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The exploration of the genetic basis of the proverb "the tasty orange, grown in southern china, would turn sour once it is grown in the north."

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ABSTRACT. *The tasty orange, grown in southern China, would turn sour once it is grown in the north" is a very popular proverb in China for thousands of years, and it has been taught to many teenagers for the philosophy behind it. However, many researches seem to prove that the proverb may be wrong scientifically. Therefore, we decide to explore this problem from many aspects. Hopefully, our research can play a good role in the popularization of science and stimulate the interest of some teenagers to biology.*

KEYWORDS: *Citrus; Poncirus; genus; kinship; trifoliata*

1. Introduction

In China," The tasty orange, grown in southern China, would turn sour once it is grown in the north." is an analogy that harness natural phenomena to vividly illustrate that the growth of the person is inseparable from the influence of the surrounding environment. This sentence has been passed down from generation to generation for many centuries for the philosophy behind it.

However, with the development of modern science, the idea of "the tasty orange, grown in southern china, would turn sour once it is grown in the north." has been questioned by more and more scientists recently, and more and more evidence seem to prove that orange and trifoliata (Poncirus) are different species (Pang Xiaoming, 2002; Yang Xiaoming, 2017; Lu Zhenhua, 2008), the ancients may have misunderstood their distinctions. (Chen Weihua, 2011; Wu Jianfang, 2008) But their researches have only included a part of the taxonomic approach, lacking comprehensive research on various system classification methods.

Therefore, our paper scientifically analysed the relationship between orange and trifoliata (Poncirus) from the perspectives of the distribution of orange and trifoliata, the discrepancy of morphology, and the evolution of species. It scientifically proves that orange and trifoliata belong to the different genera. And we went to the south and the north region of the River Huai and carried out the investigation on the

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morphology, we then downloaded the ITS sequences (a part of the DNA sequence of the mitochondrion that are highly specific to exact species) from the NCBI databases and employ Mega software to draw the evolution tree to show the relationship between the orange and the trifoliata. We also referred to many papers to identify the previous study on the relationship between the orange and the trifoliata as well. Our conclusion is that the trifoliata (Poncirus) and the orange are not the identical species.

2. Results

2.1Geographical distribution

We have drawn the following geographical distribution map by consulting the website of the Ministry of Agriculture and some related literature (Gan Jianping,1997; Guo Tianyu & Xu Rongman, 1999; Wu Jiangfang,2008).

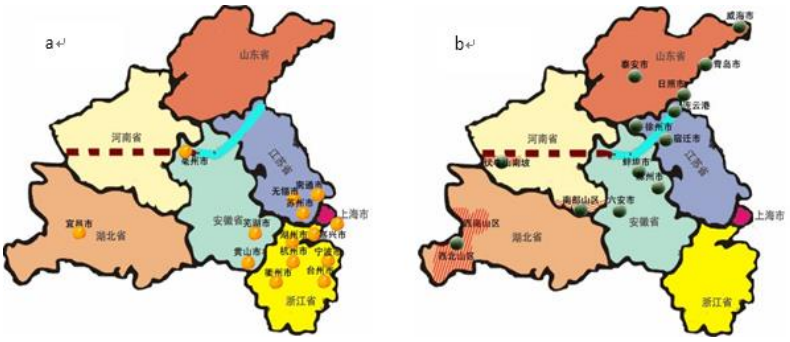

Figure 1 the distribution of the orange and the trifoliata across the region of the River Huai: a. The picture on the left is the distribution of the orange. b. The picture on the right is the distribution of the trifoliata.

From the map of the distribution above, the trifoliata is more widely distributed than the orange, such as the North of the River Huai in Rizhao City, Tai'an City, Weihai City, Shandong Province; south of the River Huai River in Suqian City, Jiangsu Province, Anhui Province The city of Zhangzhou is close to the Huaihe River, but it is rarely found in the Yangtze River Delta. In addition, the trifoliata can also grow in harsh mountain areas, such as the mountainous area of western Hubei Province and the southern slope of Funiu Mountain in Henan Province, while orange is difficult to grow in harsh environments. However, the orange it is only distributed on the south side of the River Huai, which is in line with the description of the ancients.

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Figure 2: Distribution of the orange and the trifoliata across the country (partial map of China)

Next, we studied the distribution of these two in a wider range, we found that the trifoliata is more widely distributed nationwide and distributed in both the north and the south according to the picture above. In particular, we should note that the orange can also cross the River Huai from south to grow in the northern regions of Shaanxi and Gansu.

2.2 Cladogram

Traditional taxonomy is mostly based on morphology. However, the evidence of DNA sequence of the species is more to confirm the relationship between the two species. We searched the ITS sequence of each DNA species in the NCBI database following the default operation of mega software, then we obtained the following cladogram that showed the relationship of the species within the Citrus:

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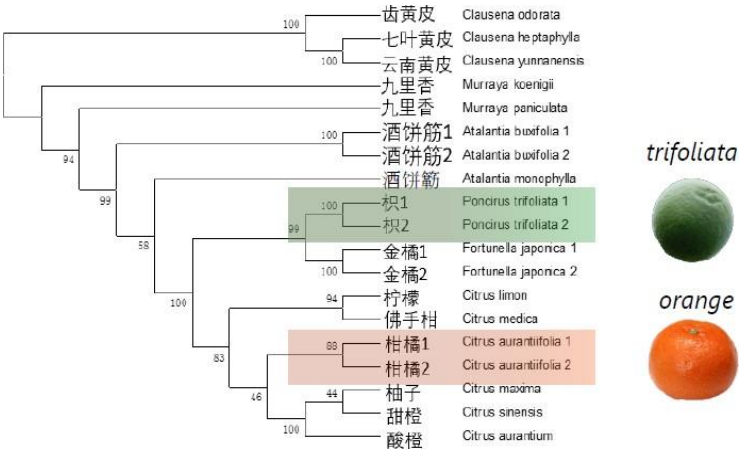

Figure 3 the cladogram of the Citrus. It can be seen that the trifoliata (green) and the orange (orange) are divided into two branches during the evolution process, which illustrates that the orange and the trifoliata are now two independent species.

In our cladogram above, the number on each branch means the credibility of the discrimination of the two species. It can be seen that the credibility within the Citrus is low, which is similar to the results in other literatures(Xie Rangjin, 2008, Yang Xiaoming, 2017, Pang Xiaoming, 2002). However, contrary to Yang Xiaoming's conclusion, the time in which the Poncirus and the Citrus clustered together preceded the time of the Fortunella and the Citrus. We thought that the reason is that the different kinds of DNA sequences we used: we use ITS sequence and they utilize the gene sequences from the nucleus. The results are naturally biased when using unlike materials, but all the literature above told us that the trifoliata and the orange can never be the same species.

2.3Morphology

We conducted an investigation to the south and north of the River Huai to study the diversity of the morphology of these species, and we fixed four relatively significant morphological differences: whole plant morphology, leaf, fruit, stem. Through field trips, we end up with the following results:

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Figure 4: the whole plant morphology of the orange and the trifoliata a. orange

tree b. trifoliata tree

Through our field investigation, we can see that the root of the orange trunk is thick, and the orange tree is an evergreen tree species, so we concluded that the orange tree is the arbor. The trunk of the trifoliata root is not obvious: It is almost branched after it is unearthed. There is no particularly obvious difference between the trunk and the branches of the trifoliata, which is the character of shrubs.

Figure 5 the leaf of the orange and the trifoliata (the orange is on the left side while the trifoliata is in the right.)

The leaf shape of the orange is the single compound leaf, and the leaf shape of the trifoliata is a three-leaf compound leaf. The leaf of the orange is twice to three times as large as the leaf of the trifoliata (The picture reflects the true size of both). The feels of the leaves of the orange are waxy, while the leaves of the trifoliata are thin and dry when touching them by hand. In addition, in a few cases, the leaves of the trifoliata will also have four small leaflets or five leaflets on one big leaf.

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Figure 6: the fruit of the orange and the trifoliata (the orange is on the left side while the trifoliata is in the right.)

The surface of the fruit of the orange is smooth, and the fruit of the trifoliata is covered with fuzz, and the fruit of the orange is two to three times larger than the fruit of the trifoliata. At the same time, we have to point out that the color of orange and the trifoliata is not a distinguishing factor. The orange in this picture was taken in November and is not fully mature, so the surface was still quite green, during our investigation process spanning one month, we found that the fruit size of orange and the trifoliata did not change substantially, but the change range in color was very large.

Figure 7 the stem of the orange and the trifoliata (the orange is on the left side while the trifoliata is in the right.)

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As we can see in the picture, there are few thorns on the stems of the orange plants, but they are very rough with many branches. However, the thorns on the stems of the trifoliata are obvious, and the stems are very smooth with no branches.

From the results above, the orange and the trifoliata have a very huge divergence in morphology, so we think that the possibility of the two being the same species is very small, they seem more likely to be two discrete species.

Combining the result of previous studies of geographical distribution difference and cladogram of DNA sequences, we can make the conclusion that the orange and the trifoliata are different species.

3. Discussion

Our work is mainly based on geographical distribution, morphological discrepancy and evolutionary relationships (through DNA sequence analysis). We use a variety of different methods that are used to study the relationships between species in the field of biology. Systematic, detailed and elaborative comparison of the relationship between the two species has been conducted, and it is highly explicit that orange and trifoliata have devided into two different species since a long time ago due to the natural factors rather than external factors such as the environment. Our research is more systematic and comprehensive than the previous studies, and our finding is more explicit, that is, the orange and the trifoliata are definitly two different species.

We draw the identical conclusions from the construction of the cladogram (a tree which can reflect the evolutionary relation) that the orange and the trifoliata are different species (Xie Rangjin, 2008, Yang Xiaoming, 2017, Pang Xiaoming, 2002), we harness the ITS sequence, the SSR sequence is utilized by Pang (Pang Xiaoming, 2002), and the whole genome DNA sequence is utilized by Yang, including the resequencing on the chloroplast DNA sequences and genomes(Yang Xiaoming,2017). Lu Zhenhua separated the outer clusters and sequenced three chloroplast DNA fragments during the construction of the cladogram (Lu Zhenhua, 2008). In addition, our cladogram has the similar tendency as the work obtained by others in the Citrus and the Poncirus, that is, the evolutionary relationship within the genus of the Citrus is disordered. However, our cladogram is different in some respects from the part of the cladogram of Yang Xiaoming,

their cladogram is rooted in the genus of the Murraya (九 里 香), however our cladogram is rooted in the Clausena(黄皮属). In Yang Xiaoming's cladogram , the Citrus and the Fortunella first come together in a branch, while the genus of the Poncirus is outside(Yang Xiaoming,2017), while in our cladogram , the Poncirus and the Fortunella gather together in the branch, and the Citrus is

outside. We analyze this phenomenon and think that the reason is that we utilize different sequences. Yang Xiaoming has applied 541 single-copy nuclear genes in series, but we utilize ITS sequences (Yang Xiaoming,2017). Different results will

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come out naturally when we use Different gene sequences, we also found this phenomenon in our practical operation. However, it must also be indicated that different researcher utilize different sequences and the conclusions obtained are somewhat different.

With regard to this cladogram, we think further that different sections of the gene are subject to different selection pressures and possess different conservatisms, so the results of different evolutionary relationships can also be obtained. We can organize the historical evolutionary tree and look up the characteristics of the various gene sequences used to construct the cladogram (such as the structural characteristics of this DNA sequence segment, the selection pressure of the traits associated with this gene, etc.), and maybe a general qualitative law can later be obtained and used to evaluate the credibility of different phylogenetic trees. Finally, due to financial constraints and time constraints, wes only covered two locations in the North of River Huai and the South of River Huai. Although the minimum requirements were met (ie, the North of River Huai and the South of River Huai were investigated), more work needs to be performed to be more representative and persuasive, we can also dig deeper into morphological research later.

In short, through the analysis of the cladogram, we found that the trifoliata and the orange are not the homogeneous species, and the Citrus genus is independent of the Poncirus. In addition, our research also proposes a novel view that the Citrus is independent, and Kumquat is inlaid in the genus. This is different from the previous opinions of Yang Xiaoming and Xie Rangjin (the Poncirus genus is independent, and the Fortunella is inlaid in the Citrus) (Xie Rangjin, 2008, Yang Xiaoming,2017). In summary, we believe that the proverb “ The tasty orange, grown in southern China, would turn sour once it is grown in the north. “is not right and misled many people. We should correct this in future education because the ancients are wrong, we may also cultivate their interest in biology and improve their critical thinking through this opportunity.

4. Method

4.1. Participants

There is no participants because this is not an interview or survey. We utilized data and photos to carry out experiment and analysis.

4.2. Materials

4.2.1 Software & Tool

We employed software: Mega 7 from the official website https://www.megasoftware.net/ and notepad official website https://notepad-plus-plus.org/ , then we downloaded the sequences from NCBI database https://www.ncbi.nlm.nih.gov/ . We choosed the option “Nucleotide” and we typed

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the term: “Citrus” and “Poncirus”. Also, we downloaded the sequences of other species (“Clausena”, “Murraya”, “Atalantia” and “Fortunella”)which were in the identical subfamilies. Then we utilized Mega 7 to build the evolution tree to analyse the relationship of these species. And we obtained our incredible results utimately, which will be showed in the result section.

4.2.2 Object

The sequences were downloaded from NCBI https://www.ncbi.nlm.nih.gov/ as above (see Software & Tool). The sequences were utilized to do the simulation analysis. We have also gone to the following sites to observe the morphology

(2018.10.14 in No. 289, Jinglingqiao Village, Zhangze Town, Songjiang District, Shanghai, China and 2018.11.10 in Xialou Village, Lingbi County, Suzhou City, Anhui Province, China)We went there to conduct on-site sampling of the oranges and trifoliata grown in the field to study their morphology, in which the latter area has no orange distribution. Then we went to Jinglingqiao Village again in 2018.11.24 to get more details in morphology.

4.3 Procedure

4.3.1 Data acquisition & evolution tree

First, we opened the NCBI official site https://www.ncbi.nlm.nih.gov/. Then we choosed the option” Nucleotide”, selected” Citrus ITS”, “Poncirus

ITS”, “Clausena ITS”, “Murraya ITS”, “Atalantia ITS” and “Fortunella ITS”, then we downloaded top 10 sequences for each species as fasta format. Then we opened these fasta files through notepad and check the sequence. We harnessed the Mega software and aligned the sequences, deleted the part which is not neat.

We also pressed “phylogeny” and then followed the default setting, we ultimately obtained the evolution tree and our work came to an end for a while in the last.

4.3.2 Field trip & Morphology

The morphology is another efficient way to identify the species. We therefore went to two places as has been said above. We utilized our camera to picture the characteristic area (We pictured their blades, stalks, fruits and the entirety). Then we compared these characteristics with the literatures (Li Runtang, 2004; Li Hongguang, 2010) and websites. Finally, we proposed our conclusion combining the above two factors (Morphology and DNA sequences)

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