

# The genetic diversity of central and peripheral populations of ratlike hamster (*Cricetulus triton*)

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**Abstract** The impact of habitat fragmentation and isolation on the genetic diversity of populations has attracted much attention in studies of meta-population and conservation biology. In this work, using the randomly amplified polymorphic DNA (RAPD) technique, we studied the genetic diversity of central, peripheral and peninsular populations of ratlike hamster, which were collected in five locations of the North China Plain and its surrounding areas, in 1999. The study revealed that, i) the genetic diversity of central population of Raoyang County > the sub-central populations of Gu'an County and Taikang County > the peripheral population of Shunyi District > the peninsular population of Mentougou District; ii) the genetic diversities of the five populations were positively correlated to the nearest distances to the peripheral line of population distribution; iii) there were significant differences of gene frequencies of some RAPD fragments among the five populations. More RAPD fragments disappeared in peripheral populations than in central or sub-central populations. The frequencies of two RAPD fragments were correlated to the latitude. This study clearly indicated that the variation of the genetic diversities of the five populations was caused by edge effect and fragmentation through the enhanced inbreeding and genetic drift, and thus supported the view that habitat fragmentation and related edge effect reduce the population genetic diversity.

**Keywords:** *Cricetulus triton*, genetic diversity, geographical variation, edge effect, ecological adaptation, RAPD.

Habitat fragmentation and isolation under the human disturbance and destruction have become a serious problem in wildlife conservation. The effect of such fragmentation and isolation on the population genetic diversity is still unknown, and is the hot spot in conservation biology<sup>[1]</sup>. Habitat fragmentation and isolation often result in central, peripheral and peninsular populations. There are two opposite views about the genetic diversities in central and peripheral populations. The first view is that, because the peripheral population is small, isolated and at the edge of suitable habitat<sup>[2-4]</sup>, and thus suffers inbreeding and genetic drift, the genetic diversity of the peripheral or isolated populations will be reduced. Eldridge et

al.<sup>[5]</sup> also believed that the genetic diversity of isolated population was small. Many prior studies showed that the genetic diversities of large populations were higher than small ones<sup>[6-8]</sup>, but there were also opposite examples<sup>[9]</sup>. Generally, the genetic diversities of endangered species are low, which often accelerates their extinction process<sup>[10,11]</sup>, but there are also few exceptions, e.g. the northern Elephant seals (*Mirounga angustirostris*) of west Pacific of America<sup>[12]</sup>. Another view is that, the peripheral population suffers high natural selection, and experiences various natural selection<sup>[4,13-17]</sup>. Strong selective pressure would increase the genetic diversities of populations, and these populations would be the sources for producing new species and should be well preserved as a priority<sup>[4,16,17]</sup>. Petit et al.<sup>[18]</sup> discovered that the genetic diversities of peripheral and central populations of a tree *Argania spinosa* in Morogo was very complicated; for small peripheral populations, the genetic diversities were smaller, but still high for large peripheral populations. Therefore, the genetic diversity of peripheral and central populations is worth studying further, and more experiments are badly needed.

Recently, studies on conservation genetics are no longer restricted to endangered species. In order to reveal the pattern and process of genetic diversity and conservation related principles, non-endangered species were encouraged to be used for genetic study<sup>[19]</sup>. Because the number of endangered species is often small and it is hard to collect their samples, they are very limited for genetic study. It is also suitable to use small animals as models to study the effect of habitat fragmentation and isolation on genetic diversity. In this work, using the RAPD technique<sup>[20-24]</sup>, we studied the genetic diversity of central, peripheral and peninsular populations of ratlike hamster (*Cricetulus triton*), aiming to test the two opposite hypotheses as to the effect of habitat fragmentation and isolation on population genetic diversity.

## 1 Materials and methods

(i) Distribution of ratlike hamster. The ratlike hamster is widely distributed in the farmland of North China, and also in southern Siberia of Russia and Korea<sup>[25]</sup>. There are 5 sub-species of ratlike hamster, i.e. *C. t. triton*, *C. t. fuscipes*, *C. t. collinus*, *C. t. incanus*, and *C. t. canus*. The body mass of adult hamster is usually 80—160 g. The life span of the hamster is about one year, and it often breeds from March to August with 1—3 litters a year, and the litter size ranges from 2 to 22, often 9 to 10<sup>[26]</sup>. The hamster prefers to live in dry habitats, mainly inhabits farmland of plain, hill or valley, and also appears in the shrubs, forest plantation, wasteland or grass nearby farmland.

## NOTES

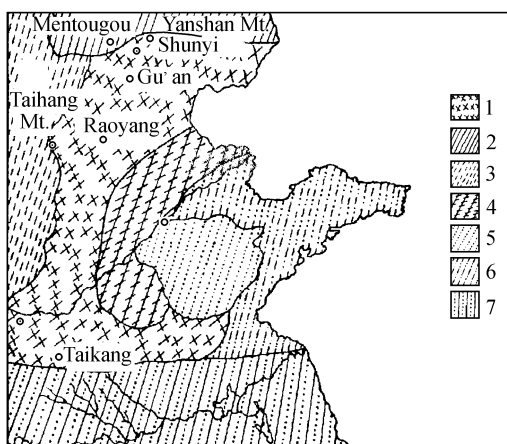


Fig. 1. The rodent community of North China Plain and its surrounding areas. 1, Ratlike hamster + Striped hamster + Striped mouse (main distribution area of the hamster *C. t. triton*); 2, Field mouse + White-bellied rat + Striped mouse + Ratlike hamster; 3, White-bellied rat + Field mouse + Striped mouse + Ratlike hamster; 4, Striped hamster + Ratlike hamster; 5, White-bellied rat + Striped hamster + Striped mouse; 6, Striped hamster + Striped mouse; 7, Striped mouse + Striped hamster + Ratlike hamster.

The study area covers the distribution area of the sub-species *C. t. triton*, i.e. the North China Plain and its surrounding areas (fig. 1). According to the classifications of rodent pest community by Ye et al.<sup>[27]</sup>, Lu et al.<sup>[28]</sup> and Hebei Plant Protection Station et al.<sup>[29]</sup> as well as other materials, the rodent community in North China Plain and its surrounding areas is shown in fig. 1. In the Mentougou area, the hamster only habits in the farmland or surrounding shrubs of the Yanshan Mountain valleys, being connected to the plain population through a narrow corridor of valley farmland. Field mouse (*Apodemus peninsulae*) and White-bellied rat (*Rattus confucianus*) are dominant species, making up to 80%—90% of the rodents caught by wooden snare-trap. Thus, the hamster population in this region is a typical peninsular population. The North China Plain is surrounded by the Yanshan Mt. in the north, by the Beitaihang Mt. in the west. Thus a clear line exists between the plain and the two mountains, which is also the edge line of ratlike hamster population. The rodent community outside of the line is dominant by Field mouse and White-bellied rat. The Shunyi district is located

nearly the edge line, only 15 km away from the edge line, and obviously the community is defined as a peripheral population. Gu'an County is 50 km away from the edge line, and the community is defined as sub-central population. Raoyang County is 130 km away from the edge line, and historically has been an area with frequent outbreaks of hamster. The Ratlike hamster is dominant, and the Striped hamster (*C. barabensis*) ranks the second. The population of this area is defined as a central population. The south edge line of *C. triton* is roughly at the north up streams of the Huaihe River with wet conditions, not suitable for hamster to live. The Striped mouse (*A. agrarius*) is the dominant species. Taikang County, 60 km from the edge line, is defined as a sub-central area.

(ii) Sample collection. From September 20 to October 5, 1999, 200—300 live traps were set in a 2—3 km transect line in five locations, including Mentougou District of Beijing, Shunyi of Beijing, Gu'an County of Hebei Province, Raoyang County of Hebei Province, and Taikang County of Henan Province (table 1). The traps were evenly set along the transect for 3 consecutive nights. The distance to the edge lines are defined as the minimum distances of the locations to the edge line of the main distribution region of *C. t. triton*. The fresh liver tissues were collected for extracting DNA.

(iii) Experimental conditions. The total DNA was extracted from about 50 mg fresh liver tissue of a ratlike hamster by referring to Salah et al.<sup>[30]</sup> with a slight modification. The extracted DNA was preserved at 4°C. The primers were bought from the Operon Co. The RAPD reaction was set by following Williams et al.<sup>[31]</sup> with a slight modification. The reaction volume was 25 µL, including Tris-HCl 10 mmol/L, pH 8.3; KCl 50 mmol/L; MgCl<sub>2</sub> 1.5 mmol/L; 4 kinds of dNTP each with 100 µmol/L; random primers each of 8 µmol/L; genome DNA of about 25 ng; Taq polymerase 1 U; a drop of mineral oil was added to seal the sample vessels. PCR was carried out in a programmable thermal controller type 9600 (Perkin-Elmer). The amplification profile was 5 min at 94°C, followed by 40 cycles. Each cycle consisted of 1 min denaturation at 94°C, 1 min annealing at 36°C and 2 min extension at 72°C. After the last cycle, there was a 10 min

Table 1 Samples of *C. triton* in this study

Site	Habitat	Longitude/(°)	Latitude/(°)	Distance to edge line/km	Trap success (%)	Sample size	Proportion of juvenile and sub-adult (%)	Proportion of females (%)
Mentougou	valley farmland	115.51	39.96	0	1	13	53.8	69.2
Shunyi	farmland	116.65	40.14	15	16	25	24.0	56.0
Gu'an	farmland	116.34	39.25	50	7.2	25	68.0	48.0
Raoyang	farmland	115.69	38.14	130	1.53	18	72.2	50
Taikang	farmland	114.86	34.07	60	3.3	11	63.6	63.6

extension at 72°C. Amplification products were preserved at 4°C, and were analyzed by electrophoresis in 1.4% agarose and detected by staining with 0.05% ethidium bromide.  $\lambda$  DNA/*EcoR* I + *Hind* III was used as the molecular marker. Three hours after electrophoresis at 50 V, the results were photographed on a UV instrument.

(iv) Data analysis. The proportion of polymorphic loci, Shannon index and Nei index were commonly used to describe the genetic diversity of populations. The proportion of polymorphic loci is the proportion of an allele whose gene frequency is smaller than or equal to 0.99<sup>[32]</sup>. Supposing an RAPD loci has two alleles, according to the Hardy-Weinberg rule, by using Kongkiatngam et al.'s method<sup>[32]</sup>, the recessive or dominant gene frequencies were estimated. Based on these gene frequencies, the genetic variation, genetic evenness within populations ( $H_{pop}$ ) and total genetic variation ( $H_{sp}$ ) were calculated by Shannon index and Nei's index. SPSS For Windows Version 8.0 was employed for statistic analysis.

### 3 Results

(i) Genetic difference in central, peripheral and peninsular populations. The proportion of polymorphic loci, Shannon index and Nei index of the five populations are shown in fig. 2. The proportion of polymorphic loci of Mentougou, Shunyi, Gu'an, Raoyang and Taikang populations are 0.594, 0.720, 0.684, 0.855 and 0.726, respectively; the loci numbers are 41, 54, 52, 65 and 53, respectively. By statistics, the proportion of polymorphic loci between Shunyi population and Mentougou populations ( $p = 0.04$ ); Mentougou and Raoyang populations ( $p = 0.000$ ), Taikang and Raoyang populations ( $p = 0.037$ ) and Gu'an and Raoyang populations ( $p = 0.025$ ) differed significantly; but not significant among the other populations.

The Shannon indices of Mentougou, Shunyi, Gu'an, Raoyang and Taikang populations are  $4.868 \pm 2.616$ ,  $5.778 \pm 2.413$ ,  $6.118 \pm 1.442$ ,  $7.642 \pm 1.666$  and  $6.253 \pm 2.316$  respectively; Nei's indices are  $0.193 \pm 0.108$ ,  $0.228 \pm 0.102$ ,  $0.250 \pm 0.070$ ,  $0.311 \pm 0.064$  and  $0.256 \pm 0.096$  respectively. By combining the results of the three diversity indices together, the genetic diversity of the central population in Raoyang > sub-central populations in Gu'an and Taikang > peripheral population in Shunyi > peninsular population in Mentougou. The results of 7 primers showed similar trends with good replications.

Based on the result of Shannon index, the most genetic variation was within population (71.2%), and a small portion of variation among populations (28.8%). Based on the result of Nei index, genetic difference among the five populations was  $0.271 \pm 0.073$ , i.e. 27.1% genetic variation was among populations, most variation was within populations (72.9%), which was similar to that revealed

by Shannon index (table 2).

(ii) Correlations between genetic diversity and distance to edge line of habitat. Fig. 3 indicates the relationship between the distance to edge line and the proportion of RAPD polymorphic loci, Shannon index and Nei index. Through statistical analysis, the distance to edge line was positively correlated to the proportion of RAPD polymorphic loci ( $r = 0.904$ ,  $p = 0.035$ ,  $n = 5$ ); to the Shannon index ( $r = 0.975$ ,  $p = 0.005$ ,  $n = 5$ ), and to the Nei index ( $r = 0.982$ ,  $p = 0.003$ ,  $n = 5$ ). This result further supported the above discovery that the genetic diversity in central population was higher than that of peripheral population. The correlations among three genetic diversities and the sample size, trap success, female ratio and age structure were not significant, indicating that they were not key factors in determining the genetic variation of the five populations, which further supported the observation that edge effects attributed these variations.

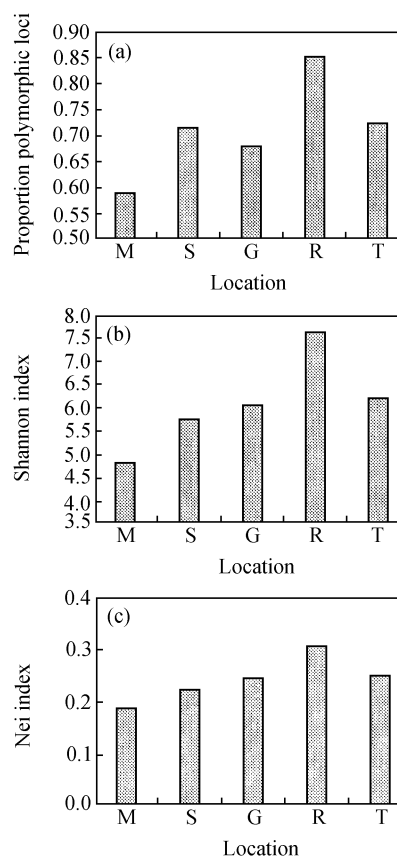


Fig. 2. The genetic diversity by the proportion of polymorphic loci, Shannon index and Nei index of the five locations. M, S, G, R, T represent Mentougou, Shunyi, Gu'an, Raoyang and Taikang, respectively.

(iii) Some RAPD loci frequencies of the five populations. The gene frequencies of some RAPD loci differed significantly among the five populations. In table 3, only RAPD fragments with significant difference of gene

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Table 2 Genetic differentiation among five populations of ratlike hamster

Index	Total genetic diversity, $H_{SP}$	Genetic diversity within population, $H_{POP}$	Proportion of genetic diversity within population, $H_{POP}/H_{SP}$	Proportion of genetic diversity among populations, $(H_{SP} - H_{POP})/H_{SP}$
Shannon	$8.492 \pm 1.755$	$6.128 \pm 1.759$	$0.712 \pm 0.086$	$0.288 \pm 0.086$
Nei	$0.336 \pm 0.065$	$0.248 \pm 0.071$	$0.729 \pm 0.073$	$0.271 \pm 0.073$

Table 3 Gene frequencies of some RAPD loci with significant difference among the five locations<sup>a)</sup>

Primer	Location					$\chi^2$ test among the five locations									
	Mentougou	Shunyi	Gu'an	Raoyang	Taikang	M-S	M-G	M-R	M-T	S-G	S-R	S-T	G-R	G-T	R-T
OPA04-A3	0.85	0.80	0.48	0.83	0.09		*		**	*		***	*	*	***
OPA04-A4	0.92	0.68	0.76	0.89	0.36				**					*	**
OPA04-A5	0.69	0.36	0.80	0.89	0.36					**	**			*	**
OPA04-A7	0	0	0.76	0.94	0.36		***	***	*	***	***	**		*	**
OPA09-B5	0	0.20	0.36	0.44	0.27		*	**	*						
OPA09-B6	0.77	0.80	0.40	0.28	0.36		*	*	*	**	**	*			
OPA18-C12	0	0.12	0.60	0.39	0.18		***	*		***	*			*	
OPB05-G11	0.23	0.12	0.36	0.56	0.82				**		**	***		*	
OPB05-G14	0.92	0.96	0.52	0.39	0.73		*	**		***	***	*			
OPH14-D2	0.54	0.16	0	0	0	*	***	**	**	*					
OPH14-D7	0.31	0.48	0	0	0		**	*	*	***	**	**			
OPH14-D11	0.85	0.80	0.52	0.17	0.27			***	**	*	***	**	*		
OPM06-E1	1.00	1.00	0.20	0.33	0.91		***	***		***	***			***	**
OPM06-E6	0	0	0.44	0.33	0.45		**	*	*	***	**	***			
OPM06-E7	0	0	0.44	0.17	0.27		**		*	***	*	**			
OPM06-E9	0	0	0.76	0.72	0.63		***	***	**	***	***	***			
OPY04-F5	0.15	0.08	0.52	0.33	0		*			**	*			**	*
OPY04-F8	1.00	1.00	0.84	0.56	1.00			**		*	**		*		*
OPY04-F12	0.62	0.24	0.76	0.83	0.27	*				***	***			**	**
OPY04-F17	0	0.16	0.28	0.33	0.27		*	*	*						

a) \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ . M = Mentougou, S = Shunyi, G = Gu'an, R = Raoyang, T = Taikang.

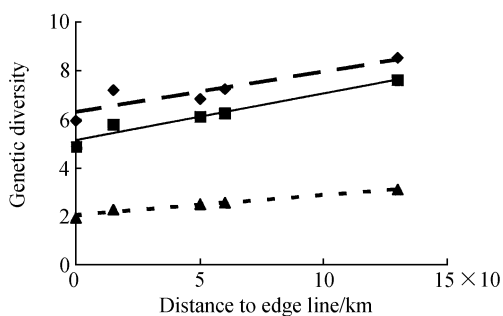


Fig. 3. The relationship among the distances of populations to the edge line ( $\times 10$  km), proportion of polymorphic loci ( $\times 10\%$ ), Nei index ( $\times 1/10$ ) and Shannon index.  $\blacklozenge$ , Polymorphic loci;  $\blacksquare$ , Shannon index;  $\blacktriangle$ , Nei index; ---, polymorphic loci; —, Shannon index; ···, Nei index.

frequency are listed. Some fragments were only found in some locations, e.g. fragments OPA04-A7, OPM06-E6, OPM06-E7 and OPM06-E9 were found in Gu'an, Raoyang and Taikang populations, but not in Mentougou and Shunyi populations; fragments OPH14-D2 and

OPH14-D7 were only found in Mentougou and Shunyi populations, but not in the other three locations; fragment OPY04-F5 was not found in Taikang population, but in the other four populations; fragments OPA18-C12 and OPY04-F17 were not found in Mentougou population, but in the other four locations.

There were only 2 or 3 fragments absent in central or sub-central populations in Raoyang, Gu'an and Taikang, but 4 fragments absent in the peripheral population in Shunyi; 6 fragments absent in peninsular population in Mentougou. This discovery further supported the above discovery that edge effects reduced the genetic diversity. However, it was noticeable that two new fragments, OPH14-D2 and OPH14-D7, were found in the peripheral populations which were not seen in central or sub-central populations.

Furthermore, we found that fragment OPA04-A3 was positively correlated to the latitude ( $r = 0.852$ ,  $p = 0.067$ ,  $n = 5$ ); while fragment OPB05-G11 was negatively correlated to the latitude ( $r = -0.950$ ,  $p = 0.013$ ,  $n = 5$ ) (fig.

4). No fragments were found to be significantly correlated to the latitude.

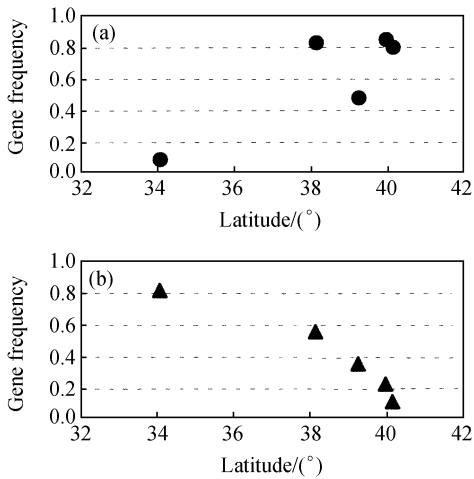


Fig. 4. The relationship between gene frequency and latitude. (a) OPA04-A3; (b) OPB05-G11.

#### 4 Discussion

At the community level, due to the edge effect, the species diversity of peripheral community is often higher than that of central ones<sup>[33]</sup>, but the genetic diversity in peripheral and central populations is still unknown<sup>[2-4,13-17]</sup>, which is the frontier of Meta-population Theory, Conservation Biology and Molecular Ecology.

The result of this study revealed that, the genetic diversity of *C. triton* was the highest in central population, higher in sub-central populations, lower in peripheral population and the lowest in peninsular population; the population genetic diversity was positively correlated to the distance of the population to its distribution edge line. This conclusion strongly supports the hypothesis that due to inbreeding and genetic drift, peripheral and isolated small population owns lower genetic diversity<sup>[2-4]</sup>. We found more absent RAPD fragments in peripheral or peninsular population, and thus obtained direct evidence of genetic loss due to inbreeding and genetic drift (table 3). Though this observation did not support the other hypothesis that the strong environmental pressure and verified selective pressures increase the genetic diversity<sup>[4,14-16]</sup>, the view of the hypothesis that strong selective pressure may produce new genes, and then help species differentiation may be correct<sup>[4,17]</sup>. This is because in this study we found two new RAPD fragments in peripheral and peninsular populations, which were not found in central population. These two fragments might be the result of adaption to mountain environment because they were not found in the south peripheral population.

We also found that the frequencies of two RAPD loci were much correlated to the latitude. The frequency of fragment OPA04-A3 was higher in high latitude; while the

frequency of fragment OPB05-G11 was lower in high latitude. We speculated that these two fragments might be related to adaptation to temperature ingredient along the latitude. In fact, the temperature varied greatly among the five locations, e.g. the yearly temperature averages in Mentougou, Shunyi, Gu'an, Raoyang and Taikang are 7°C, 11.5°C, 12°C, 12.2°C and 14°C, respectively. The impact of precipitation would be small because the regional difference was not large. The annual precipitations in Mentougou and Raoyang were relatively small, ranging from 500 to 550 mm; the annual precipitation in the other locations were from 600 to 650 mm. Slatkin<sup>[34]</sup> believed that the difference of gene frequencies indicated that these genes experienced a different selection and variation. The biological reason of these differences is unclear unless further studies are carried out by using molecular techniques. Ehrlich and Roughgarden<sup>[35]</sup> reported that, there were two LDH alleles in a fish (*Fundulus heteroclitus*) living in the semi-salt coastland of West Pacific of America, which changed along latitude, one dominant in south waters, and the other dominant in north waters. It was proved that the LDH enzymes coded by these two alleles were adaptive to water temperature.

The RAPD technique is a widely used gene marker method<sup>[36,37]</sup>. It is hard to identify the heterogeneity of a RAPD band without resorting to other techniques. It is impossible to get the gene frequency just depending on the RAPD bands. However, by referring to the method put forward by Kongkiatngam et al.<sup>[32]</sup>, the allele gene frequency can be calculated. Lewontin<sup>[38]</sup> first used Shannon index for studies on human genetic diversity. Chalmer et al.<sup>[39]</sup> first used it for analyzing RAPD data. Wei et al.<sup>[40]</sup> estimated the Shannon index of both phenotype and gene type by using RAPD data through calculating the gene frequency by referring to Kongkiatngam et al.<sup>[37]</sup>. They concluded that the Shannon index of gene type estimated by using the Kongkiatngam et al.'s method was more reasonable than the index of phenotype frequency. In our study, we estimated the gene frequencies first by using Kongkiatngam et al.'s method, and then to estimate the Shannon index and Nei index by using these gene frequencies. The result indicated that both indices gave similar trends of genetic diversity of the five locations. However, the Shannon index seemed more sensitive. Thus the Shannon index is quite reliable in estimating the genetic diversity. Nei<sup>[41]</sup> suggested that the proportion of polymorphic loci was some arbitrary, the Nei index was better than the proportion of polymorphic loci. In our study, we found that the proportion of polymorphic loci showed similar trends to the Shannon index and Nei index, and more similar to the Shannon index. Therefore, this index is also suitable for describing the population genetic diversity.

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