Phylogenetic relationships in the taxons of the order Anseriformes determined by the specificity of immunoglobulins

Sigita Slavėnaitė,
Aniolas Sruoga

Institute of Ecology,
Akademijos 2,
LT-2600 Vilnius, Lithuania

The paper deals with application of immunoglobulin specificity for phylogenetic investigations in the tribe Mergini from the order Anseriformes. Blood sera from smew (*Mergus albelus*), goosander (*Mergus merganser*), long-tailed duck (*Clangula hyemalis*), common goldeneye (*Bucephala clangula*), common scoter (*Melanita nigra*) and also polyspecific antisera obtained by immunisation of Chinchilla breed rabbits with the above sera were used in our investigations. The species-specific and non-specific antigenic determinants were detected in the species studied. Species-specific antigenic determinants were detected between the reaction of antigens and antibodies from the same species and non-specific between the reaction of antigens and antibodies from the blood sera of different species. Phylogenetic interpretation of antigenic heterospecificity was based on the equation of Dino and Georgi (1982). The data on genetic distances obtained by this equation showed the following course of genetic similarity: long-tailed duck, smew, goosander, common scoter, common goldeneye. The greatest genetic similarity was observed between long-tailed duck and goosander (D = 0.6636). Goosander and common goldeneye were most remote (D = 0.8450).

Key words: Mergini, Anseriformes, phylogeny, immunoglobulins

INTRODUCTION

In recent years, while solving disputable problems of animal phylogeny, systematics and domestication, contemporary geneticists seek to evaluate the whole complex of morphological, physiological, palaeontological, embryological, etiological, biochemical, immunological, etc. features determining the uniqueness of a species. The methods of immunogenetic analysis allow to follow the evolution of the chemical structure of proteins according to variations in their antigenic specificity. Therefore the immunogenetic parameters to which antigenic differences of individuals under investigation belong are of great value for determination of interspecies and intraspecies relationships on various taxonomical levels. Those parameters are also important for the observation of molecular evolution phenomena. Therefore, the objective of our work was to investigate the genetically determined antigenic differentiation of blood sera from individuals belonging to the sea ducks (Mergini tribe) of the order Anseriformes.

Waterfowl of the order Anseriformes are spread almost continent-wide, they habituate in various ecological conditions. The current taxonomic grouping of the order Anseriformes is derived principally from the classic work (Delacour, Mayr, 1945). There this order has been subdivided into suborders: Anhimae and Lamellirostres. Systematics generally agreed upon the composition of the tribe Mergini as an monophyletic group. Exceptions of this consensus were the failure to segregate the Mergini from the other diving ducks (Aythini) (Phillips, 1924) and tribal separation of the eiders from other Mergini (Delacour, 1959; Cramp, Simmons, 1977). Earlier morphological studies provide differing, fragmentary and often phenetic assessment of phylogeny within the tribe (Johnsgard, 1964). Also a preliminary, species-level phylogenetic analysis of the mergansers from the tribe Mergini using morphological characters has been presented (Livezey, 1986).

In our work we supply a phylogenetic interpretation of antigenic heterospecificity of the following species from the tribe Mergini of the order Anseriformes: smew (*Mergus albelus*), goosander (*Mergus merganser*), long-tailed duck (*Clangula hyemalis*), common goldeneye (*Bucephala clangula*), common scoter (*Melanita nigra*).

MATERIALS AND METHODS

Blood serum proteins of five species (smew), goosander, long-tailed duck, common goldeneye, common scoter from the tribe Mergini of the order Anseriformes have been used for investigations of antigenic heterospecificity. The blood of the birds that had perished after accidental trapping in the fishing nets was used for experiments. The sera were obtained by centrifugation at 1000–1500 rpm for 5 min. Then immunisation was carried out according to Viazov's method. For this purpose Chinchilla breed rabbits as recipients and the above sera as donors were used. The obtained polyspecific antisera and sera of the species under investigation were used for determination of species-specific and non-specific antigen–antibody reactions. Species-specific antigen–antibody reactions took place between the antigens and antibodies obtained after immunisation of rabbits with the serum from the same species. Thus, species non-specific antigen–antibody reactions were formed between the antigens and antibodies obtained after immunisation of rabbits with the serum from the different species. These reactions were detected using the method of double diffusion into agar gel according to Ouchterlony (Vaičiūnienė, 1997) and estimated as the number of precipitation lines.

The phylogenetic interpretation of Mergini tribe species antigenic heterospecificity was based on the Dino and Georgi equation (Dino, Georgi, 1982): genetic distance = 1 – X. There X is a non-weighed, simple matching coefficient obtained by dividing the number of species-specific antigen–antibody reactions and the total amount of antigen–antibody reactions.

RESULTS AND DISCUSSION

The species-specific and species-non-specific antigenic determinants were detected in the blood sera of the all species studied. They were expressed as precipitation lines formed in the crossed antigen–antibody reactions. The data on antigenic heterospecificity are supplied in Table 1. They show that the number of species-specific antigenic determinants in all species fluctuated from 6 to 9. Common scoter has the largest and long-tailed duck the least amount of species-specific antigenic determinants. The number of species-non-specific antigenic determinants fluctuated from 1 to 5. It is worth noting that antigens of common goldeneye formed one precipitation line with polyspecific antisera of two duck species, goosander and common scoter. At the same time antigens of common scoter blood serum formed four precipitation lines with polyspecific antisera of both merganser species.

Phylogenetic interpretation of antigenic heterospecificity of the species under investigation was based on the experimentally confirmed presumption that the structural similarity of molecules from the comparable taxons reflects their phylogenetic similarity (Wiley, 1981). Thus, according to the Dino and Georgi equation, genetic distances were calculated and data listed in Table 2. These data show the following course of Mergini tribe species genetic similarity: long-tailed duck, smew, goosander, common scoter, common goldeneye. The greatest genetic similarity was observed between long-tailed duck and goosander (D = 0.6636). Goosander and common goldeneye were most remote (D = 0.8450).

In our investigations of antigenic heterospecificity, homologous proteins from Mergini tribe species were detected. The evolutionary significance of antigenic heterospecificity has been determined by investigation of the role of genetic mechanisms in immunologic reactions. Many investigations have shown that the homologous genes also determine homologous proteins. The obtained data are actual for the further phylogenetic analysis of such a tribe, because detection of structural differences in biopolymers provides the possibility to estimate the similarity of comparable species genotypes. However, most data of the previous phylogenetic analysis of such tribes are based on investigations carried only on external features, which are determined by a great number of genes. Molecular studies of the phylogeny of the tribe Mergini are also limited. The composition of integumental lipids confirmed a moderate distance between common eider (*Somateria mollisima*) and several other genera (Jacob, Glaser, 1975). A clear distinction between the eiders and other Mergini was also found using feather proteins (Brush, 1976). A moderately large genetic distance between two

Table 1. **The species-specific (bold) and non-specific (normal print) antigenic determinants of the Mergini tribe species**

Antigens from	Antibodies against				
	Mergus albelus	*Clangula hyemalis*	*Mergus merganser*	*Melanita nigra*	*Bucephala clangula*
Mergus albelus	**7**	2	2	1	3
Clangula hyemalis	3	**6**	4	3	2
Mergus merganser	5	3	**8**	2	2
Melanita nigra	4	1	4	**9**	3
Bucephala clangula	3	2	1	1	**8**

Table 2. **Genetic distances among Mergini tribe species detected from antigenic differences**

Genetic distances among	Antigens from					Average
	Mergus albelus	*Clangula hyemalis*	*Mergus merganser*	*Melanita nigra*	*Bucephala clangula*	
Mergus albelus and *Clangula hyemalis*	0.700	0.75				0.7250
Mergus albelus and *Mergus merganser*	0.777		0.800			0.7889
Mergus albelus and *Melanita nigra*	0.875			0.6923		0.7835
Mergus albelus and *Bucephala clangula*	0.700				0.7272	0.7171
Clangula hyemalis and *Mergus merganser*		0.600	0.7272			0.6636
Clangula hyemalis and *Melanita nigra*		0.857		0.750		0.8035
Clangula hyemalis and *Bucephala clangula*		0.750			0.800	0.7750
Mergus merganser and *Melanita nigra*			0.800	0.6923		0.7461
Mergus merganser and *Bucephala clangula*			0.800		0.890	0.8450
Melanita nigra and *Bucephala clangula*				0.75	0.890	0.8200

species of *Bucephala* and the two other Mergini sampled (*Melanita* and *Clangula*) has been reported on the grounds of electrophoresis of 13 proteins (Patton, Avise, 1985). Analysis of phylogenetic relationships among 8 species of the tribe Mergini in the multilayer polyacrylamide gel system using 8 polymorhic loci of non-specific proteins (prealbumin1, prealbumin2, albumin, postalbumin, pretransferrin, posttransferrin, macroglobulin, and transferrin) also confirmed a comparatively large distance between *Bucephala clangula* and *Melanita nigra* and a similar course of genetic similarity (Slavėnaitė et al., 1998). A recent comparison of Anseriformes using DNA hybridization included only a single representative of the Mergini (*Melanita*) and therefore provided no insight into relationships within the tribe (Sibley, Ahlquist, 1990).

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Sigita Slavėnaitė, Aniolas Sruoga

IMUNOGLOBULINŲ SPECIFIŠKUMO PANAUDOJIMAS FILOGENETINIŲ RYŠIŲ NUSTATYMUI ŽĄSINIŲ (ANSERIFORMES) BŪRIO TAKSONOMINIUOSE LYGMENYSE

S a n t r a u k a

Straipsnyje aptariamos imunoglobulinų klasės baltymams priklausančių antikūnų specifiškumo panaudojimo galimybės žąsinių (Anseriformes) būrio jūrinių ančių (Mergini) tribos filogenetiniams tyrimams. Darbui panaudoti mažojo dančiasnapio (*Mergus albelus*), didžiojo dančiasnapio (*Mergus merganser*), ledinės anties (*Clangula hyemalis*), klykuolės (*Bucephala clangula*) ir nuodegulinės anties (*Melanita nigra*) kraujo serumai. Šių rūšių kraujo serumais imunizavus šinšilų veisles triušius gauti polispecifiniai antiserumai. Dvigubos difuzijos agaro gelyje metodu pagal Ouchterlony Mergini tribos rūšių kraujo serumai bei polispecifiniai antiserumai panaudoti rūšiai specifinėms ir nespecifinėms antigeninėms determinantėms nustatyti. Filogenetinė antigeninio heterospecifiškumo interpretacija atlikta, remiantis Dino ir Georgi formule (1982). Pagal šią formulę gautus genetinių distancijų duomenis nustatyta tokia Mergini tribos rūšių genetinio panašumo eiga: ledinė antis, mažasis dančiasnapis, didysis dančiasnapis, nuodegulinė antis ir klykuolė. Genetiškai artimiausios buvo ledinė antis ir didysis dančiasnapis (D = = 0,6636). Klykuolė ir didysis dančiasnapis buvo labiausiai genetiškai nutolę (D = 0,8450).

Raktažodžiai: Mergini, Anseriformes, filogenija, imunoglobulinai