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DISTRIBUTIONS OF TRIPLET CODONS IN MESSENGER RNA SECONDARY STRUCTURES*

ZHANG Jing[①②] GU Bao-hong[③] PENG Shou-li[④] LIU Ci-quan[②③]

(①*Department of Statistics, Yunnan University, Kunming* 650091)
(②*Laboratory of Cellular and Molecular Evolution, Kunming Institute of Zoology, the Chinese Academy of Sciences, Kunming* 650223)
(③*Modern Biological Center, Yunnan University, Kunming* 650091)
(④*Center for Nonlinear Complex System, Department of Physics, Yunnan University, Kunming* 650091)

Abstract Analysis of the secondary structures of mRNAs which encode mature peptides shows that the location of each codon in mRNA secondary structure has a trend, which appears to be in agreement with the conformational property of the corresponding amino acid to some extent. Most of the codons that encode hydrophobic amino acids are located in stable stem regions of mRNA secondary structures, and vice versa, most of the codons that encode hydrophilic amino acids are located in flexible loop regions. This result supports the recent conclusion that there may be the information transfer between the three-dimensional structures of mRNA and the encoded protein.

Key words Triplet codon, Amino acid, mRNA secondary structure

Since triplet codons were determined, a lot of studies have been made on the structure of the genetic code and its function. As far as the structure of code is concerned, RNY pattern was proposed (where R denoting purine, Y pyrimidine, and N any nucleotide) (Crick, 1968; Eigen *et al*., 1979) for a comma-less code based on physicochemical properties of nucleic acids and optimal codon-anticodon interactions, which was also supported by the statistical analysis in present rRNAs (Eigen, 1985). It has been noticed that RNY patterns are abundant in current genes. This is often interpreted as the molecular track of a primeval comma-less code. However, Wong *et al*. (1986) argued on the basis of mutational rate consideration that observed RNY preferences should not have survived by random mutational events without selective interference.

The results mentioned above only involve the distribution of codons in DNA single strand. In fact, codon distribution also shows some characteristics connecting with biological function in double strands. Analyzed the codon distributions of sense and antisense strands in a polynucleotide double strand, Konecny *et al*. (1993, 1995) argued in quantitative terms that the genetic code is "adapted" to simultaneous coding of two proteins in both complementary nucleic acids, i.e. informational adaptation. For instance, silent mutations in the sense strand correspond to conservative ones in the antisense. It is also not difficult to see that codons for hydrophobic amino acids in one strand are complementary to codons for the hydrophilic amino

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acids in another, and codons for charged (hydrophilic) amino acids are prevalently complemented by codons for uncharged. This structure of genetic codons results from the functional pseudo-symmetry of the triplets (Konecny, 1995).

mRNA is the direct template of protein synthesis, so it should be more significant to study the corresponding relation between mRNA sequence and the encoded protein because of the existence of the non-coding intervening nucleotide sequence in eukaryotic DNA. RNA molecule can fold back onto itself in the structure stabilized by hydrogen bonds between complementary bases. The biological functions of RNA molecules are always associated with their fold structures. Such a fold structure, i.e.secondary structure, contains stems (base-pairing or double regions) and loops (single-stranded regions). The stem of RNA secondary structure is similar to a DNA double strand, except G pairing with U in RNA. Some elementary studies on codon distributions in RNA secondary structure have been made (Hasegawa *et al.*, 1979; White *et al.*, 1972; Konecny, 1995), which reveal some structural features of codons and the encoded amino acids. However, these work only considered the situation of "triplet-codon pairing" actually, namely the three bases of a codon in one strand of a stem just pair with the three bases of another codon in the complementary strand. So it was concluded that RNY pattern codons are mainly situated in stem regions and the silent mutations in one strand of a stem correspond to the conservative ones in the complementary strand, similar to those in DNA. In fact, the stems of an RNA secondary structure do not always be paired by "triplet-codon". However, they are paired by base complementary. Therefore more detailed studies are needed about the distribution of codons, the influences of silent mutations and other structural features in RNA secondary structure.

Recently, we have made a comparative study on the secondary structures of some mRNAs and the three-dimensional structures of the encoded proteins. Some positive correlation has been found (Zhang *et al.*, 1997). This result suggests that mRNA perhaps carries three-dimensional genetic information (spatial structural information) besides one-dimensional linear information. In order to further understand how the three-dimensional information is contained in an mRNA secondary structure and how it is transferred to protein, we analyzed the secondary structures of 86 mRNAs which encode the mature peptides. Some distribution features of the triplet codons in mRNA secondary structures were found. They appear to be in agreement with the conformational properties of the coded amino acids. Here the conformational property of an amino acid means the conformation in protein taken by the amino acid and its backbone. This result is in favor of the conclusion of the correlation between the three-dimensional structures of mRNA and protein.

1 mRNA samples and their secondary structures

The predominantly used thermodynamic folding algorithm for the secondary structures of all kinds of RNAs was originally proposed by Zuker *et al.* (1981). The prediction accuracy of Zuker's algorithm is higher as far as the current knowledge, especially to smaller RNA molecules. For example, the accuracy for tRNA is 96%, and for 5S rRNA is 88% (Jaeger *et al.*, 1989; Zuker, 1989; Zuker *et al.*, 1991). It is obvious that the prediction accuracy is higher as RNA is smaller. We also used Zuker's algorithm and PCFOLD program to predict the secondary structure of mRNA. PCFOLD program has a limitation of nucleotide length<

425, so we have to choose mRNA samples according to this limit.

mRNA samples were mainly taken from EMBL nucleotide sequence database released 37 December 1993, involving mammals, invertebrates, vertebrates, synthetics and NCBI backbone. We only considered the mRNA sequences which encode the mature peptides and whose nucleotide lengths are less than 425, so 67 samples have been collected in this database. In addition, we have also collected 19 samples in GenBank database released by December 1996, and they have been used to make the comparative analysis of the three-dimensional structures with their corresponding proteins. The nucleotide lengths of about 70% of the 86 samples are less than 300. Therefore, the prediction accuracy of their secondary structures should be higher.

2 Loop codons and stem codons in an mRNA secondary structure

Every mRNA sample was folded with PCFOLD program to obtain its secondary structure. All triplet codons of every sample were sorted into stem codons and loop codons according to their locations in the mRNA secondary structure. By a stem codon we mean that its three bases are all paired and there is no any bulge base in the complementary region. All other triplet codons are called loop codons. It should be mentioned that the codons whose bases are paired completely but with bulge bases in complementary (such as the codon UCC at 3'end in Fig.1) are defined as loop codons, because RNA backbone occurs bending at this location besides the normal propeller-like twist. From our study of the correspondence between the three-dimensional structures of mRNA and protein, loop codon regions in mRNA secondary structure are generally related with the turns or the connecting peptides of the protein (Zhang *et al.*, 1997; Liu *et al.*, 1998).

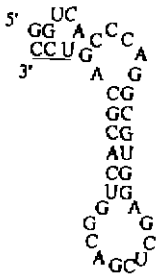

Fig.1 A segmet of the mRNA secondary structure of 1EXG

3 The distributions of all kinds of triplet codons in mRNA secondary structures

Eigen *et al.* (1985) grouped triplets in 5S rRNAs into RNY, RNR, YNY and YNR, and found their occurring frequencies as following: RNY > RNR > YNY > YNR. We found that this relation is primarily remained in mRNAs after analyzing 86 samples, see Table 1, where we grouped codons in more detail than Eigen. But to different species, there are some differences. It can be seen from Table 1 that the occurring frequencies of RNR pattern are higher than RNY's in mammals and vertebrates; the frequency of YNY is higher than RNY's in vertebrates; and the frequency of YNR > YNY in synthetics. These differences maybe result from the evolution of species.

The distributions of all patterns of the triplets in the nucleotide sequences have supplied a lot of meaningful information, which are helpful for us to understand the origin and the structures of genes. However, considering the actual state (fold structure) of RNA molecules in living cells, the location distribution of every pattern of the triplet codons in the secondary structures of mRNAs should also be considerable. Our analysis shows that some meaningful distribution features do exist, which seem to be just related to the rules establishing RNA secondary structures. Table 2 lists the ratio of the two numbers for every pattern of codons locating in the loop regions and in the stem regions respectively, which is called the loop-stem ratio. The loop-

stem ratio, to a certain extent, reflects the probability for a codon locating in the stem region or in the loop region. The bigger the loop-stem ratio is, the bigger the probability for a codon locating in the loop regions is; or vice versa. According to our above definition of a loop-codon and a stem-codon, the loop-stem ratio of all triplet codons in an RNA secondary structure is generally bigger than one. To our 86 samples, it is 2.34 on the average. It can be seen from Table 2 that the ratios have some differences to different species. The loop-stem ratio of RRR pattern is highest in the overall result, next is RRY. The loop-stem ratios of RRR in mammals and in synthetic are also higher. However, the loop-stem ratio of RRR is lower than the loop-stem ratios of YYY and RYY in invertebrates; YYY's is highest and those of RRY and YRY are all lower than the average.

From the statistical results, the loop-stem ratio of the codon seems to be influenced by its occurring frequency to some extent. In overall, the loop-stem ratios of the triplets primarily increase with the increment of their occurring frequencies (Fig.2). But the increment rate is small when the frequencies are less than 15.74. The increment rate of loop-stem ratio becomes big when the frequencies > 15.74. That is, the loop-stem ratios of RRY, RYY and RRR are associaed with their frequencies closely. The situation in mammals is almost similar to that in overall. Most of the curve of vertebrates is in agreement with that of overall. However, in invertebrates and synthetics, there are bigger deviations compared with other species in the middle parts, especially in synthetics (Fig.2).

4 The locations of triplet codons in mRNA secondary structures and the conformational properties of amino acids

Synonymous codon usage biases to various secondary structures in proteins has been detected (given in elsewhere). Therefore, it should be needed to consider the effect of codon usage in studying protein folding, not only to consider amino acids itself. Here, combined with the conformational properties of amino acids, we investigate the distributions of codons in mRNA secondary structures. 61 triplet codons are grouped into 5 sets according to the loop-stem ratios of individual codons (Table 3). Generally, if the loop-stem ratio of a codon is lower, the codon is frequently located in stem region of mRNA secondary structure; inversely, if the loop-stem

Table 1 Occurring frequencies (%) of all kinds of codons in the mRNAs of different species

Species	RRY	RYY	RRR	RYR	YYY	YRY	YYR	YRR
Overall①	16.74	15.74	17.57	10.58	11.93	11.88	9.2	6.35
MAM	16.17	13.61	22.86	11.28	12.59	8.71	7.78	7.00
INV	16.79	17.44	13.76	11.05	13.00	14.84	7.58	5.53
VRT	13.57	13.31	17.29	11.86	12.56	17.78	8.27	5.36
SYN	16.76	14.86	17.71	8.19	10.09	8.76	14.48	9.14

①MAM denotes mammals, INV invertebrates, VRT vertebrates and SYN synthetics.

Table 2 Ratio of the numbers for every pattern of codons locating in loops and in stems (loop-stem ratio)

Species	RRY	RYY	RRR	RYR	YYY	YRY	YYR	YRR	Average
Overall	2.59	2.19	3.54	2.07	2.09	2.07	2.30	1.89	2.34
MAM	3.33	1.69	4.25	2.02	1.61	2.61	2.03	2.21	2.47
INV	1.98	3.60	2.85	3.64	4	1.40	2.68	2.64	2.85
VRT	2.52	2.64	2.97	1.81	2.16	1.97	2.20	1.36	2.21
SYN	3.89	1.79	5.64	2.07	3.82	2.29	4.43	2.43	3.30

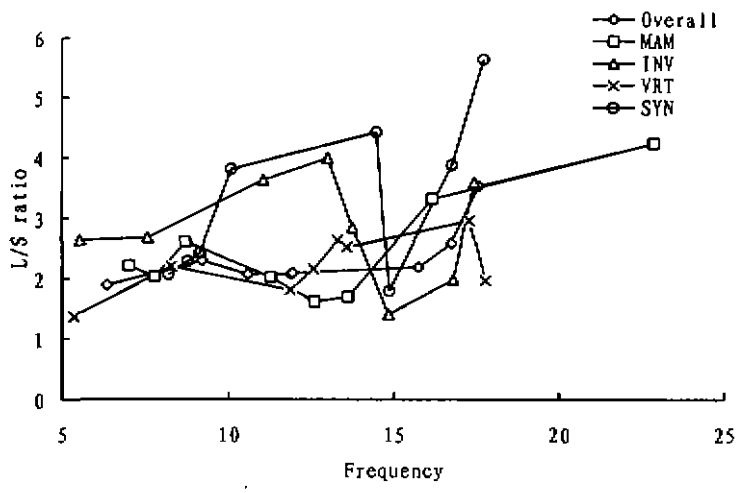

Fig. 2 The relationship of loop-stem ratios of all kinds of pattern codons and their occurring frequencies

ratio of a codon is higher, the codon is frequently located in loop region.

At first, putting the first two sets together, we analyze the case of L/S≤2.34 (the average value of all samples, Table 2). It has been found that most of the codons with L/S≤2.34 encode the amino acids that are hydrophobic or prone to be inside proteins. It is well known that Phe, Leu, Val, Ala and Gly are hydrophobic amino acids and that Trp has the hydrophobic trend although it is of polarity (Lai *et al*., 1993). It can be seen from Table 3 that the amount of their codons come to about 61% in this set of codons. The loop-stem ratio of Trp is the lowest (0.83), i.e. the frequency is high for its bases occurring in stem regions. In addition, Glu generally forms salt bridge and Cys forms disulfide bond inside proteins. Therefore, summed up, about 71% of the codons with L/S≤2.34 encode the amino acids with hydrophobicity or the trend situated inside proteins. Arg, Ser and Gln are hydrophilic amino acids; their codons are only about 29% in this set of codons. However, it should be noted that Arg has hydrophobic trend although it is charged (Lai *et al*., 1993). So the proportion of codons coding for the amino acids with hydrophobicity or the trend situated inside proteins amounts to 86%.

The situation is just reverse in the set of codons with L/S>4, i.e. most of these codons encode the amino acids that are hydrophilic or prone to be located at the surfaces of proteins. About 85% of this set of codons code for Asn, Thr, Gln, Tyr, His, Lys and Arg, which are of polarity or charged (i.e. hydrophilic). The codons of Leu, Ile are only about 15%.

In the set of codons with 2.34<L/S≤3, codons UUA, CUU, AUU, AUC, CCC, CCA and CCG code for hydrophobic amino acids Leu, Ile and Pro. The codons coding for hydrophilic amino acids are AGU, AGC (Ser), ACG (Thr), GAC (Asp) and CGA (Arg) (Asp and Arg are polar). In the set of codons 3<L/S≤4, the codons coding for hydrophobic amino acids are CUC (Leu), AUG (Met) and CCU (Pro); and the codons coding for hydrophilic amino acids are UCA (Ser), UAU (Tyr), CAC (His), GAU (Asp) and GAA (Glu) (Asp and Glu are polar). It can be easily seen that half of the codons whose loop-stem ratios are between 2.34 and 4 code for hydrophobic amino acids, and another half of them code for hydrophilic or polar amino acids. Of course, Arg and Glu have the trend situated inside pro-

teins.

Table 3　Codons and corresponding amino acids classified according to the loop-stem ratios of codons

Loop-stem ratio (L/S)	Triplet codon (s)	Amino acid	Loop-stem ratio (L/S)	Triplet codon (s)	Amino acid
L/S≤1	UCG	Ser	2.34<L/S≤3	GAC	Asp
	UGG	Trp		CGA	Arg
	GGU	Gly	3<L/S≤4	CUC	Leu
	CGG	Arg		AUG	Met
1<L/S≤2.34	UUU, UUC	Phe		UCA	Ser
	UUG, CUG	Leu		CCU	Pro
	GUU, GUC, GUA, GUG	Val		UAU	Tyr
	UCU, UCC	Ser		CAC	His
	GCU, GCC, GCA, GCG	Ala		GAU	Asp
	CAG	Gln		GAA	Glu
	GAG	Glu	L/S>4	AAU, AAC	Asn
	UGU, UGC	Cys		CUA	Leu
	AGG, CGU, CGC	Arg		AUA	Ile
	GGC, GGA, GGG	Gly		ACU, ACC, ACA	Thr
2.34<L/S≤3	UUA, CUU	Leu		CAA	Gln
	AUU, AUC	Ile		UAC	Tyr
	AGU, AGC	Ser		CAU	His
	CCC, CCA, CCG	Pro		AAA, AAG	Lys
	ACG	Thr		AGA	Arg

5 Discussion

As far as genetic code is concerned, many rules have been found in it, such as the rules of the degeneracy. In addition, there are also some meaningful patterns of base distributions related to the properties of amino acids. For examples, the codons whose middle bases are U encode hydrophobic amino acids, and the codons whose middle bases are A encode hydrophilic amino acids. Some of these rules have been explained from the stereochemical theory. From DNA or mRNA linear sequences, the distributions of codons also show some characteristics, such as the 3-periodicity (Voss, 1992) in coding sequences and the bias of the codon usage (Lió, 1994). All of these rules suggest that the assignment and the distribution of codons in genes are not random. In another hand, the non-random distribution of codons is also embodied in the three-dimensional structures of nucleic acids molecules. This is noticed in the pseudo-symmetry of the function in DNA double strands (Konecny, 1995). Mature mRNA is a direct template of protein synthesis. It is generally thought that mRNA only provide the linear information to the protein until now. However, mRNA has a specific fold structure. The functions of RNAs are associated with the three-dimensional structures.

In the consideration of mRNA secondary structure, generally, a triplet codon can occur at any location in an RNA secondary structure. Considering the conventional base-pairing rule of RNA (G≡C, A=U and G−U) and the base-pairing stacking interaction which influence the free energy of the RNA secondary structure, there is a certain location bias for every codon in

the secondary structure. We can roughly calculate the probability of every codon occurring in stem region or in loop region. Suppose $N_1N_2N_3$ (N_i denotes any base, $i = 1, 2, 3$) is a triplet codon, only the base-pairing form is concerned, its probability occurring in stem region (three bases are paired) can be calculated with

$$P_s\ (N_1N_2N_3) = P\ (N_1)\ P\ (N_2)\ P\ (N_3) \qquad (1)$$

where

$$P\ (N_i) = \begin{cases} 1/3, & \text{if } N_i = \text{A or C} \\ 2/3, & \text{if } N_i = \text{G or U} \end{cases}$$

However, we should note that the interactions of base-pairs are not the same to different base-pairs, so are the stacking interactions of the paired bases. For example, G≡C pairing is more stable than A=U pairing; A=U pairing is more stable than G−U pairing. In addition, there are also some other factors, such as the occurring frequencies of codons, to influence the location of the codons in mRNA secondary structure. So it is necessary to consider these interactions and the influence factors in order to reflect the probability more accurately. This can be achieved by adding certain weights. Thanks to the thermodynamic data available, the weight concerning the interactions and the stacking of base-pairing can be determined easily. But the weight concerning the other factors is more complex. These problems will be discussed elsewhere in detail.

According to (1) and combined with base-pairing stacking energy (Turner *et al.*, 1987), we can roughly estimate the probability of some codons occurring in stem region or in loop region. For instance, the codons containing base "A" should occur in loop regions more frequently. And the more a codon contains the base "A", the smaller the probability for it locating in the stem region is, or the bigger the probability for it locating in the loop region is. Similarly, the more a codon contains the base "G", the bigger the probability for it locating in the stem region is. This is in good agreement with the statistical result (Table 3). The loop-stem ratios of AAA and AAG (Lys) are 10.15 and 6.09 respectively; the loop-stem ratios of AAU and AAC (Asn) are 5.67 and 7.41 respectively; and that of GUU (Trp) is 0.82, etc.

In RNA secondary structure, the stem region is comparatively stable, inversely, the loop region is flexible and has a bigger contacting area with solvent. It is known that the conformational property of Lys is flexible and prone to be on the surfaces of proteins. The conformational property of Asn is prone to be in the turn regions of proteins. And Trp has the position trend inside proteins. So the location distributions of these codons are accordance with the conformational trend of the corresponding amino acids.

Our result shows that the distributions of the codons in mRNA secondary structures are not random. These non-random distribution regulations are associated closely with the conformational properties of the amino acids. This supports our conclusion that there is correlation between the three-dimensional structures of mRNAs and proteins. From the viewpoint of information transfer, it is suggested that the conformational properties of amino acids maybe result from the distribution features of the codons in mRNA secondary structure.

It should also be mentioned that there are two particular codons which encode hydrophobic amino acids in the case of loop-stem ratio>4. The codon CUA (Leu) and AUA (Ile) have higher loop-stem ratios (15 and 4.4 respectively) and they occur in the lowest frequency in

their cognate codons (encoding Leu and Ile respectively) in our samples. In effect, they just belong to the suppressed codons defined by Lió *et al*. (1994) according to the terms of strong and weak bases. It is known that the synonymous codon usage of an amino acid has bias. This bias is reflected in the secondary structures of proteins in particular. This result suggests that the codon usage bias may be related to the structural need of the protein. More detailed study on this aspect will be given in another paper.

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三联体密码子在mRNA二级结构中的分布

张 静[①②] 顾宝洪[③] 彭守礼[④] 刘次全[②③]

(①云南大学统计系 昆明 650091)

(②中国科学院昆明动物研究所细胞与分子进化开放研究实验室 昆明 650223)

(③云南大学现代生物中心 昆明 650091)

(④云南大学物理系非线性中心 昆明 650091)

摘 要 对编码成熟肽的mRNA二级结构的分析显示，每个密码子在mRNA二级结

构中的位置有一定的倾向性，这种倾向性似乎与相应氨基酸的构象性质相一致。大多数编码疏水氨基酸的密码子位于mRNA二级结构中较稳定的茎区；反之，大多数编码亲水氨基酸的密码子位于柔性的环区。这个结果支持了最近得到的关于mRNA与蛋白质之间存在着三维结构信息传递的结论。

关键词 三联体密码子，氨基酸，mRNA二级结构
中图分类号 Q522.2，Q755

高黎贡山小熊猫生态对策的初步研究

A PRELIMINARY STUDY ON THE BIONOMIC STRATEGIES OF THE RED PANDA IN GAOLIGONG MOUNTAINS

关键词 小熊猫，生态对策，高黎贡山
Key words Red panda (*Ailurus fulgens*), Bionomic strategies, Gaoligong mountains
中图分类号 Q959.838

小熊猫（*Ailurus fulgens*）产于喜玛拉雅－横断山脉地区，是地史保存下来的第三纪孑遗动物。由于长期的生存竞争和自然选择结果，使其营养生态位由高质量、竞争大的肉食转向低质量、竞争小但营养成分稳定的竹类。其头骨、臼齿和前掌都已特化，适于咀嚼和抓食竹子。但由于其消化道仍是典型的食肉型，只能从竹子细胞的可溶性物质中获取能量。而竹子的植物纤维含量高，细胞可溶性物质含量低，小熊猫必然要采取某种优化的生态对策来保证自身生存、繁衍的能量需要。本研究旨在从生境和食物的选择方面对该地区小熊猫的生态对策进行探讨。

1 研究地点

高黎贡山自然保护区位于云南西部98°34′～98°50′E，24°56′～26°09′N，北接青藏高原东缘，南北走向。受青藏高原的屏障作用，该地区5月至10月上旬为雨季，10月下旬至次年4月为旱季。山体切割剧烈，海拔1 090～3 916 m。植被从干热河谷稀树灌丛、常绿阔叶林、针阔混交林、高山暗针叶林、高山灌丛草甸直至流石滩。

限于人力和经费，经过预研，研究区域选在片马丫口附近，海拔2 800～3 400 m约30 km^{2}的区域内。该区域地势复杂陡峭，气候随海拔、坡向变化较大。丫口年均气温7℃，最高12.5℃（8月），最低－0.5℃（2月），12月至次年3月为积雪期。植被主要有：(1)温凉性针叶林，分布于海拔2 800～3 200 m。乔木主要是云南铁杉（*Tsuga dumisa*）、华山松（*Pinus armandi*），呈零星片状分布，间有栎类、桦木及冷杉，成层现象明显。林下灌丛有箭竹、杜鹃，盖度随林形和地势而异，一般达50%，高者可达90%。(2)寒温性灌丛，分布在海拔3 200～3 400 m，以箭竹、花楸和杜鹃为主，其上层间有苍山冷杉（*Abies delavayi*），下层草本多为蕨类与禾本科植物。

研究区域内小熊猫所食竹类为：(1)矩鞘箭竹（*Fargesia arbiculata*），分布在海拔3 000～3 400 m，高1.5～2.5 m，密度5～66株/m^2，并随海拔上升而增高。(2)云龙箭竹（*F. papyrifera*），分布于海拔1 800～3 200 m，高2～5 m，密度4～12株/m^2。

2 研究方法

沿山脊在东西坡海拔2 800～3 400 m间分别设4条样带，共8条。按一定时间间隔（约2个月）沿样带搜巡并记录各样带上小熊猫活动痕迹（采食残迹、粪便）及相应生境情况（海拔、坡度、竹子密度、

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