兽类学报1998, 18 (4): 304~310
Acta Theriologica Sinica

INTERSPECIFIC DIFFERENCES OF ISOZYME SYSTEMS BETWEEN *APODEMUS DRACO* AND *APODEMUS PENINSULAE*[*]

FU Biqian CHEN Wei GAO Wu
ZHANG Kexin YUAN Hong RUI Chaosheng
(*Department of Biology, Capital Normal University, Beijing*, 100037)
LI Juhuai
(*Beijing Academy of Agricultural Sciences, Beijing*, 100081)

Abstract

Apodemus draco and *A. peninsulae* are the two species quite similar in morphological characters. In order to explore some diagnostic means of the biochemical taxonomy of the two species, LDH, EST and SOD isozymes were isolated from leg muscles, breast muscles, hearts, kidneys, livers, spleens, lungs and brains of *A. draco* and *A. peninsulae* and were analyzed by isoelectric focusing with PAGE. Distinct interspecific differences were found in EST and SOD electrophoregrams, but not found in LDH electrophoregrams. It was suggested that *A. draco* and *A. peninsulae* could be unequivocally distinguished by the presence or absence of A_2 band in EST isozymes or by the variations of isoelectric point (pI) of main bands in SOD isozymes.

Key words *Apodemus draco*; *Apodemus peninsulae*; EST isozymes; LDH isozymes; SOD isozymes; Biochemical taxonomy

The taxonomic relations within the genus *Apodemus* are unstable and of considerable interest because of the difficulty of classifying species by morphological techniques. Within the genus, *A. draco* and *A. peninsulae* are the two chief members of the subgenus *Sylvaemus* in China, and quite similar either morphologically or ecologically. The difficulty has been existing in differentiating them, even though some varied morphological taxonomic criteria had been proposed (Allen, 1940; Corbet, 1978; Feng et al., 1986). Moreover, some morphological differentiation between the two species, such as that in the proportion of length of body to the length of tail, may be significant in the south, but slight in the north of China.

In the lately decades, the studies on isozymes have proposed a lot of important information for analyzing genetic variations, evolutionary and taxonomic relationships of natural populations. However, only a little information concerning *A. draco* and *A. peninsulae* is at present available (Ma et al., 1989; Wilson et al., 1993).

* We are greatly indebted to Professor Ma Yong (Institute of Zoology, the Chinese Academy of Sciences) and Professor Li Shaowen (Beijing University) for their help to the study and constructive suggestions of the manuscript.
Received 23 July 1997, Accepted 2 June 1998

The present research aims at studying the differences of some isozyme systems between *A. draco* and *A. peninsulae*, and exploring some diagnostic means of the biochemical taxonomy of the two species.

MATERIALS AND METHODS

1. Experimental animals

Four specimens of *A. draco* were collected from Mentougou district of Beijing and seven specimens of *A. peninsulae* collected from Changping county of Beijing. The animals were collected from August to October, 1994, as well as all adult and healthy.

A. agrarius, which belongs to the other subgenus (*Apodemus*) of the genus *Apodemus*, was used as a reference, for it's generally agreed that this species is a distinctive one. It was expected that the electrophoresis results of *A. agrarius* would be helpful to analyzing the degree of the isozymic differentiation between *A. draco* and *A. peninsulae*.

2. Sample preparation

Eight tissues (leg muscle, breast muscle, heart, kidney, liver, spleen, lung and brain) of the three species were homogenized in an ice bath, with 1 g tissues in 2 ml cane sugar solution (20% w/v). The homogenates were then centrifuged at 5 000 g for 10 min at 4℃ and the supernatants removed and used for subsequent analysis.

3. Isoelectric focusing

Isoelectric focusing with PAGE was carried out as described by Li et al. (1986a, b). The ampholine was manufactured by the Chinese Military Academy of Medical Sciences, Beijing, with pH 3.0～9.5. The electrophoresis was performed for 5 h in a refrigerator kept at 4 ℃, with an initial current of 12 mA and initial voltage of 38 v, and a final voltage of 580 v.

4. Staining and fixing procedures

The staining solution and incubating procedure utilized to visualize enzymes followed the methods of Liu et al. (1985) for LDH, Ma et al. (1989) for EST, and Wendel et al. (1989) for SOD.

The determining of pH, the fixing and drying of gel slab were all performed as described by Li et al. (1986a, b).

The experiments were repeated several times and no significant intraspecific differences were found.

RESULTS

1. Lactate dehydrogenase

As the other mammals, tissue specificities observed in LDH isozymes from the species under study were distinct. Among all LDH zymograms of the eight tissues examined, those of brains and kidneys are the clearest and the most complete, therefore, were studied thoroughly.

In the zymograms of brains, as shown in Plate I- a, the bands could be divided into five classes according to their pI: class A between pH 5.6～5.7, class B between pH 5.8～6.1, class C between pH 6.2～6.3, class D between pH 6.4～6.7, and class E between pH 6.8～7.4. Similar patterns in class A, B, C and E were observed for *A. draco* and *A. peninsulae*. Both of the species have two bands in class A and C, three in class B, four in class E; the pI of the corresponding bands from different species is the same. The chief variations between the two species lie in class D: in *A. draco* four bands could be recognized clearly although D_1 and D_2 are near to each other, whereas in *A. peninsulae* D_1 and D_2 merge into a single whole.

As for the kidney zymogram of *A. peninsulae*, D_1, D_2 and D_3, D_4 merge respectively, and the B_3 band is slighter than that of *A. draco*.

Similar patterns could be found in *A. agrarius*, with four bands in class D both in brain and kidney, and with slighter B_3 in kidney.

2 Esterase

Esterase could be divided into α-EST and β-EST according to the different substrates utilized in the experiments, the former with α-Naphthol acetate and the latter with β-Naphthol acetate. Both α-EST and β-EST are multiple in each of the eight tissues of the species examined.

The α-EST zymograms of the leg muscle, breast muscle and lung tissues for the species studied are shown in Plate I- b. Interspecific differences were seen very great in the anodic area (class A) with a pI ranging between pH 3.5～5.1. Within this class, four dark bands (A_1, A_2, A_3, and A_4) and one light band (A_5) could be recognized for *A. draco*, with A_3 and A_4 usually being joint together into a predominant band; whereas, A_2 is absent and A_3, A_4 are lighter for *A. peninsulae*. As regards the other area of the zymograms, the bands are usually lighter, and there are no significant interspecific differences except the activity of some corresponding bands from various species.

Similar interspecific variations could be observed in the α-EST zymograms of hearts, spleens and brains for *A. draco* and *A. peninsulae*. In kidneys and livers, however, things are different: the bands within class A are usually light both in *A. draco* and in *A. peninsulae*, although A_2 is present in the former; the activity differences between the corresponding bands from various species are very obvious in the intermediate parts of α-EST zymograms between pH 5.6～6.3, where some bands are dark and connected in *A. peninsulae* but light and separated in *A. draco*.

Although markedly different as discussed above, the two species are the same in pI of A_1 bands, which locates at pH 3.6, and different from *A. agrarius* in which the pI of A_1 bands locates at pH 4.0. It takes a lot of work to do to determine whether the variation in pI of A_1 bands represents an important difference between the two subgenus.

In the β-EST zymograms, the presence or absence of A_2 band is still the most distinct difference between *A. draco* and *A. peninsulae*.

3 Superoxide dismutase

It was observed in the experiment that the SOD zymograms are quite similar for various tissues of the same species. The kidneys, lungs and leg muscles patterns are shown in Plate I-c. It should be noted that in this photograph the bands are achromatic because of negative staining reaction.

As shown in Plate I-c, eight bands (six anodic bands, one intermediate band as well as one cathodic band) could be recognized in *A. peninsulae*, but the sixth (SOD-6) is absent in *A. draco*. SOD-4 migration is more anodic in *A. peninsulae* than in *A. draco*, so is SOD-8. With the differences as mentioned above, most of bands are only slightly present and difficult of recognition, thus making it almost impossible, in fact, to use them for taxonomic comparison. SOD-5 from *A. peninsulae* and SOD-3 from *A. draco* are, in contrast, predominant in all tissues examined and markedly species specific in pI: the former at pH 6.4, but the latter at pH 5.8. It is obvious that the two bands could be considered diagnostic for the two species.

Comparing the zymograms of the two species to those of the control *A. agarius*, it could be observed that the differences in pI of main bands are significant yet for the species of various subgenus.

DISCUSSION

Among the three isozyme systems studied, LDH is an important system and often used in analyzing evolutionary and taxonomic relationships of natural populations. As regards the comparision on the characters of LDH among species within the same genus or even the same subgenus, a lot of work has been done (Page, 1973; Rainboth, 1974; Basaglia, 1991; Cammarata et al., 1991; Shi et al., 1984; Nie et al., 1995; Li et al., 1987; Wang et al., 1989; Zhou et al., 1990; Zhang et al., 1992). It could be found from these studies that the effect of LDH isozymes on the biochemical taxonomy might be different for different groups of animals.

The results of the present work showed that the interspecific differences in LDH zymograms are slight, not only between *A. draco* and *A. peninsulae* but also between them and *A. agrarius*. Therefore, LDH isozymes seem not to be satisfactory enough for the biochemical taxonomy of the genus *Apodemus*.

For studying evolutionary and taxonomic relationships of populations, EST isozymes may prove particularly useful, as they do not constitude a single isozymic series, but rather a whole train of such series (Arnason et al., 1966). From previous work, it seems that the EST zymograms are often markedly different in the different species of the same genus or subgenus, or even in the different mutants of the same species (Miao et al., 1980; Ye et al., 1984; Li et al., 1986a; Huang et al., 1985, 1988; Wang et al., 1992). It was on the basis of the comparative studies on the EST zymograms of the genus *Apis* that Li et al. (1986a) proved there are six independent species within the *Apis* rather than four species that was thought before. From the results of our work, EST isozymes were turned out to be of diagnostic value for *A. draco* and *A. peninsulae*, partic-

ularly by the presence of the A_2 band in *A. draco*.

Superoxide dismutase, also being called tetrazolium oxidase (TO), is an antioxidiant enzyme catalyzing the detoxification of the superoxide radical. It is just less than three decades since the enzyme was used in judging phylogenetic relationships and solving taxonomic problems (Johnson et al., 1970; Page et al., 1973). By examining the patterns of seven isozyme systems and of general proteins from some tissues of two species of the genus *Mullus*, Cammarata et al. (1991) found the SOD isozyme as well as general proteins of the muscle species-specific. Moreover, He et al. (1993) researched the significance and effect to apply the analytic method of SOD isozymes to mammalian taxonomy, with bats and murids as materals, and thought SOD isozyme is a very useful tool enzyme to distinguish new species or evolution of mammals. Based on the results obtained in our experiments, SOD isozymes proved to be good indicators of the biochemical taxonomy of the genus *Apodemus*.

In a word, as discussed above, *A. draco* and *A. peninsulae* are markedly different in their electrophoregrams of some isozymes even though they are quite similar in morphological characters. They could be unequivocally distinguished from each other by the presence or absence of A_2 band in EST isozymes and by the variation of pI of main bands in SOD isozymes. Furthermore, by analyzing the statistical relations between biochemical characters and morphological characters, it is possible to find some good distinguishing morphological characters so as to use them in the taxonomy of the two species.

REFERENCE

Allen G M. 1940. The Mammals of China and Mongolia. Vol. 2. New York: *Amer Mus Nat Hist*, 939～950.

Arnason A, Pantelouris E M. 1966. Serum esterase of *Apodemus sylvaticus* and *Mus musculus*. *Comp Biochm Physiol*, **19** (1): 53～61.

Basaglia F. 1991. Interspecific gene differences and phylogeny of the Sparidae family (Perciformes, Teleostei) estimated from electrophoretic data on enzymatic tissue-expression. *Comp Biochem Physiol*, 99B (3): 495～508.

Cammarata M, Parrinello N, Arculeo M. 1991. Biochemical taxonomic differentiation between *Mullus barbatus* and *Mullus surmuletus* (Pisces, Mullidae). *Comp Biochem Physiol*, 99B (3): 719～722.

Corbet G B. 1978. The Mammals of the Palaearctic Region: a taxonomic review. London: British Museum (Natural History), 132～133.

Feng Zuojian, Cai Guiquan, Zheng Changlin. 1986. The Mammals of Xizang, China. Beijing: Science and Technology Press, 330～340.

He Xinxia, Zhou Yucan, Shao Lingxiang. 1993. A test on the way of superoxide dismutase isozymes to mammalian taxonomy. *Acta Theriologica Sinica*, **13** (4): 296～299.

Huang Shengmin, Ding Linhua, Pan Shuying, Zhang Hanyun, Li Shaolan, Yang Lianxi. 1985. Studies on esterase isoenzymes in *Attacus cynthia ricini* sp. *Zoological Research*, **6** (3): 287～291.

Huang Shengmin, Qin Changgeng, Pan Shuying, Wang Xiuyong, Li Xiaohui, Tian Shukui. 1988. Comparative studies on the electrophoregram of esterase isozyme and lactate dehydrogenase of *Carassius auratus gibelio* Bloch and *Carassius* sp. *Zoological Research*, **9** (1): 69～78.

Johnson A G, Utter F M, Hodgins H O. 1970. Interspecific variation of tetrazolium oxidase in *Sebastodes* (Rockfish). *Comp Biochem Physiol*, **37** (2): 281～285.

Li Baoguo, Chen Fuguan. 1987. A comparative study of the karyolypes and LDH isozymes from some zokors of the

subgenus *Eospalax*, Genus *Myospalax*. *Acta Theriologica Sinica*, **7** (4): 275～282.

Li Shaowen, Meng Yupin, Chang Zhongbin, Li Juhuai, He Shaoyu, Kuang Bangyu. 1986a. Comparative study of esterase isozymes of six species of *Apis*. *Acta Scientiarum Naturalium Universitatis Pekinensis*, (4): 53～56.

Li Shaowen, Meng yupin, Chang J T, Li Juhuai, He Shaoyu, Kuang Bangyu. 1986b. A comparative study of esterase isozymes in 6 species of *Apis* and 9 genera of *Apoidea*. *J Apicult resear*, **25** (3): 129～133.

Liu Guofu, Wen Deqi, Hu Xiaomei. 1985. Lactate dehydrogenase isoenzymes of the pika and the plateau zokor. *Acta Theriologica Sinica*, **5** (3): 223～228.

Ma Lailing, Chen Yuexian, Li Shaowen, Li Juhuai. 1989. Studies on the esterase and malate dehydrogenase isozyme zymogram of *Apodemus peninsulae* of the region qin Ling. *Acta Theriologica Sinica*, **9** (1): 63～67.

Miao Jianwu, Jiang Wenbin, Huang Shengmin. 1980. Studies on comparison of esterase isozyme of six species mosquitoes in China. In: Shanghai Institute of Entomology, Academia Sinica, editor. Contributions from Shanghai Institute of Entomology. Vol. 1. Shanghai: Shanghai Scientific and Technical Publishers, 89～92.

Nie Liuwang, Guo Chaowei, Wu Xiaobing. 1995. The LDH isozyme PAGE analysis of four tissues of five species in Colubridae. *Zoological Research*, 16 (1): 31～36.

Page L M, Whitt G S. 1973. Lactate dehydrogenase isozymes, malate dehydrogenase isozymes and tetrazolium oxidase mobilities of darters (Etheostomatini). *Comp Biochem Physiol*, 44B (2): 611～623.

Rainboth Jr W J, Whitt G S. 1974. A analysis of evolutionary relationships among shiners of the subgenus *Luxilus* (Teleostei, Cypriniformes, Notropis) with the lactate dehydrogenase and malate dehydrogenase isozyme systems. *Comp Biochem Physiol*, 49B (2): 241～252.

Shi Yingxian, Li Shipeng, Gao Qingsheng, Huang Zhujian, Gao Yuru, Liu Weixin, Ma Lianke, Chen Lidong. 1984. Comparative studies on serum protein, hemoglobin and LDH between *Alligator sinensis* and *A. mississippiensis*. *Acta Herpetologica Sinica*, **3** (2): 21～24.

Wang Aimin, Zhang Xiaolan, Li Hongye, Liao Lifu, Jiang Wei. 1992. A comparative analysis of α and β esterase isozymes in *Lagurus luteus* and *L. lagurus*. *Hereditas* (*Beijing*), **14** (3): 29～32.

Wang Guilan, Tang Chengkui, Xu Jinju. 1989. Comparative study on contents of lactate dehydrogenase isozymes from normal serums of four Tasa in the genus *Macaca*. *Zoological Research*, **10** (sup): 143～149.

Wendel J F, Weeden N F. 1989. Visualization and interpretation of plant isozymes. In: Soltis D E and Soltis P S, editors. Isozymes in plant biology. London: Chapman and Hall, 5～45.

Wilson D E, Reeder D M. 1993. Mammal species of the world: a taxonomic and geographic reference (2nd ed). Washington and London: Smithsonian Institution Press, 569～572.

Ye Binghui, Shen Shibi. 1984. A comparative study on the isozyme patterns of esterase and malic dehydrogenase of 4 indoor species of cockroaches. *Zoological Research*, **5** (4): 325～328.

Zhang Weidao, Zhao Ru. 1992. A study of lactate dehydrogenase isozymes of two species in genus *myotis*. *Hereditas* (*Beiging*), **14** (6): 12～15.

Zhou Yucan, Shao Lingxiang, He Xinxia. 1990. Comparative study of superoxide dismutase and lactate dehydrogenase isozymes in the tissues from three kinds of Murids. *Acta Theriologica Sinica*, **10** (4): 299～303.

中 文 摘 要

中华姬鼠与大林姬鼠的同工酶差异

傅必谦　陈　卫　高　武　张可心　袁　虹　芮朝胜
（首都师范大学生物系，北京，100037）
李举怀
（北京市农林科学院，北京，100081）

中华姬鼠（*Apodemus draco*）和大林姬鼠（*Apodemus peninsulae*）是形态学上十分相似的两种鼠类。为了对两种姬鼠的分类提供生物化学方面的依据，采用聚丙烯酰胺凝胶等电聚焦电泳方法比较和分析了两种姬鼠的LDH 同工酶、EST 同工酶和 SOD 同工酶的差异。结果表明，两种姬鼠的LDH 同工酶酶谱基本相似，而 EST 同工酶和 SOD 同工酶酶谱则存在明显的种间差异。根据 EST 同工酶A_2带的有无和 SOD 同工酶主带等电点的差别，能将两种姬鼠很容易区分开来。

关键词　中华姬鼠；大林姬鼠；LDH 同工酶；EST 同工酶；SOD 同工酶；生化分类

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（3）甘孜州主要采伐利用的森林是冷杉、云杉林，这不是黑熊的栖息环境，因而黑熊的栖息地保存较好；尽管70年代以前黑熊作为农牧业的害兽，奖励群众猎杀，但因食物充足、交通不便、地广人稀、人口密度低等因素仍然保存有丰富的资源和一定的种群数量。

5　保护对策

（1）做好保护野生动物的宣传教育工作，充分利用当地群众信奉藏传佛教的宗教信仰“不杀生”，保护好黑熊资源。

（2）加强法制建设，认真宣传贯彻《野生动物保护法》。当地群众有狩猎习惯，尤其黑熊胆是名贵药材，熊掌是山珍，仍有不法分子偷猎；各地应加强野生动物保护法的贯彻落实，一旦发现违法者，坚决依法严惩。

（3）加强黑熊资源的调查研究。尽管目前甘孜地区的黑熊资源较丰富，我们仍然要保护好黑熊的栖息地，严禁乱砍滥伐。同时研究黑熊的生态特性、探索种群变化动态及发展趋势，提出相应的对策，保护资源。

关键词　黑熊；数量；分布；保护

Key words　Asiatic black bear (*Selenarctos thibetanus mupinensis*); Number; Distribution; Protection

彭基泰（四川省甘孜州林业局，康定，626000）
PENG Jitai (*Forestry bureau of Ganzi Prefecture, Sichuan Province, Kangding*, 626000)