Investigation of interspecies consanguinity in the tribe Mergini from the order Anseriformes

S. Slavėnaitė,
A. Sruoga,
E. Mozalienė

Institute of Ecology,
Akademijos 2,
LT-2600 Vilnius, Lithuania.
E-mail:igl@ekoi.lt

The polymorphic loci of prealbumin1, prealbumin2, albumin, postalbumin, pretransferrin, posttransferrin, macroglobuline, transferrin and the indicator of population similarity according to L. A. Zhivatovsky were used for determination of the genetic consanguinity of six Mergini species from the order Anseriformes. The results have shown the greatest similarity between *Clangula hyemalis* and *Mergus merganser* (R = = 0.9891) and the greatest difference between *Melanita nigra* and *Mergus albelus* (R = 0.8040). The following sequence of genetic similarity of the six Mergini species has been determined according to the aggregate of allele frequencies of 8 polymorphic loci: Long-tailed Duck (*Clangula hyemalis*), Smew (*Mergus albelus*), Goosander (*Mergus merganser*), Harlequin Duck (*Histronicus histronicus*) Common Scoter (*Melanita nigra*), Common Goldenye (*Bucephala clangula*).

Key words: Anseriformes, Mergini, genetic similarity, polymorphism, electrophoresis

INTRODUCTION

A maximum number of features determining the unique character of a species must be evaluated in order to estimate phylogenetic divergence and convergence. These features are the result of genes' activity and show interspecies consanguinity. The species in various taxonomic levels are compared according to cytologic, morphophysiologic, etiologic, embryiologic and other features. Evaluation of individuals according to structural differences of their macromolecules (proteins, enzymes, DNA) also is very popular for interspecies consaguinity investigations. For this purpose the immunogenetic methods of typification of antigenic structures, two-dimensional electrophoresis allowing to identify a great variety of individual proteins, restricting analysis of DNA fragments, investigation of polymorphism of microsatellite DNA, investigation of genetic polymorphism of DNA by random amplified polymorphic DNA polymerase chain reaction are used. The use of molecular markers also facilitates investigations of morphophysiologic variability, there interspecies relationships could be confined by evaluation of genotype frequencies and allele concentrations in the appropriate loci [1].

Thus, the abundance of features characterising the uniqueness of a species determines the variety of methods for the investigation of interspecies relationships; the initial data in the majority of cases are related with the frequencies of morphs (genes, genotypes, phenotypes) in the groups of individuals under study. In our investigations, for evaluation of interspecies consanguinity of the Mergini tribe according to the frequency of morphs, we used the indicator of similarity according to L. A. Zhivatovsky. This indicator was used because of the following advantages: 1. Insignificant changes of widely spread morphs and also disappearance or upraise of rare morphs have a little impact on the digital value of this indicator. 2. The indicator of similarity does not depend on the parameters characterising different morphs. 3. The contribution of a morph to the digital value of such parameter is proportionate to the frequency of the morph [2].

The order Anseriformes of waterfowl comprises about 150 species of ducks, geese, mergansers, and phalamhedies all over the world. The current taxonomic grouping of the order Anseriformes derive principally from the classic work [3] and to a lesser extent from earlier investigations [4–7], where systematists generally agreed upon the composition of the tribe Mergini as of a monophyletic group. Earlier morphological studies provide a differing, fragmentary and often phenetic assessment of interspecies relationships within the tribe [8, 9].

MATERIALS AND METHODS

Blood sera of the following species of the tribe Mergini have been used for investigations: Long-tailed Duck

(*Clangula hyemalis*), Goosander (*Mergus merganser*), Common Scoter (*Melanita nigra*), Smew (*Mergus albelus*), Common Goldeneye (*Bucephala clangula*), Harlequin Duck (*Histronicus histronicus*).

The blood samples were obtained during expeditions and put into tubes with heparin in order to prevent coagulation. The plasma was obtained after erythrocite sedimentation during centrifugation at a speed of 1500 rpm. Electrophoretic investigation of blood serum proteins was made by using a multi-layer system of polyacrilamide gel [10, 11]. The gels were prepared [12] and the fractionation of isozymes was performed at the direct current of 110 mA (260 V). The arrangement of protein fractions in the electrograms and protein classification were assessed by protein electrophoretic mobility [13]. The allelic variants were resolved by a direct side-by-side comparison of migrating proteins on the same gels. Non-specific proteins were screened by using multi-layer 7.5% polyacrilamide gels with Tris-EDTA-borate (pH 8.3–8.4) and Tris-glycine (pH 8.3–8.4) buffers. The following parameters were used for determination of biochemical polymorphism: the frequency of genes, the parameter of genetic similarity (r) according to L. A. Zhivatovsky ($r = \Sigma\sqrt{p_i q_i}$, there p and q are the frequencies of morphs of comparable populations according to the I number).

RESULTS AND DISCUSSION

Electrophoretic analysis of non-specific proteins of individuals from Mergini tribe species reveals diallelic genetic systems of prealbumin1 (Pr1), prealbumin2 (Pr2) albumin (Al), postalbumin (Pa), pretransferrin (Prtf), posttransferrin (Ptf), macroglobuline (Mc) and one polyallelic system of transferrin (Tf). The data of the analysis of gene frequencies of the species studied are provided in Table 1. The greatest differences according to the frequency of alleles have been detected between Goosander (*Mergus merganser*) (F = 0.25, S = 0.75) and Common Scoter (*Melanita nigra*) (F = = 1, S = 0) and also between Common Goldeneye (*Bucephala clangula*) (F = 1, S = 0) and Smew (*Mergus albelus*) (F = 0.3333, S = 0.6667) in the diallelic genetic systems of Pr2 and Prtf, respectively. Statistically reliable differences have been detected among the majority of allele frequencies in the polyallelic genetics of transferrin in all species of the tribe Mergini.

Based on the frequency of alleles in 8 polymorphic loci, calculation of genetic similarity of Mergini tribe species has been made according to the indicator of L. A. Zhivatovsky. The value of this indicator never exceeds 1. It equals to 1 only in the cases when the species compared are equal according to frequency of morphs, and it is equal to zero when the populations have no common morphs. This statement has been confirmed by our data provided in Table 2, indicating that *Mergus albelus* and *Melanita nigra* as well as *Mergus merganser* and *Melanita nigra* are most proximate according to indicator *r* which equals to 1.0 in the diallelic genetic systems of Ptf and Mc, respectively. A great genetic similarity has been detected among *Mer-*

Table 1. **Gene frequency of the Mergini tribe species**

Species	Pr1	Pr2	Al	Pa	Prtf	Ptf	Mc	Tf
Clangula hyemalis	S = 0.4138 F = 0.5862	S = 0.5862 F = 0.4138	S = 0.5690 F = 0.4310	S = 0.5172 F = 0.4828	S = 0.4483 F = 0.5517	S = 0.3621 F = 0.6379	S = 0.4655 F = 0.5345	a = 0.3108 b = 0.1897 c = 0.344 d = 0.1552
Mergus merganser	S = 0.5278 F = 0.4722	S = 0.75 F = 0.25	S = 0.3611 F = 0.6389	S = 0.6111 F = 0.3889	S = 0.3889 F = 0.6111	S = 0.5278 F = 0.4722	S = 0.5 F = 0.5	a = 0.3056 b = 0.1944 c =0.2222 d = 0.2778
Mergus albelus	S = 0.5833 F = 0.4167	S = 0.5833 F = 0.4167	S = 0.4167 F = 0.5833	S = 0.6677 F = 0.3333	S = 0.6667 F = 0.3333	S = 0.5 F = 0.5	S = 0.4167 F = 0.5863	a = 0.625 b = 0.2917 c = 0.0833
Melanita nigra	S = 0.5714 F = 0.4286	S = 0.0 F = 1.0	S = 0.5714 F = 0.4286	S = 0.4286 F = 0.5714	S = 0.2143 F = 0.7857	S = 0.5 F = 0.5	S = 0.5 F = 0.5	a = 0.5 b = 0.5
Histronicus histronicus	S = 0.4375 F = 0.5625	S = 0.625 F = 0.375	S = 0.3125 F = 0.6875	S = 0.875 F = 0.125	S = 0.357 F = 0.643	S = 0.625 F = 0.375	S = 0.625 F = 0.375	a = 0.4375 b = 0.4375 c = 0.125
Bucephala clangula	S = 0.45 F = 0.55	S = 0.35 F = 0.65	S = 0.5 F = 0.5	S = 0.55 F = 0.45	S = 0.0 F = 1	S = 0.45 F = 0.55	S = 0.35 F = 0.65	a = 0.25 b = 0.5 c = 0.25

gus albelus and *Melanita nigra* as well as *Histronicus histronicus* and *Bucephala clangula* in the diallelic system of Pr1 (r = 0.9999). Higher differences according to indicator r have been detected between *Bucephala clangula* and *Mergus albelus* in the diallelic genetic system of Prtf, as well as between *Melanita nigra* and *Histronicus histronicus* in the diallelic genetic system of Pr 2, where the values of indicator r were 0.5733 and 0.6123, respectively. The calculated common indicator R representing the genetic similarity of Mergini tribe species according to aggregate frequencies of the 8 polymorphic loci shows the greatest similarity between *Clangula hyemalis* and *Mergus merganser* (R = 0.9891) and the greatest difference between *Melanita nigra* and *Mergus albelus* (R = 0.8040) (Table 3). The following sequence of genetic similarity of 6 species of the tribe Mergini has been determined according to aggregate allele frequencies of 8 polymorphic loci: Long-tailed Duck (*Clangula hyemalis*), Smew (*Mergus albelus*), Goosander (*Mergus merganser*), Harlequin Duck (*Histronicus histronicus*), Common Scoter (*Melanita nigra*), Common Goldenye (*Bucephala clangula*).

The obtained results show a comparison of species genetic similarity at molecular level. The main advantage of phylogenetic investigations carried out at molecular (proteins and DNA) level is that they allow to compare the genotypes of the organisms. The data of such analysis not only complement conventional phylogenetic investigations carried out by comparing the living organisms according to their external features but essentially differ from them. However, the results of molecular studies of the tribe Mergini are limited [14]. The composition of integumental lipids confirmed a moderate distance between Common Eider (*Somateria mollisima*) and several other genera [15]. A clear distinction between the eiders and other Merginis using feather proteins was also found [16]. A moderately large genetic distance between two species of *Bucephala* and the two other Mergini samples (*Melanita* and *Clangula*) determined by electrophoresis of 13 proteins has been reported [17]. A recent comparison of Anseriformes using DNA hybridization included only a single representative of the Mergini (*Melanita*) and therefore provided no relationships within the tribe [18].

Also, the polymorphic proteins detected in our investigations as molecular markers could be useful for further investigations of relationships among separate

Table 2. **The indicator of similarity (r) of the Mergini tribe species**

Loci	Comparison of two species (*) from the Mergini tribe according to indicator of similarity (r)											
	1 and **2**	**1** and **3**	**1** and **4**	**1** and **5**	**1** and **6**	**2** and **3**	**2** and **4**	**2** and **5**	**2** and **6**	**3** and **4**	**3** and **5**	**3** and **6**	**4** and **5**	**4** and **6**	**5** and **6**
Pr1	0.9934	0.9854	0.9874	0.9996	0.9993	0.9983	0.9989	0.9958	0.9969	0.9999	0.9893	0.9910	0.9909	0.9925	0.9999
Pr2	0.9846	0.9999	0.6432	0.9991	0.9715	0.9841	0.6432	0.9907	0.9154	0.6455	0.9990	0.9722	0.6123	0.8062	0.9614
Al	0.9779	0.9883	0.9998	0.9659	0.9975	0.9984	0.9774	0.9986	0.9901	0.9879	0.9940	0.9964	0.9653	0.9974	0.9815
Pa	0.9955	0.9887	0.9960	0.9283	0.9994	0.9987	0.9830	0.9516	0.9970	0.9713	0.9684	0.9931	0.8795	0.9925	0.9308
Prtf	0.9988	0.9953	0.9682	0.9956	0.7412	0.9603	0.9815	0.9994	0.7817	0.8893	0.9507	0.5733	0.9872	0.8863	0.8018
Ptf	0.9860	0.9902	0.9901	0.9648	0.9959	0.9996	0.9992	0.9951	0.9969	0.1	0.9920	0.9987	0.9920	0.9987	0.9844
Mc	0.9993	0.9999	0.9993	0.9870	0.9930	0.9978	1.0	0.9920	0.9884	0.9978	0.9790	0.9991	0.9920	0.9883	0.9614
Tf	0.9841	0.8451	0.7021	0.9641	0.9798	0.8111	0.7087	0.8238	0.8198	0.9409	0.9821	0.9214	0.9409	0.8535	0.9751

**1* – Long-tailed duck (*Clangula hyemalis*), *2* – goosander (*Mergus merganser*), *3* – smew (*Mergus albelus*), *4* – common Scoter (*Melanita nigra*), *5* – harlequin Duck (*Histronicus histronicus*), *6* – common Goldenye (*Bucephala clangula*).

Table 3. **The common indicator of similarity (R) of the Mergini tribe species**

Species	*Clangula hyemalis*	*Mergus merganser*	*Mergus albelus*	*Melanita nigra*	*Histronicus histronicus*	*Bucephala clangula*
Clangula hyemalis	–	0.9891	0.9741	0.9107	0.9755	0.9597
Mergus merganser		–	0.9685	0.9110	0.9683	0.9357
Mergus albelus			–	0.8040	0.9818	0.9311
Melanita nigra				–	0.920	0.9394
Histronicus histr.					–	0.9495
Bucephala clangula						–

systems of proteins and enzymes, among allelic and nonallelic genes and for other purposes.

Received
3 September 2002

References

1. Parker P. Ecology 1998; 79(2): 361–82.
2. Живатовский ЛА. Журнал общей биологии 1979; XL(4): 590–6.
3. Delacour J, Mayr E. Wilson Bull 1945; 57: 3–55.
4. Phillips JC. A Natural History of the Ducks. Vol. 1 Boston, 1922.
5. Phillips JC. A Natural History of the Ducks. Vol. 2. Boston, 1923.
6. Phillips JC. A Natural History of the Ducks. Vol. 3. Boston, 1925.
7. Phillips JC. A natural history of the ducks. Vol. 4. Boston, 1926.
8. Johnsgard PA. Wildfowl 1962; 13: 130–48.
9. Johnsgard PA. Condor 1964; 66: 113–29.
10. Davis BJ. Disc electrophoresis-11. Annals of the New York Academy of Sciences, 1964; 121: 404–27.
11. Brewer GJ. An Introduction to Isozymes Technique, New York: Academic Press, 1970.
12. Harris H & Hopkinson DA. 1977. Handbook of Enzyme Electrophoresis in Human Genetics. Amsterdam – North Holland Publishers.
13. Kuryl J, Gasparska J. Comp Biochemical Physiology 1985; 80B(2): 309–19.
14. Sruoga A, Mozalienė E, Paulauskas A, Slavėnaitė S, Bentkuvienė J. Genetic variability of proteins in waterfowl tribes of the order Anseriformes. *Proceedings of the Latvian Academy of Sciences.* 1995. Vol. 5/6: A 41.
15. Jacob J, Glaser A. Biochem Syst Ecol 1975; 2: 215–20.
16. Brush AH. J. Zoology 1976; 179: 467–98.
17. Patton JC, Avise JC. Genetica 1985; 68: 129–43.
18. Sibley CG, Ahlquist JE. Phylogeny and Classification of Birds: a Study in Molecular Evolution. New Haven, 1990: 66–73.

S. Slavėnaitė, A. Sruoga, E. Mozalienė

TARPRŪŠINIO GIMININGUMO NUSTATYMAS ŽĄSINIŲ (ANSERIFORMES) BŪRIO MERGINI TRIBOJE

S a n t r a u k a

Polimorfiniai prealbuminų1, prealbuminų2, albuminų, postalbuminų, pretransferinų, postransferinų ir transferinų lokusai kaip molekuliniai žymenys bei populiacijų panašumo rodiklis pagal L. A. Živatovskį (R) buvo panaudoti tarprūšinio giminingumo nustatymui tarp 6 Mergini tribos, priklausančios žąsinių (Anseriformes) rūšių būriui. Didžiausias genetinis panašumas buvo nustatytas tarp *Clangula hyemalis* ir *Mergus merganser* (R = 0,9891), o labiausiai skyrėsi *Melanita nigra* ir *Mergus albelus* (R = = 0,8040). Pagal 8 polimorfinių lokusų alelių dažnių visumą nustatyta tokia Mergini tribos rūšių genetinio panašumo seka: ledinė antis (*Clangula hyemalis*), mažasis dančiasnapis (*Mergus albelus*), didysis dančiasnapis (*Mergus merganser*), (*Histronicus histronicus*), nuodėgulė antis (*Melanita nigra*), klykuolė (*Bucephala clangula*).