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REVIEW

Top-geoherbs of traditional Chinese medicine: common traits, quality characteristics and formation

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Abstract **Top-geoherbs used in China are always featured with high qualities, and they grow in specified areas with specific environment. Recently, researches on top-geoherbs have attracted increasing attention in China and other countries. In order to have a thorough knowledge of top-geoherbs, this article reviews the concept, historical evolution, common trait and quality characteristics of top-geoherbs, and explains the forming mechanism including genetic mechanism and environmental mechanism. In addition, it introduces the influence of human factors on the quality of top-geoherbs. Finally, it proposes some problems that should be paid attention to in the researches on top-geoherbs.**

Keywords top-geoherbs; common traits; quality characteristics; formation; biological mechanism

Introduction

Geoherb is a term used by ancient Chinese to describe the inner species variation of Chinese medicinal materials relevant to the geographical variation, and top-geoherb (Dao-di Herbs) is the population of a geoherb in specific areas (top-geoherbal habitat) featured with proverbial superior qualities and popularly used in traditional Chinese medicinal clinical practice [1–3]. Geoherblism is the generic term of various merits possessed by top-geoherbs. As a concept of quality of traditional Chinese medicine (TCM) established by usage in TCM industry in China, top-geoherb can be considered as a distinctive standard distinguishing high-quality TCM used by ancient doctors of TCMs. The usage amount of top-geoherbs is the largest in TCM, and its economic value is the highest. Among the 500 TCMs, there are 200 TCMs with obvious geoherblism, whose usage amount makes up 80% of the total usage of TCMs [4]. *Geoherbs in China* edited by Shilin Hu recorded 160 common top-geoherbs consisting of 132 plant medicines, 20 animal medicines and 8 mineral medicines.

After the establishment of the People's Republic of China in 1949, the research development of top-geoherbs has absorbed high attention of the government. Many scholars developed researches on problems related to the scientificity of top-geoherbs [1–3,5–24]. Some literatures, such as *Geoherbs in China* [1] and *Illustration of the Chinese herbs in the place of the genuine* [25] edited by Shilin Hu, are one of the most important achievements in the researches on top-geoherbs of the age. Papers published by Luqi Huang about the genetic mechanism [12], environmental mechanism [10], and model hypothesis [9] of geoherblism of Chinese materia medica represented the latest development of the researches on top-geoherbs. Recently, the concept of top-geoherbs has been paid attention to by the western world [26–28], but the researches on top-geoherbs have been carried out mainly in China, which results in some disturbances hampering foreign scholars to understand top-geoherbs. On the basis of reviewing the evolution of the concept of top-geoherbs and introducing their common traits and quality characteristics, the present paper mainly introduces the achievements of study on the forming mechanism of top-geoherbs, and discusses several key problems in the future study on top-geoherbs.

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Concept and historical evolution of top-geoherbs

The meaning of top-geoherbs was reflected as early as in *Shen Nong's Herbal*, in which the importance of origin was emphasized. Among the 365 kinds of herbs included in *Shen*

Nong's Herbal, many kinds could be distinguished as top-geoherbs because of their names, such as Bajitian (*Radix Morindae Officinalis*), Shujiao, Shuzao, Qinjiao, Qinpi, Wuzhuyu, Ejiao, Daizheshi, etc. (Ba, Shu, Qin, Wu, Dong'e, Daizhou are all old place names around Western Zhou Dynasty). Although every herb was recorded without detailed growing area, the records such as growing in valleys, in marshs, in ponds, in hills, in fields and in flat land were shown. It seemed that the details of herds in *Shen Nong's Herbal* were shown with ancient authentic colors. *Huangdi's Inner Classic*, a contemporary medicine monograph as *Shen Nong's Herbal*, expounded the meaning of top-geoherbs at theoretical level, and *Shanghan Lun* (*Treatise on Cold-induced Febride Diseases*) was a medicine monograph which first recorded lots of top-geoherbs such as Ejiao, Badou, Daizheshi, etc. [29].

Collective Notes to the Canon of Materia Medica written by Hongjing Tao in the Southern Song Dynasty made clear the good quality of top-geoherbs. The quality of more than 40 top-geoherbs was described by number one, best, finest, etc. in this book. This book discussed the influence of top-geoherbs and non-top-geoherbs on the clinical effect for the first time, and indicated the confusion and adverse results caused by materials obtained locally [29].

Subsequent books, such as *Xinxiu Bencao* (*Newly Revised Canon of Materia Medica*) in the Tang Dynasty, *Supplement to Thousand Golden Prescriptions* written by Simiao Sun in the Tang Dynasty, and *Zhenglei Bencao* (*Classified Materia Medica*) written by Shenwei Tang in the Song Dynasty, all had a large number of records about top-geoherbs and top-geoherbs habitats. The word, top-geoherb, was first used in *Mudan Ting* in the end of the Ming dynasty, and *Bencao Pinhui Jingyao* at that time recorded 916 plant medicines, of which 268 were top-geoherbs including 32 kinds in Sichuan, 27 kinds in Guangdong, 8 kinds in Henan, and 199 other kinds in other areas. In the same period, *Compendium of Materia Medica* written by Shizhen Li also recorded many top-geoherbs [29].

Zongwan Xie, a modern pharmacognosist, considered that top-geoherbs referred to the medicinal materials growing in habitat with particular natural conditions and ecological environment. The production of top-geoherbs was usually concentrated, and their cultivation techniques and harvesting processing usually had certain stress. Therefore, these drugs were usually of better quality and better curative effect than drugs belonging to the same species but from other habitats [2]. Luqi Huang *et al*.[10] considered that top-geoherbs, the products of the natural environment, geographical environment and people, was a kind of typical products of geographical indications. He propounded natural factors and human factors effecting the formation of top-geoherbs should be distinguished, and modern biologic methods and techniques should be applied to thoroughly study the forming mechanism of top-geoherbs, and to reveal natural factors closely related to geoherblism.

The common traits and quality characteristics of top-geoherbs

The common traits

Specified quality standard and superior clinical effects

In the long process of adapting to unique habitat in the locality, top-geoherbs generally represented specificity in some aspects such as appearance, texture and chemical constituents of materia medica. Taking *Lycium barbarum* L. as an example, it differed from other non-geoherbs in many traits such as full and big seeds, red surface, freshly, seedless, sweet and bitter. The rhizome of top-geoherb of *Rhizoma Atractylodis Lanceae*, from Maoshan, Jiangsu, contained generous spots of oil cavity. The content of anthraquinone derivatives in rhubarb from Qinghai Province was higher, and this top-geoherb had obvious purgative effects; but non-geoherb from Heilongjiang with high content of tannin had the effect of checking diarrhea. The content of effective constituents in top-geoherb of *Cortex Fraxini Szaboanae* from Shaanxi was higher than that from Sichuan; the content of phenols in top-geoherb of *Cortex Magnoliae Officinalis* from Sichuan was six times higher than that from Jiangxi. The differences of top-geoherbs in chemical constituents might finally induce different clinical effects. For example, the antibacterial activity of top-geoherb of *Herba Andrographitis Paniculatae* from Guangzhou was better than that from Fujian and Anhui [4]. Unique quality characteristics resulted in unique clinical effects for top-geoherbs, which were universally well received by doctors of Chinese medicine and patients [1,2,4].

Obvious regionalism

Top-geoherbs and non-top-geoherbs were usually considered as the same species with distinct traits. Top-geoherbs could not be formed without specified habitat, although the seeds or seedlings of them were used. Environmental factors, involving soil, water, light, temperature, topography and so on, played an important role in the formation of top-geoherbs' traits in that top-geoherbs and non-top-geoherbs had similar genetic background. Accordingly, place names were often applied to top-geoherbs to indicate the genuine locations, such as Chuan Beimu (*Bulbus Fritillariae Cirrhosae*), Hang Baizhi (*Radix Angelicae* from Hangzhou), Mao Cangzhu (*A. lancea* (Thunb.) DC.), Huai Dihuang (*Radix Rehmanniae* from Henan), Guan Fangfeng (*Radix Saposhnikoviae* from north-east in China), which were well-known at home and abroad because of their good qualities [2].

Abundant culture connotation

Top-geoherbs were not only the product of nature, but also the product of development of social productivity. With regard to cultivated materia medicas, techniques of producing and processing, such as cultivation, breed, harvesting, processing,

were one of the important factors effecting the traits formation of top-geoherbs. These techniques reflected the technical level of local cultivation of materia medica, and were selective for various traits, accumulation and distribution of active constituents in drugs. The longer the cultivating history was, the more mature the techniques were, and the more outstanding the traits of top-geoherbs were. In addition, the application of top-geoherbs could reflect the realization and comprehension of knowledge and theory of clinical characteristic, toxic and side-effect, and it was one of the important signs of the medical level. In general, the formation of top-geoherbs was relevant to characteristic geographical environment, culture background and TCM theory of China. As an important feature of top-geoherbs, culture connotation made top-geoherbs different from other natural products, and also made it a kind of special natural products with intellectual property rights [4].

High economic value

Because of special habitat suitable for the growth development and quality formation of the original plants, top-geoherbs had relative larger growing scale in genuine areas. In addition, they had favorable competitive advantage and higher economic value due to advanced cultivation and processing techniques, lower production cost, excellent quality, and good market reputation. Generally, the price of top-geoherbs was higher than that of non-top-geoherbs of the same species. For example, the price of *Pericarpium Zanthoxyli Bungeani* from Sichuan was two times higher than that from other areas. Most top-geoherbs based on growth, processing and sale were the important economy mainstay of the local villages and mountainy, and promoted the development of the local professions such as tourism and export [4].

Quality characteristics of top-geoherbs

The bioactive natural products, especially their compound types, compositions and contents in Chinese herbal medicines, were the genuine core of geoherblism as a top-geoherb. Top-geoherbs could produce chemical types different from that in other populations within species and present excellent therapeutic effect depending on their unique chemical constituents. In an open complicated system, the formation of chemical type of top-geoherbs (one of the phenotypes) was the product which had adapted to environment for a long time and indicates self-adapting characteristic [4]. Initially, researchers attempted to identify top-geoherbs via their typical chemical components. But with researches on top-geoherbs becoming more deeply, they realized that differences in chemical constituents between top-geoherbs and non-top-geoherbs might not be the presence or absence of one or more constituents, but rather definite content or alternation of ratio of some components.

To prove that top-geoherbs had unique compound ratio, Lanping Guo *et al*.[13] collected 47 rhizomes of *A. lancea* in 7 populations, and 6 naphtha components in the rhizomes were determined by gas chromatography-mass spectrometry (GC-MS). Cluster analysis of the 6 compounds indicated that these samples were divided into two kinds, and the chemical constituents in top-geoherbs were different from that in non-top-geoherbs of *A. lances* at population level (Fig. 1). The essential oil in top-geoherbs was lower than that in non-top-geoherbs ($P < 0.01$), but the components yielding more than 1% (% of the total oil) were higher. The content of hinesol and β-eudesmol was more in non-top-geoherbs, and the content of atractylon and atractylodin was more in top-geoherbs ($P < 0.001$). Atractylon, hinesol, β-eudesmol and atractylodin in top-geoherbs presented a certain ratio, that is (0.70–2.00):(0.04–0.35):(0.09–0.40):1.00. Yunping Liu *et al*. [30] discovered that essential oil in top-geoherbs and non-top-geoherbs of *Herba Agastaches Rugosae* also altered in proporation. Recently, the idea that difference in chemical constituents among top-geoherbs represented quantitative alternation rather than qualitative alternation has achieved scholars' consensus gradually. Therefore, multicomponent chemical fingerprint has been applied in the researches on top-geoherbs [31].

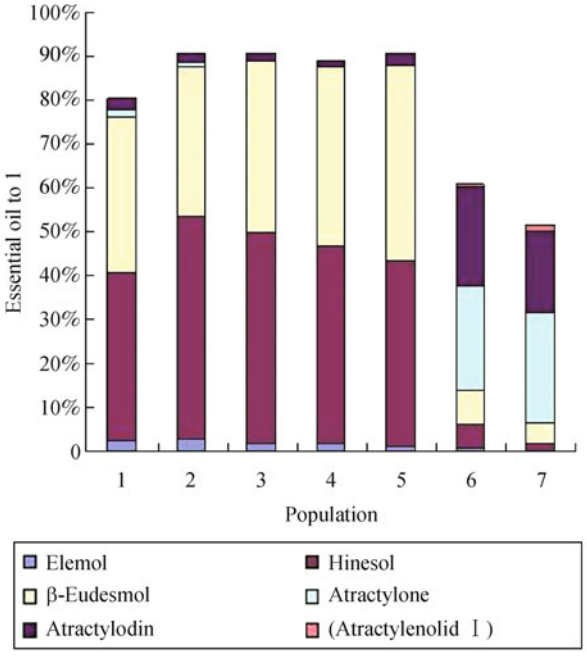

Fig. 1 The differences in essential oil of *A. lancea* at individual level and population level. 1–5. Non-top-geoherbs; 6–7. Top-geoherbs.

Because the quantitative alternation and matching changes between top-geoherbs and non-top-geoherbs were tiny, routine analysis methods had great difficulties in information extraction. Near infrared spectrum had maintained a rapid

development, and matching chemometrics, it displayed hugeous advantage in the analysis of unique chemical constituents in top-geoherbs, which could enhance the extraction progress of chemical characteristic of top-geoherbs [17].

Biology mechanism of the formation of top-geoherbs

In biology, the phenotype of top-geoherbs was determined by autologous genotype, and was influenced by specified habitat. The formation of top-geoherbs was the product of interaction of genotype and habitat, and it could be expressed by the formulation of "phenotype = genotype + environmental modification." Phenotype, the total of the observed structure and function features, included the character, microstructure, the content of active components and so on. Genotype means the variation of top-geoherbs in gene level, and environmental modification means any unhereditary alternation of phenotype resulting in habitat. Now, the researches on the formation of top-geoherbs mainly focus on two aspects: heredity and environment.

Genetic mechanism

What is the genetic basis for top-geoherbs? Is there a "genuine gene" that could distinguish top-geoherbs? Does top-geoherbs show up obvious genetic differentiation in population level? Researches on the molecular mechanism of top-geoherbs, in view of the above questions, could reveal the genetic differentiation of populations at molecular level, determine genotype characteristic and the effect of environment on gene expression, and then reveal the contribution of genetic factors to the formation of top-geoherbs [12].

In view of the above problems about top-geoherbs, several scholars carried out a large number of researches which achieved rich outcome in the following related fields: genetic diversity and molecular authentication, genetic differentiation and evolutionary genetics, geographical variation and environmental suitability, germplasm resource assessment and cultivar breeding, functional gene expression and regulation, transgene and bio-safety evaluation, etc.[12]. Scholars have gradually realized that top-geoherbs and non-top-geoherbs displayed certain continuity and transition, and it was a process of quantitative alternation related to geographic distance from non-top-geoherbs to top-geoherbs. Differentiation among populations, which had not yet reached isolated level, existed between the two kinds of materia madicas.

On the basis of analysis of genetics research on top-geoherbs, Luqi Huang *et al*. [12] concluded that top-geoherbs presented the following characteristics proven by successful researches: (1) The more obvious the geoherblism was, the more obvious the genetic differentiation was. Lanping Guo *et al*. [32] analyzed 47 individuals of *A. lancea* from 3 populations, which were divided into 5 sub-populations of non-geoherbs and 2 sub-populations of top-geoherbs by random amplification of polymorphic DNA (RAPD) with 20 random primers in order to study the genetic structure. The results showed that 77 bands were polymorphic among the total of 94, monomorphicbands was 81.91%, Shannon's information index was 0.4132 and Nei's gene diversity was 0.2743 in *A. lancea*. Genetic distances between individuals of top-geoherbs were smaller than that among the non-geoherbs. All of the 11 individuals of top-geoherbs from 2 sub-populations of top-geoherbs clustered together. Nested analysis showed that variations within populations, between sub-populations and between *A. lancea* were 76.74%, 11.58% and 11.68%, respectively. It indicated that there were genetic variations between top-geoherbs and others, and genetic factors played an important role in forming top-geoherb of *A. lancea*. (2) The differentiation of gene or haplotype frequencies between top-geoherb populations and non-top-geoherb populations was an important character of the top-geoherbs, which decided the genetic diversity pattern of top-geoherbs. *Scutellaria baicalensis* (Huangqin or Chinese skullcap) was a typical top-geoherb in Chinese traditional medicine, whose top-geoherb area was Chengde of Hebei Province and nearby areas in China. QingjunYuan *et al*. [26] used molecular phylogeography to analyze chloroplast haplotypes of 602 and 451 individuals of Chinese skullcap *S. baicalensis* representing 28 wild and 22 cultivated populations across top-geoherb and non-top-geoherb areas, respectively. They found that the genealogical structures and frequencies of 25 chloroplast haplotypes of *S. baicalensis* presented a significant divergence: haplotype G, B and C, which connected to other 10, 6, and 4 haplotypes by one mutation, respectively. The haplotype distributions of wild populations were noticeably structured. The ancestral lineage formed by haplotype G with closely connected haplotypes was mainly restricted to the central range of this species, Chengde of Hebei Province and nearby areas, which was exactly the geoherb area of *S. baicalensis* (Fig. 2). So this lineage could be named as geoherb lineage. The results showed that the genetic differentiation between top-geoherb populations and other populations was noticeable. Mantel test analyses showed that genetic distance was significantly correlated with geographical distance (r = 0.4346, $P < 0.001$) in wild populations, which was a typical isolation by distance genetic pattern (Fig. 2). The results suggested that the haplotype frequencies in top-geoherb area were significantly different from that in non-top-geoherb areas. The authors concluded that the geoherblism of *S. baicalensis* is correlated with the genetic diversity pattern and haplotype or gene frequencies of populations in this top-geoherb. (3) Lots of studies showed that the biosynthesis of these natural products usually are regulated by transcriptional control of the relevant enzymatic genes, such as the precursor of Taxol [33], artemisinic acid (precursor of Artemisinin) [34],

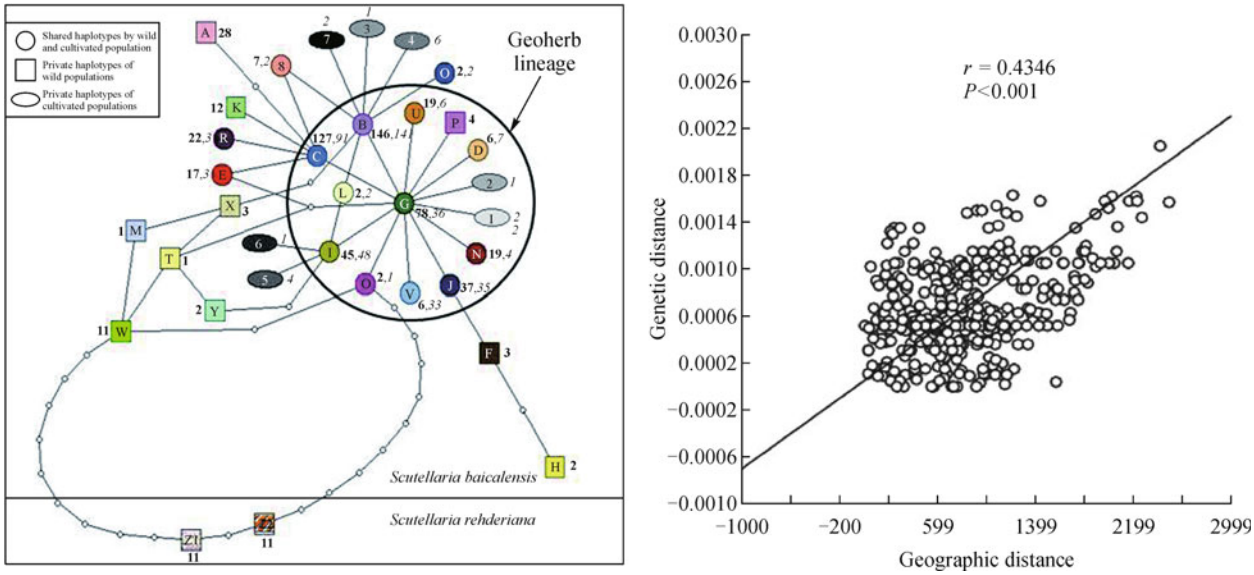

Fig. 2 Nested cladogram and frequencies of chloroplast haplotypes and scatterplots of genetic distances against geographic distances in *Scutellaria baicalensis*. The bold and italic numbers besides haplotypes represent the number of wild and cultivated individuals with certain haplotype, respectively. Open small circles represent inferred interior nodes that were absent in samples. Each branch indicates one mutation.

scopolamine [35] and etc. So, the geoherblism of top-geoherbs, showed a kind of quantitative inheritance traits regulated by multiple minor genes at the individual level. Tanshinones were abietane-type norditerpenoid quinone natural products found in the Chinese medicinal herb *Salvia miltiorrhiza* Bunge. Specifically, they were found as bioactive lipophilic pigments in the intensely red rhizome (root), which was called Danshen in traditional Chinese medicine. Danshen was regarded as a model top-geoherb by our research group who tried to explain its geoherblism by clearing tanshinones biosynthesis regulation under the multiple minor genes. The total RNA was extracted in fresh Danshen from authentic origin, and a high quality cDNA library has been constructed. The first microarray chip of Chinese herbal medicines was manufactured from ~8700 random cDNA inserts, which ranged in size from 0.5 to 2.5 kb. This was used to compare mRNA levels from induced and control *S. miltiorrhiza* hairy root cultures or in different growth stages [36,37]. The full length cDNA sequences including SmAACT, SmHMGR, SmDXR, SmCMK, SmIPPI, SmFPS, SmGGPPS, SmCPS, SmKSL and SmCYP001 in tanshinones biosynthetic pathway were isolated from *S. miltiorrhiza* hairy root using rapid amplification of cDNA ends (RACE) and polymerase chain reaction (PCR) [38–40]. For the next gene expression profiling assays, YanfangYang *et al*. [41] considered that the Actin and Ubiquitin were the most stable whereas EF1a and 18S did not favor normalization of quantitative real-time polymerase chain reaction (qRT-PCR) results as internal controls in different tissues from *S. miltiorrhiza*. Zhubo Dai *et al*. [42] found that the SmHMGR2 was a novel and important enzyme involved in the biosynthesis of diterpenoid tanshinones, and overexpression of it could increase enzyme activity and enhance the production of tanshinones in *S. miltiorrhiza.* Wei Gao *et al*. [27] described the cloning of two diterpene synthase genes (SmCPS, SmKSL) from *S. miltiorrhiza* and the characterization of the corresponding enzymes. Characterization of the SmCPS revealed a (+)-copalyl diphosphate synthase. The participation of a (+)-CPP synthase was previously unrecognized, as the tanshinones have been aromatized, eliminating the stereochemical features present in the diterpene precursors. Characterization of the SmKSL revealed a new diterpene synthase with the product being a new compound, which has been named miltiradiene. The new diene is related to pimaradiene, but has a 1,4-cyclohexadiene arrangement that nicely sets the stage for aromatization of the C-ring, a feature seen in the tanshinones. Based on the tanshinones and other known diterpenoids such as ferruginol, miltirone and neocryptotanshinone accumulated in *S. miltiorrhiza*, a biosynthetic pathway was proposed for tanshinones (Fig. 3).

Environmental mechanism

Quantitative trait regulated by multiple minor genes could be influenced by environment, which is the greatest feature. The phenotype of quantitative trait was resulted from the interaction of heredity and environment, that was phenotype = gene + environment + interaction of gene and environment [43]. This shows that genes had potency to develop some definite phenotype, but it was not the necessary condition to

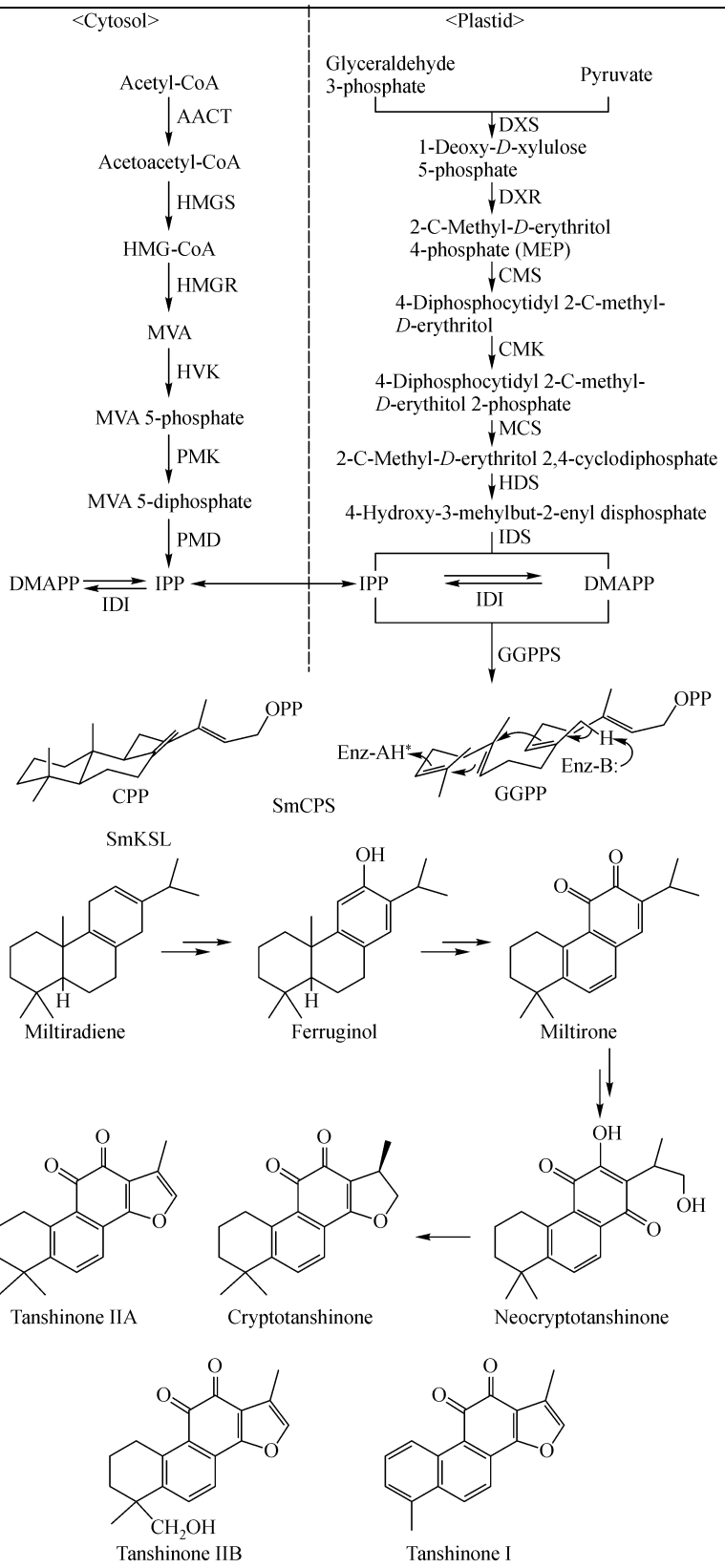

Fig. 3 The biosynthetic pathway of tanshinones. DXS: 1-deoxy-*D*-xylulose 5-phosphate synthase; DXR: 1-deoxy-*D*-xylulose 5-phosphate reductoisomerase; CMS: 4-diphosphocytidyl-2C-methyl-*D*-erythritol synthase; CMK: 4-diphosphocytidyl-2C-methyl-*D*-erythritol kinase; MCS: 2-C-methyl-*D*-erythritol 2,4-cyclodiphosphate synthase; HDS: hydroxy-2-methyl-2-(*E*)-butenyl 4-diphosphate synthase; IDS: isopentenyl diphosphate/dimethylallyl diphosphate synthase; IDI: isopentenyl diphosphate isomerase; AACT: acetoacetyl-CoA thiolase; HMGS: 3-hydroxy-3-methylglutaryl synthase; HMGR: 3-hydroxy-3-methylglutaryl reductase; MVK: mevalonic acid kinase; PMK: phosphomevalonate kinase; PMD: mevalonic acid diphosphate decarboxylase; GGPPS: geranylgeranyl-pyrophosphate synthase; SmCPS: *Salvia miltiorrhiza* copalyl diphosphate synthase;SmKSL: *S. miltiorrhiza* kaurene synthase-like.

determine these phenotypes. They could determine a series of possibilities depending on the habitat. The reason that top-geoherbs could not form without definite habitat was that traits reflecting their good qualities, that was, geoherblism, was quantitative trait regulated by multiple minor genes. Lanping Guo *et al*. [44] used canonical correlation and stepwise regressive analysis to get leading factors affecting the content of the essential oil components of *A. lancea*, and 6 regressive models between climate factors and 6 main essential oil components. They found that high temperature was the limiting factors for *A. lancea* and the interaction of temperature and precipitation was the key factor of forming the essential oil components of *A. lancea*. They used GIS software IDIRIEIW to extract the climate characteristic in Mt. Maoshan, the top-geoherbal habitat, and found that habitat of *Mao-A. lancea* was characterized as highest temperature, short drought season and more precipitation, and the forming of *Mao-A. lancea* related to the high temperature stress [15]. They also found that top-geoherbs of *A. lancea* faced nutrient stress, and proved that high temperature and K shortage stress could make essential oil compounds more similar to the top-geoherbs than others by experiments in green house.

Effective constituents were the material basis that could determine the curative effect of materia medicas. Some effective constituents, few or none of which existed under normal conditions, were only produced when external stimuli (for example, drought, freeze, injury) were achieved, and these constituents belonged to abnormal secondary components called phytoalexin [3].

According to analysis on the influence of environment on top-geoherbs, Luqi Huang *et al*. [11] pointed out that environmental stress may be beneficial for the formation of top-geoherbs.

Influence of human factors on the quality of top-geoherbs

Besides the influence of heredity, environment and their interactions, seed selection, raise seedling, cultivation, harvest and processing of top-geoherbs are the perfect combinations of the hard, wise work of local human and natural environment for hundreds of years. Therefore, to a great extent the good quality of top-geoherbs is a work combining environment, materia medica with human, of which human factors had indispensable influence [10]. The effect of processing, a pretreatment method for natural drugs characteristic of traditional Chinese medicines, on geoherblism has always been concerned. Researches indicated that processing could enhance effect, decrease toxicity, transform the active fraction and acting time, and etc. The formation of most top-geoherbs was inseparably related to their unique processing methods, and different materia medicas needed different processing methods. For example, the top-geoherbs of *Radix Rehmanniae* need to be steamed and exposed to the sun alternatively for 9 times [45]. After processing, the chemical composition was more relevance to the characteristic of top-geoherbs. Comparing the essential oil compound of *A. lancea* between the raw materials and stirred-frying with bran ones, Jie Zhou *et al*. [46] found that the content of total oil was decreased after being processed, especially the contents of β-eudesmol and hinesol, but the number of the components were increased, which indicated that processed *A. lancea* was closer to top-geoherbs than the non-processed ones.

Problems in researches on top-geoherbs

High attention should be paid to the research scale

Top-geoherbs are formed in the biologic evolution process, and their genetic essence is the change of gene frequency at population level. In other words, top-geoherb at gene level represents a probability, which is significant only at population level. For individual level, non-top-geoherbs and top-geoherbs are both continuous and uninterrupted, and it is a process of quantitative and gradual change [5]. Scale effects considered that when the observed, tested, analyzed or simulative spatial and temporal scales changed, the system characteristic would also change [47]. Therefore, researchers should pay high attention to scale effects, and related observations must be carried out in populations, which must be based on the actual areas of top-geoherbs rather than artificial areas. Here, scale effects involve both ecological scales and genetic scales, that is, when researches on molecular mechanism of top-geoherbs are carried out at population level, the research scope should be determined in populations, and more importantly, high attention should be given to gene evolution speed when selecting the related genes [48]. Likewise, various molecular techniques are suitable for molecular tests with different variation speeds respectively, and careful selection is required based on the objective.

The theory and method of quantitative genetics should be fully utilized

As stated above, top-geoherbs represent the changes of gene frequency at population level, and quantitative inheritance regulated by multiple minor genes at individual level, which causes top-geoherbs to represent quantitative characteristics at phenotype, population and gene levels. When studying quantitative variability of morphology, it is difficult to decide which gene works, and is only from the standpoint of quantitative inheritance. Owing to quantitative genetic characteristics, the methods and techniques of quantitative genetics may be the important measure to study the molecular mechanism of top-geoherbs in future from the aspects of: genetic material basis, continuous variation of traits, scale

problem, mean component and variation component, interaction and linkage, gene and effective factor, selective advance, etc. It is particularly noteworthy that study on quantitative trait locus (QTL) becomes the subject of the forming molecular quantitative genetics and the most powerful weaponry for studying quantitative trait heredity [12,49].

Interaction of gene and environment is the key point

According to the theory of quantitative inheritance, the final expressing form of quantitative characteristic of morphology is caused by various factors, and its development is effected by many aspects, involving direct additive genetic effect or nonadditive genetic effect, maternal genetic effect, interaction of gene and environment, random effects of environment, and so on. Here, the interaction of gene and environment is the important feature of polygenic inheritance, and its main styles are: (1) environment can affect genetic structure through the selection of some population; (2) the interaction of genotype and environment in development presents nongenetic effect [12,43]. The interaction of genetic variation and ecological environment greatly enriches the diversity and germplasm resources of the original species, and provides biologic basis for the formation of quality of top-geoherbs. Therefore, research on the interaction of genetic variation and ecological environment is the key point to reveal the molecular mechanism of top-geoherbs, which will become the hot spot and bottleneck of researches on top-geoherbs.

The perfect communication of natural science and humanities will perfectly reveal scientific connotation of top-geoherbs

As a specific intellectual property right, geographical indications can identify a good as originating in the territory of a member, or a region or locality in that territory, where a given quality, reputation or other characteristics of the good is essentially attributable to its geographical origin. The products of geographical indications have the following features: (1) the usual structure in Chinese geographical indications is that "geographic name + trade name"; (2) the name should indicate the real provenance of the commodities, such as champagne, Shaoxing yellow wine, West Lake Longjing tea; (3) the commodities or service expressed by geographical indications have unique quality, reputation or other features; (4) geographical indications have close connection with the quality, reputation or other features of their marking commodities, which is the most essential feature of geographical indications, and they can recognize other similar products. An ordinary geographic name can develop into geographical indication because the quality, reputation or other features of commodities are decided by specific natural environment and human factors in provenance [10].

Top-geoherbs are a kind of typical products of geographical indications according to their features. Besides therapeutic effect in clinic, the good qualities of top-geoherbs also include their appearance traits, even their transmission mode, market reputation, and etc. In other words, top-geoherb is not a net concept of natural science, and except for the attribute of natural science, it has culture attribute and economic attribute. Through the utilization of modern science and technology to reveal the biologic, chemical and pharmacological features and laws, one of the important tasks confronting researches on top-geoherbs is to refer to the identification, preservation of geographical indications for products to carry out the identification and preservation related to intellectual property rights [10].

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