Study of morphological variations between Pacific herring *Clupea pallasii* and Atlantic herring *Clupea harengus*

LIU Ming[1] JIA Ning[1] ZHAO Lin-lin[1] ZHUANG Zhi-meng[2]
JIN Xian-shi[2] GAO Tian-xiang[1]*

([1]Key Laboratory of Mariculture, Ministry of Education, Ocean University of China, Qingdao 266003, China)
([2]Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao 266071)

ABSTRACT Twenty-three morphological parameters were analyzed using ANOVA, cluster analysis and discriminant analysis to study the morphological variations between *Clupea pallasii* and *Clupea harengus*. ANOVA results showed that there were extremely significant differences ($P<0.001$) in the means of 14 morphological parameters, greater variance between populations than within populations. Cluster analysis suggested that all of the 82 samples were divided into two clusters. Discriminant analysis based on 23 parameters showed that there were extremely significant differences between the two species ($P<0.001$); discriminant analysis with 11 selected parameters gave a discriminant formula with accuracy of 100%. However, the coefficients of difference of 23 parameters suggested that the differentiations between the two species were still under the level of different geographic populations within species according to Mayr's 75% Rule. We postulated that the small scale divergence in morphology between the two species might be caused by a recent separation and similar ecology environment.

KEY WORDS Pacific herring *Clupea pallisii* Atlantic herring *Clupea harengus* Morphological variations ANOVA Cluster analysis Discriminant analysis Coefficient of difference

中图分类号 Q959.4 **文献识别码** A **文章编号** 1000-7075(2009)06-0081-07

太平洋鲱和大西洋鲱间的形态变异研究

刘 名[1] 贾 宁[1] 赵林林[1] 庄志猛[2] 金显仕[2] 高天翔[1]*

([1] 中国海洋大学教育部海水养殖重点实验室,青岛 266003)
([2] 中国水产科学研究院黄海水产研究所,青岛 266071)

摘 要 为阐明太平洋鲱和大西洋鲱种间形态上的差异,应用单因子方差分析、聚类分析和判别分析方法对两种鱼的 23 个形态学参数进行了研究。单因子方差分析结果表明:14 个形态参数平均值存在极显著差异($P<0.001$),且种群间差异大于种内差异。聚类分析结果显示所有 82 个个体明显聚成两支。所有 23 个形态参

The study was supported by Ocean University of China-Students Innovation Training Program, National Basic Research Program (2006CB400607) and State 863 High-Technology R&D Project of China (2006AA09Z418)

* Corresponding author: Prof. Gao Tian-xiang. E-mail: gaozhang@ouc.edu.cn
Author: Liu Ming (1982-), Male, Doctor. E-mail: shelleyliuming@hotmail.com

数的判别分析结果表明，两种间的形态差异极显著（$P < 0.001$）；利用挑选后的 11 项参数判别分析，得出判别等式，两种鱼间的形态差异仍然极显著（$P < 0.001$），判别准确率 100%。所有 23 个形态参数的差异系数全部小于 Mayr 的 75% 法则临界值，表明两个种间形态学上虽然存在一定分化，但分化程度尚未达到亚种水平。作者推测两个种间形态上低水平的分化可能由于分化的时间较短以及相似的生活环境造成的。

关键词 太平洋鲱 *Clupea pallasii* 大西洋鲱 *Clupea harengus* 形态差异 单因子方差分析 聚类分析 判别分析 差异系数

Introduction

Pacific herring (*Clupea pallasii* Valenciennes, 1847) and Atlantic herring (*Clupea harengus* Linnaeus, 1758) are two members of *Clupea*, Clupeiformes. Both of them are important economic fish species in the North Pacific and North Atlantic. Pacific herring is distributed north of 35° N from the Yellow Sea to California in the Pacific, and Atlantic herring is distributed both sides of the Atlantic from 33° N to 80° N (Zhang 1997). Both of them are pelagic, schooling and coastal species. They usually live in cold waters at a range of temperatures between 6～10 ℃. Every year mature adults migrate inshore and spawn in sheltered inlets, sounds, bays, and estuaries rather than along open coastlines. There is a latitudinal cline in spawn timing within the range of the two species, which was thought to coincide with "local spring", a period of increasing plankton productivity (Lassuy 1989; Kobayashi 1993).

Pacific herring was first described as a subspecies of Atlantic herring, because of morphological similarity between the two species. The acknowledged morphological difference is that Atlantic herring generally has a greater number of vertebrae (Domanico *et al.* 1996; Tang 1991). However, the numbers have an overlapped range (54～57) in many populations of the two species. Pacific herring and Atlantic herring began to be recognized as distinct species based on genetic variation (Grant 1986; Robins *et al.* 1991). An average genetic distance of 0.27 was found between the two species using allozyme analysis (Grant 1986). In recent years, more studies were focused on the divergence between the two species in genetics. An estimate of 2.6% sequence divergence was obtained from the analysis of mtDNA restriction site data (Kornfield *et al.* 1985), and a 1.3% divergence was also got by ribosomal DNA sequence variation (Domanico *et al.* 1996). However, the morphological variation between the two species is not yet clear.

In the present study, we are interested in finding more differentiations both in meristic and morphometric characters of these two species, which would be expected to illuminate the divergence degree in morphology. Furthermore, we hope to test the conformity between morphological variations and genetic variations, and try to learn the impact factors associated with divergence degree in morphology. ANOVA, cluster and stepwise discriminant analysis were used to study the morphological variations between two populations of the two species, then coefficients of difference (CD) were calculated and their morphological differences were defined according to Mayr' s 75% Rule (Mayr *et al.* 1953).

Materials and Methods

Samples collection

Thirty-four individuals of *C. pallasii* and forty-eight individuals of *C. harengus* were analyzed in this study. *C. pallasii* samples were collected from the Yellow Sea of China in December, 2007; and *C. harengus* samples were from Gulf of Maine in Canada eastern coast in June, 2003. All the individuals were mature.

Measurement

Twenty-four morphological characters, including six meristic characters and eighteen morphometric characters, were analyzed. The meristic characters included vertebrae numbers, dorsal fin rays, pectoral fin rays, anal fin rays, upper gill rakers and lower gill rakers. The morphometric characters are shown in Fig. 1.

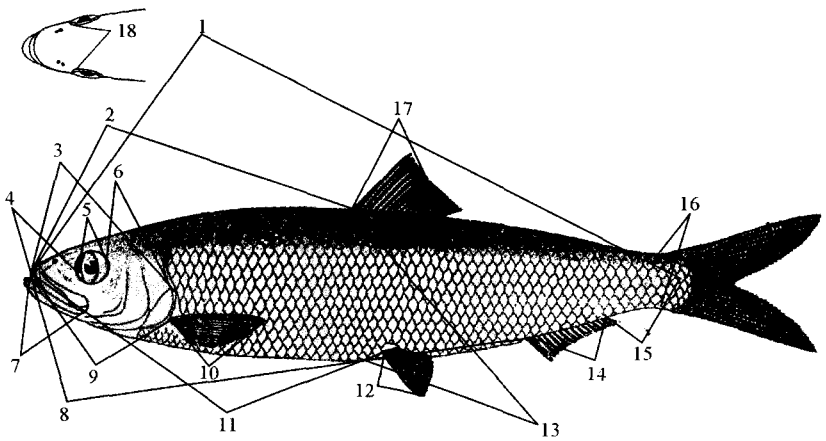

Fig. 1　The parameter measurements of all 18 morphometric characters

1. Standard length 2. Predosal length 3. Head length 4. Snout length 5. Orbit diameter 6. Postorbital length 7. Upper jaw length 8. Prepelvic length 9. Prepectoral length 10. Length of pectoral fin 11. Prepectoral length 12. Prepelvic length 13. Body depth 14. Length of anal fin-base 15. Length of caudal peduncle 16. Depth of caudal peduncle 17. Length of dorsal-fin base 18. Intarorbital width

To avoid the influence of body size difference on analysis, 11 morphometric characters were converted to ratios by dividing by their standard length, body depth and head length. A total of 23 parameters were used in statistical analyses, which are shown in Table 1.

Table 1　The description of means, ANOVA and coefficients of difference for *C. pallasii* and *C. harengus* based on 23 parameters.

Parameter	Pacific herring (Mean±SD)	Atlantic herring (Mear±SD)	*F* value	*P*	CD
Vertebrae numbers	53.59±1.18	55.92±1.05	88.172	0.000	1.045
Dosal fin rays	17.38±0.85	17.29±0.94	0.199	0.657	0.050
Pectoral fin rays	16.38±1.28	16.90±0.72	5.347	0.023	0.260
Anal fin rays	15.91±1.38	15.50±0.92	2.628	0.109	0.178
Upper gill rakers	17.24±1.21	18.94±1.60	27.309	0.000	0.605
Lovwer gill rakers	42.88±1.43	42.06±2.14	3.790	0.055	0.230
Orbit diameter	1.075±0.097	1.099±0.074	1.541	0.218	0.140
Interorbital width	0.922±0.106	0.893±0.083	1.864	0.176	0.153
Length of dorsal-fin base	2.968±0.276	2.504±0.171	87.907	0.000	1.038
Length of pectoral fin	3.312±0.218	3.053±0.194	31.894	0.000	0.629

Parameter	Pacific herring (Mean±SD)	Atlantic herring (Mear±SD)	*F* value	*P*	CD
Length of pelvic fin	2.288±0.231	1.968±0.143	60.009	0.000	0.856
Length of anal fin-base	2.656±0.309	2.339±0.174	35.024	0.000	0.656
Snout length/Head length	0.361±0.045	0.308±0.036	36.035	0.000	0.654
Postorbital length/Head length	0.429±0.039	0.443±0.030	3.258	0.075	0.203
Upper jaw length/Snout length	1.155±0.197	1.483±0.220	48.182	0.000	0.787
Head length/Standard length	0.220±0.021	0.218±0.010	0.237	0.628	0.065
Predo sallength/Standard length	0.491±0.019	0.528±0.017	88.606	0.000	1.028
Prepectoral length/Standard length	0.213±0.015	0.212±0.013	0.156	0.694	0.036
Prepelvic length/Standard length	0.547±0.024	0.574±0.017	35.927	0.000	0.659
Preanal length/Standard length	0.761±0.034	0.792±0.017	30.325	0.000	0.608
Length of caudal peduncle/Standard length	0.119±0.013	0.102±0.013	36.592	0.000	0.664
Body depth/Standard length	0.212±0.021	0.236±0.014	36.854	0.000	0.686
Depth of caudal peduncle/Body depth	0.376±0.042	0.308±0.020	96.550	0.000	1.097

Data analysis

All the parameters of each individual were treated firstly with Microsoft Excel, then analyzed with SPSS 13.0. Three kinds of statistical analysis including ANOVA, cluster analysis and discriminant analysis were conducted. Coefficient of difference (CD) was calculated according to Mayr *et al.* (1953). If CD of one parameter < 1.28, then we can classify the differences between them into different geography population within species (Mayr *et al.* 1953). Between-groups linage method and city-block distance were used for the hierarchical cluster analysis. Stepwise discriminant analysis was used to establish a simple discriminate formula to distinguish the two species in this study.

Results

One-way ANOVA

One-way ANOVA analysis was conducted for all twenty-three parameters of the two species. Both the *F* value, which reflected variance between populations to that within populations, and significance test *P* were given in Table 1. The standard length ranged between 19.45～29.10 cm in Pacific herring, and 17.52～23.28 cm in Atlantic herring. The body depth ranged between 3.66～6.22 cm in Pacific herring, and 4.00～5.82 cm in Atlantic herring. Both of them were divided to eliminate body size difference. In the table, the *F* values of fourteen parameters were big and extremely significant ($P < 0.001$), and one parameter was significant ($P < 0.05$). Most of these parameters were for morphometric characters.

The coefficients of difference between the two species were also shown in Table 1. All the coefficients of difference were smaller than 1.28, which is the threshold value of subspecies.

Cluster analysis

Although the coefficients of difference between the two species were small, the cluster analysis showed

that all the individuals were separated and clustered into two groups based on the twenty-three parameters (Fig. 2).

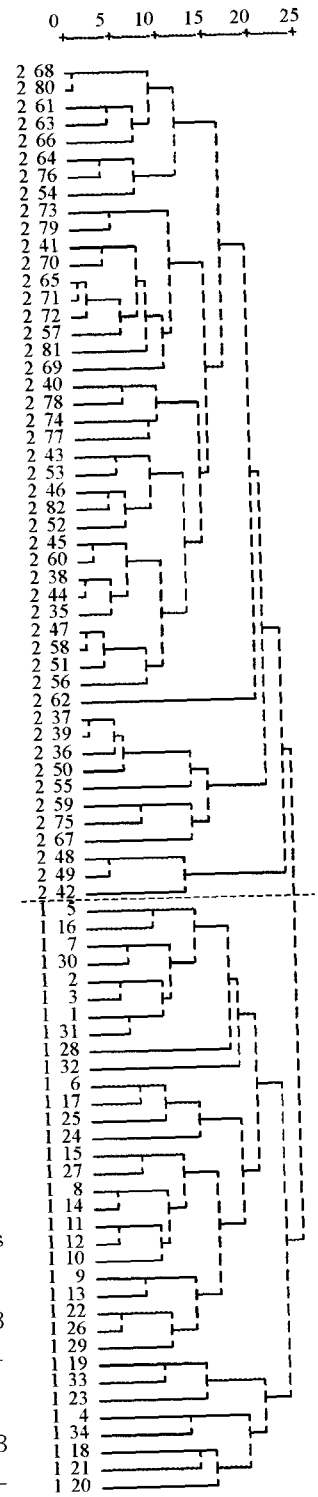

Fig. 2　Clustering dendrogram of *C. pallasii* and *C. harengus* based on 23 parameters
1. *C. pallasii*　2. *C. harengus*

Discriminant analysis

When the twenty-three parameters were used in the discriminant analysis, the accuracy of discriminant was 100%, and the effect of discriminant was extremely significant ($P < 0.001$). To enhance the convenience of the discriminant formula, eleven parameters, which contributed more to the morphological variation, were selected from those twenty-three parameters, to produce a new discriminant formula. The eleven parameters were given in Table 2 according to the F value.

Table 2　Variables (ranged by F test values) with high contribution in discriminant analysis

Variable	Parameter	F value
X1	Vertebrae numbers	114.91
X2	Length of dorsal-fin base	109.23
X3	Length of pelvic fin	101.80
X4	Length of caudal peduncle/Standard length	97.90
X5	Depth of caudal peduncle/Body depth	96.55
X6	Snout length/Head length	95.56
X7	Pectoral fin rays	93.61
X8	Upper gill rakers	93.59
X9	Prep elvic length/Standard length	91.98
X10	Head length/Standard length	88.06
X11	Predosal length/Standard length	85.25

The accuracy of discriminant based on the eleven parameters was the same (100%) as that based on the 23 parameters, and the effect of discriminant was also highly significant ($P<0.001$).

Discriminant formulae of the two species based on the 11 parameters were as follows:

Clupea pallasii: Y1 = 67.995X1 + 119.537X2 + 51.432X3 1405.776X4 + 417.629X5 + 28.802X6 + 32.889X7 + 28.261X8 + 1647.264X9 + 1118.048X10 + 2284.794X11 3705.299

Clupea harengus: Y2 = 73.105X1 + 108.406X2 + 37.932X3 1840.442X4 + 344.189X5 79.886X6 + 36.421X7 + 30.584X8 + 1760.289X9 + 936.139X10 + 2416.447X11 4007.773

Calculated by the above formula, the individual of herring can be identified to species; the capital Y denotes which species it belongs to.

Discussion

In the present study, the samples of Pacific herring and Atlantic herring have shown some significant

morphological differentiations according to ANOVA, and they were clustered into two groups by cluster analysis. However, all the coefficients of difference were lower than 1.28, the value that defines subspecies, indicating that the variation was only at the level of different geographical races. Our study failed to find the significant morphological characters which can distinguish the two species. Contrast to morphometric characters, meristic characters were more precise in taxonomic studies. Further studies in comparative anatomy should be carried out to find the proofs in meristic characters. Nevertheless, the discriminant analysis offered criteria to discriminate the two species based on eleven morphological characters in our study.

According to our results, the divergence in morphology between Pacific herring and Atlantic herring were obviously lower than in genetics from allozyme analysis (Grant 1986). Such situations can also be seen in several other marine fish species. *Lateolabrax maculates* and *L. japonicus* were treated as a species before 2001 because of their similarity in external features, but a 22.6% of net genetic distance in mtDNA sequence variation was detected between the two species (Liu *et al.* 2006). Moreover, no differentiation in morphology was found yet for the China and Japan populations of *Pennahia argentata*, but a 67.46% of variation between the two groups implied the divergence has reached subspecies level at least (Han *et al.* 2008). The impact factors may be various, but here we postulated our results might be caused by two reasons: (1) a recent separation (temporal); (2) similar ecology environment (spatial).

(1)Recent separation

According to Mayr, a new species develops if a population which has become geographically isolated from its parental species acquires characters which promote or guarantee reproductive isolation when the external barriers break down during the period of isolation (Mayr 1942). The accumulation of morphological variations was usually slower than that of genetic variations, because the mutation occurred in genetics first, then passed on to the morphological characters. Thus large scale divergence in morphology usually happens later, which need a long period especially when the environments were similar, than in genetics.

In previous studies, Pacific herring and Atlantic herring were thought to have a common ancestor which lived in Arctic Ocean (Svetovidov 1952; Grant 1986; Kobayashi 1993). Studies of phylogenetics also showed the two species had the closest genetic relationship (Cheng *et al.* 2006; Lavoue *et al.* 2007). Grant (1986) estimated the divergence time between the two species was 3.6～6.6 million years ago using allozyme variation, and Domanico *et al.* (1996) also concluded a divergence of 3.1 million years ago based on sequence variation of ribosomal DNA. However, a more late divergence about 1 million years ago was deduced by mtDNA sequence variation (Kornfield *et al.* 1985; Domanico *et al.* 1996). Those results might indicate that the divergence time was insufficient to cause interspecies' divergence in morphology.

(2) Similar ecology environment

It has been documented that some oceanographic factors such as salinity, depth and water temperature could influence the morphological characters of fish species (McHugh 1954). Distinct geographic environments accelerate the accumulation of morphological variations, while similar environments put it off. Both Pacific herring and Atlantic herring are cold water species and distribute along the coasts of middle and high latitude. Their optimum temperatures range between 6～10 ℃, and their distributions are in the isohaline of 31～33. Although small differentiations in morphology were detected among some different geographic populations (Liu *et al.* 2007), the difference degree was even far less than the differentiations between Pacific herring and Atlantic herring in the present study. The similar ecological mechanism and habitat might have played an important role in keeping the divergence in morphology at a limited level.

References

Cheng, Q. Q., and Lu, D. R. 2006. Phylogenetic analysis and relative-rate test of nine Clupeidae fishes (Osteichthyes: Clupeiformes) inferred om cytochrome b gene sequence of mitochondrial DNA. Marine Fisheries, 28:5～12

Domanico, M. J., Phillips, R. B., and Schweigert, J. F. 1996. Sequence variation in ribosomal DNA of Pacific *Clupea pallasi* and Atlantic herring *Clupea harengus*. Can. J. Fish. Aquat. Sci. 53:2 418～2 423

Grant, W. S. 1986. Biochemical genetic divergence between Atlantic, *Clupea harengus*, and Pacific herring, *C. pallasi*. Copeia. 3:714～719

Han, Z. Q., Gao, T. X., Takashi, Y., and Sakurai, Y. 2008. Deep phylogeographic break among white croaker *Pennahia argentata* (Sciaenidae, Perciformes) populations in North-western Pacific. Fisheries Science, 74:770～780

Kobayashi, T. 1993. Biochemical analyses of genetic variability and divergence of populations in Pacific herring. Bull. Natl. Res. Inst. Far Seas Fish. 30:1～33

Kornfield, I., and Bogdanowicz, S. M. 1985. Differentiation of mitochondrial DNA in Atlantic herring, *Clupea harengus*. Proceedings of the Stock Identification Workshop, Panama City Beach, Fla. 187～188

Lassuy, D. R. 1989. Species profiles: Life histories and environmental requirements of coastal fishes and invertebrates (Pacific Northwest) Pacific herring. United States Fish and Wildlife Service, Biology Report, 82 (11.126). U. S. Army Corps of Engineers, TR-EL-82-4. 1～18

Lavoue, S., Miya, M., Saitoh, K., Ishiguro, N. B., and Nishida, M. 2007. Phylogenetic relationships among anchovies, sardines, herrings and their relatives (Clupeiformes), inferred from whole mitogenome sequences. Mol. Phylogenetics and Evolution, 43:1 096～1 105

Liu, J. X., Gao, T. X., Yokogawa, K., and Zhang, Y. P. 2006. Differential population structuring and demographic history of two closely related fish species, Japanese sea bass (*Lateolabrax japonicus*) and spotted sea bass (*Lateolabrax maculatus*) in Northwestern Pacific. Mol. Phylogenetics and Evolution, 39:799～811

Liu, M., Wang, Y. J., Gao, T. X., Yanagimoto, T., Zhuang, Z. M. and Sakurai, Y. 2007. Morphological comparison of Pacific herring *Clupea pallasii* in China and Japan. Periodical of Ocean Univ. of China, 37 (Suppl.):131～136

Mayr, E., Linsley, E. G., and Usinger, R. L. 1953. Methods and Principles of Systematic Zoology. MeGraw Hill Book Company, New York

Mayr, E. 1942. Systematics and the Origin of Species. Columbia University Press, New York

McHugh, J. L. 1954. Geographical variation in the Pacific herring. Copeia, 2:139～151

Robins, C. R., Bailey, R. M., Bond, C. E., Brooker, J. R., and Lachner, E. A. 1991. A list of common and scientific names of fishes from the United States and Canada. American Fisheries Society, Bethesda, Md

Svetovidov, A. N. 1952. Clupeidae, Fauna USSR. Akademii Nauk SSSR Zoologicheskogo Instituta, 48:1～331 (Translated by Israel Program for Scientific Translation, Jerusalem.)

Tang, Q. S. 1991. Herrings in Yellow Sea. In: Marine Fishery Biology (J. Y. Deng, & C. Y. Zhao, eds), 296-356. Agriculture Press, Beijing

Zhang, S. Y. 1997. J. Animals of China (Osteichthyes Clupeiformes). Science Press, Beijing