land has led to almost exhausted adult golden camellia species. Only seedlings remain. Therefore, effective measures should be taken immediately to protect golden flower tea.

3.2. Extraction of Total DNA from Camellia Sinensis

In this study, the modified CTAB method was used to extract the total DNA of the samples. The experimental steps are as follows:

(1) Disinfect and clean the tweezers and scissors used in the experiment with 98% alcohol. Use

the sterilized tweezers to pick up an appropriate amount of dry four-season golden flower tea leaves and place them in a 2 mL centrifuge tube, cut them with scissors, and put in 3 steel balls. , cover the lid, number the centrifuge tubes, place the centrifuge tubes in the cell crusher, and grind twice, 1 min each time, after each grinding, carry out the next grinding at an interval of 30 s [15];

(2) Add 0.8 mL of 2xCTAB and 6 μL of mercaptoethanol to the polished centrifuge tube, cover with the lid and turn it upside down until the four-season camellia leaf powder and the reagent are mixed evenly;

(3) Put it in a water bath at 65 ℃ for 2 hours, take out the centrifuge tube every 15 minutes, and turn it upside down until the powder and the reagent are mixed evenly again;

(4) After the ice bath, centrifuge at 12000r/min for 10min, carefully pour off the liquid in the centrifuge tube, leaving a white precipitate (this white precipitate contains the total DNA of Camellia sinensis);

(5) Add 300uL of 70% alcohol, invert the centrifuge tube upside down, make the 70% alcohol fully contact the white precipitate, wash the white precipitate, centrifuge at 12000r/min for 10min, and carefully pour out the liquid in the centrifuge tube;

(6) Add 300uL of anhydrous alcohol, invert the centrifuge tube upside down, make the anhydrous alcohol fully contact the white precipitate, wash the white precipitate thoroughly, centrifuge at 12000r/min for 10min, carefully pour out the liquid in the centrifuge tube, and get Centrifuge tubes for total DNA [16];

(7) Place the centrifuge tube containing the total DNA (white precipitate) in an oven to dry at 37 ℃. After drying, add 50 μL of 1xTE solution to dissolve the white precipitate (total DNA), and store it in a -20 ℃ refrigerator.

3.3. Total DNA Detection

The obtained total DNA of Camellia sinensis is detected by agarose gel electrophoresis, and the steps are as follows:

(1) Glue preparation: Mix 10ml of 50xTAE solution with 490ml of ultrapure water, mix evenly, configure into 1xTAE solution, then weigh 31.5g of urea into a beaker, and mix quickly with a glass rod; it can be taken out several times during the period Shake and cool to 50-60 ℃ (the outer bottle body can be rinsed with cold water to achieve rapid cooling), add 3 μL of non-toxic nucleic acid dye, mix well; place the beaker in a water bath, heat at 65°C, and then quickly add 37.5 μL TEMED and 250 μL of 10% ammonium persulfate can be quickly stirred with a glass rod for more than ten seconds (the bottle mouth should not face people during stirring to prevent the liquid from splashing) [17-18].

(2) Glue filling: Use a glass rod to slowly pour the polyacrylamide gel into the glass plate from the upper left of the glass plate. The glue filling process needs to be uniform and continuous (to prevent the generation of air bubbles and affect electrophoresis); the glue filling is completed. Then, slowly insert the comb (once the comb is inserted, do not take it out, otherwise it will cause the failure of making the glue hole), and finally place the two plates horizontally.

(3) Spotting and electrophoresis: Use a 10 μL pipette to pipette 4 μL of the total DNA of Four Seasons Camellia sinensis and mix it with 1 μL of 6x LordingBuffer, carefully point it into the spotting well, and carefully point 3 μL DNA Ladder Marker into a new spotting well As a control, the voltage was set to 1500V for electrophoresis. The electrophoresis time depends on the length of the PCR amplification product (the length of about 100bp, the indicator electrophoresis to the third knob; the length of about 150bp, the indicator electrophoresis to the fourth knob; the length of about

200bp, the indicator electrophoresis runs out of the plate; By analogy, if the fragment is more than 200bp, the electrophoresis time will increase by 5min for every 10bp increase in the fragment length).

(4) Silver dyeing, color development and photography

After electrophoresis, gently pry one corner of the concave plate with a knife to peel off the two plates. Slowly put the square plate into a 60 ℃ preheated silver staining tank, shake it gently with a shaker for about 10 minutes, and take it out and put it in a clean water tank. Then transfer to a preheated color developing tank at 60 ℃, continue to shake with a shaker for about 10 minutes, until the 10bp DNA Ladder and the product band are clearly visible, take out the square plate and wash it with clean water, and finally take pictures and mark the data picture [19].

3.4. Amplification and Sequencing of Chloroplast DNA Fragments

In this study, a fragment of a small single-copy region (SSC) of chloroplast DNA (cpDNA) was selected, including four genes: ndhF, rpl32, trnL-U4G, and CCSA. The cpDNA fragment SSR primers were used for amplification and sequencing. The amplification system and reaction procedure are shown in Table 1. Then, use Chromas version software to check the sequence peak map of chloroplast fragments to see if the peak map is well sequenced, easy to interpret, and free of random peaks. If the data is good, you can go to the next step of analysis; if there is a disorder of the sequence, it needs to be re-amplified and then sent for sequencing . The chloroplast sequencing fragments used in this study need to be spliced with SeqMan software, and the entire spliced sequence is compared with MUSCL software, and finally corrected and manually adjusted by MEGA software.

Table 1. cpDNA fragment amplification system and reaction procedure

Reaction system		Reaction program	
Reagent	Dosage(μL)		
10*Buffer	3.0	90 ℃	5min
dNTP	3.5	92 ℃	50s
Primer-F	1.0	75 ℃	40s
Primer-R	0.5	78 ℃	60s
LA/r-Taq	1.0	65 ℃	15min
DNA	1.5	15 ℃	∞
DdH_2O	33	-	-
Total	43.5	-	-

4. Analysis of Experimental Results

4.1. Genetic Diversity Analysis

The AMOVA analysis was performed on the chloroplast data of golden camellia. As shown in Table 1, the inter-population variation accounted for 95.22%, and the intra-population variation accounted for 4.18%. The inter-population variation was greater than the intra-population variation. Significant level (FsT=0.9624, P<0.001). At the species level, the coefficient of genetic differentiation (FsT) was 0.9714, and P<0.001 was extremely significant, indicating that there was greater genetic differentiation among populations.

Table 2. Results of Genetic Variation (AMOVA) Analysis

Species	Source Of Variation	Degrees Of Freedom	Sum Of Variance	Variation Composition	Percent Variation
Camellia species	Between populations	17	37.548	94.87	F_{ST}=0.9715 (P<0.001)
	Within the population	96	2.32	5.13	F_{ST}=0.9624 (P<0.001)
	Total	113	39.868	-	-

In addition, the results of SAMOVA analysis based on cpDNA are shown in Figure 1. The Fct value continues to increase with the increase of the number of groups K. When K=5, the Fct value gradually maintains a certain value (0.967), and then the Fct value increases with K As the value increases, it gradually decreases, and the variation is very small, and the difference is not obvious.

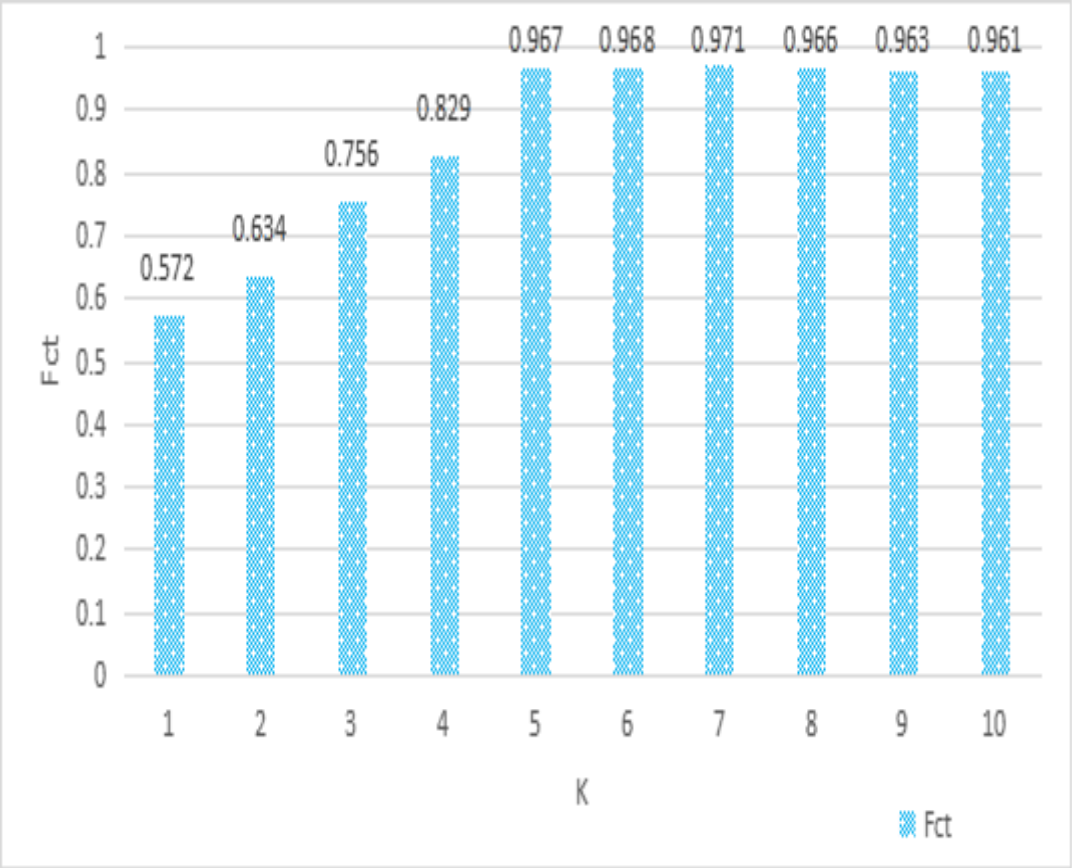

Figure 1. SAMOVA grouping analysis of cpDNA of Camellia sinensis

4.2. Microsatellite Marker Data Analysis

The 6 pairs of primers (TER2, TER5, TER7, FLA11, FLA14, FLA17) used in this study detected 107 alleles in the population studied individuals, that is, each pair of primers detected an average of 18 alleles ,as shown in Table 3. We performed a total of HWE balance test on the obtained data, and found that there were two deviations from the balance, namely TER5 and FLA14. In this study, the fixed exponent F was positive for most of the populations, indicating that there is a severe selfing

phenomenon in the population, and selfing can cause insufficient heterozygotes, thereby causing the locus to deviate from the balance.

Table 3. Genetic diversity information

	N_T	N_A	N_E	H_o	He	F_{is}	F_{sT}
TER2	14	6.1	1.576	0.621	0.572	0.106	0.472
TER5	16	4.3	1.863	0.374	0.493	0.072	0.516
TER7	23	5.7	2.479	0.538	0.345	0.064	0.384
FLA11	18	4.6	1.875	0.464	0.383	0.127	0.275
FLA14	16	3.5	1.475	0.673	0.541	0.134	0.543
FLA15	20	2.8	1.761	0.406	0.296	0.056	0.481
Mean	18	4.5	1.838	0.513	0.438	0.093	0.445

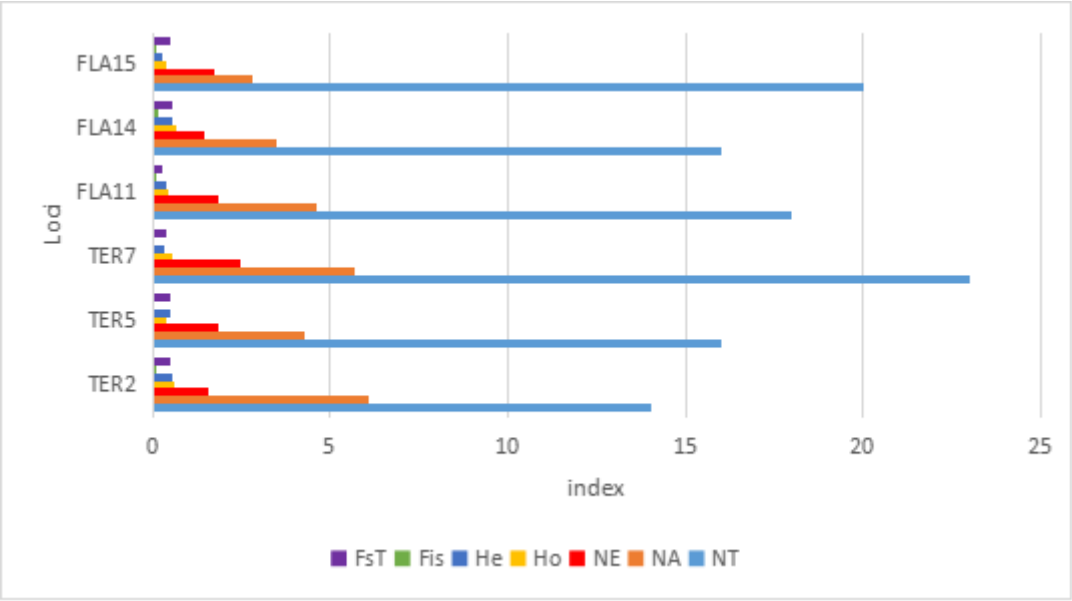

Figure 2. Locus genetic diversity

The linkage disequilibrium of the six microsatellite loci in Camellia sinensis was tested, and the results showed that each locus was relatively independent and had no linkage. Other parameter information is shown in Figure 2. The average number of alleles (N_A) in the six populations is between 2.8 and 6.1, with a mean of 4.5; the effective number of alleles (N_E) is between 1.475 and 2.479, with a mean of 1.838; observation The degree of heterozygosity (H_o) was between 0.374-0.673, with a mean value of 0.513; the expected degree of heterozygosity (H_e) was between 0.296-0.572, with a mean value of 0.438.

5. Conclusion

In this paper, taking Camellia species as an example, the genetic diversity of this species was studied by DNA sequencing and microsatellite markers. Camellia japonica has a medium level of genetic diversity and a high level of genetic differentiation among populations through experiments. The protection strategy of Camellia japonica is focused on maximizing the preservation of genetic

diversity and strengthening the protection of various groups. Protecting the existing environment of the golden camellia population and suppressing the deterioration of the ecological environment as much as possible can not only maintain the genetic diversity of rare species, but also protect our living environment.

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Data Availability

Data sharing is not applicable to this article as no new data were created or analysed in this study.

Conflict of Interest

The author states that this article has no conflict of interest.

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