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# **BULGARIAN ANTARCTIC RESEARCH**

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# RAPD analysis of Gentoo penguins' populations

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## ABSTRACT

The possibility for the use of the molecular-genetics RAPD-PCR method for studying penguins' populations was estimated. Based on the similarity indexes (S), intragroup similarity indexes ( $S_w$ ) were calculated between specimens in every group and a high level of relationship between penguins of two Antarctic Islands was detected ( $F_{st} = 7,89\%$ ). The most informative primer (OPM-02) was chosen for interspecific comparison of the genetic diversity of penguins. The characteristics obtained may be used for the description of the genomic profiles of penguins.

## KEY WORDS

Penguins, markers, PCR, RAPD, primer, polymorphism.

## INTRODUCTION

The Antarctic environment is characterized by extreme conditions, widely changing. Indeed, understanding the molecular, physiological and behavioral mechanisms by which Antarctic organisms adapt to them would provide information concerning the evolutionary and biological basis of the development of the animal world and the survival of animals in extreme polar conditions. Penguins are an important part of the Antarctic ecosystem and the study of their biology is an important contemporary issue of international Antarctic research communities.

There are two species of penguins that live in Antarctica (on the continent). Ukrainian Antarctic station "Academic Vernandsky" is located on the Antarctic Peninsula. Five species

of penguins live there, and three of them nest (Adelie, Gentoo and Chinstrap) (Cabot J. et al. 1992; Williams 1995). The research of Gentoo penguins (*Pygoscelis papua*) is carried out with the support of the international INTAS project and the Ukrainian Antarctic Centre.

The aim of the present work was to evaluate the use of the RAPD analysis for penguins' population research.

## MATERIAL AND METHODS

### 2.1. SOURCE OF GENOMIC DNA

Blood samples of penguins were collected at Piterman and Livingston Islands by the Ukrainian team during the 8 Antarctic expeditions in the summers of 2002-2004. The blood samples with heparin were kept at  $-20^{\circ}\text{C}$ . Genomic DNA was isolated by the modified salt extraction method (Maliuta et al. 1996). Also 20 probes were extracted by the standard phenol-chlorophorm method. We did not find any differences in DNA analysis obtained by both methods. Therefore, for routine extraction we used the salt extraction method as an inexpensive and simple one. The concentration of DNA probes was determined using a spectrophotometer based on absorbance at 260 and 280 nm, respectively, and was defined more accurately by electrophoresis in 0,8% agarose gel.

### 2.2. AMPLIFICATION CONDITIONS

A RAPD-PCR analysis was carried out as the modified method of Operon. A series of random primers 10 bp each has 60-70% GC contents (OPA, OPM, OPP (Operon Technologies, Alameda, CA, USA)) (Mishta et al. 2002) and an original primer (11 mer) were used for the RAPD analysis (Mishta et al. 2002).

A reaction mixture was prepared in a volume of 25 ml containing 100 ng of genomic DNA, PCR buffer (67 mM Tris-HCl, pH 8,8; 16 mM  $(\text{NH}_4)_2\text{SO}_4$ ; 0,1% Tween-20; up to 0,1 M b-mercaptoethanol; up to 5 mM  $\text{MgCl}_2$ ; 30 pmol primer; 200 mM each of dNTP and 1 U of Taq DNA polymerase (Fermentas). Amplification was performed in a AMPLY 4 Biokom programmed for 4 cycles of 1 min 20 sec at  $94^{\circ}\text{C}$ , 1 min at  $40^{\circ}\text{C}$ , 2 min at  $72^{\circ}\text{C}$ ; then for 36 cycles of 1 min at  $94^{\circ}\text{C}$ , 1 min at  $36^{\circ}\text{C}$ , 2 min at  $72^{\circ}\text{C}$  with the last cycle of 5 min at  $72^{\circ}\text{C}$ , using the fastest available transitions between each temperature.

### 2.3. AGAROSE GEL ELECTROPHORESIS AND VISUALIZATION OF BAND PATTERNS

Amplified products were analyzed by electrophoresis in 2-2,5% agarose gel and detected by staining with ethidium bromide (Maniatis et al. 1982). Plasmid pUC 19 digested by restriction endonuclease *Mva*I and plasmid pUC 19 digested by *Msp*I

were used as a molecular size standard. A negative control without any DNA template was included in each amplification batch.

#### 2.4. STATISTICAL ANALYSIS OF AMPLIFIED PRODUCTS

RAPD profiles were analyzed by RFLP Scan 3.12. (Scanalytics). Each band was considered as a RAPD marker. All the amplifications were repeated two times for analysis and only reproducible bands were considered. The amplification products of 150-1500 bp were scored across all samples as a binary matrix 1 when present or 0 when absent. Accordingly to obtained results the following indexes were estimated for every group of Gentoo penguins:

- 1.) Mean number of amplified fragments (N)

$$N = \frac{1}{n} \sum_{i=1}^n x_i, \quad x_i - \text{overall number of fragments in individual } i, \quad n - \text{overall number}$$

of individuals in a group;

- 2.) Percentage of polymorphic loci (P)

$$P = \frac{\frac{1}{n} \sum_{i=1}^n p_i \cdot 100}{N}, \quad \text{where } N - \text{mean number of fragments in a group, } n - \text{overall}$$

number of individuals in a group,  $p_i$  - overall polymorphic loci number in individual  $i$  of the group.

- 3.) Similarity indexes (index Chekanowski) (S)

$S = 2Fab / (Fa + Fb)$ , where  $Fab$  – number of the same fragments of specimens  $a$  and  $b$ ;  $Fa$  and  $Fb$  – overall fragments number of individuals  $a$  and  $b$ .

4.) Based on the values  $S$  intragroup similarity indexes APS ( $S_w$ ) and intergroup indexes ( $S_b$ ) (Maliuta et al. 1996; Morozova et al. 2002) were calculated between individuals of Gentoo penguins in every group;

$$S_w = \sum S / n, \quad n - \text{number of specimens in a group;}$$

$$S_b = \sum S_w / n, \quad n - \text{number of groups;}$$

- 5.) Coefficient of populations' subdivision:  $F_{st} = \frac{(1 - S_b)}{(2 - S_b - S_w)}$

Values are expressed as means  $\pm$  SD. Statistical significance was accepted at values of  $P < 0,05$ .

## RESULTS AND DISCUSSION

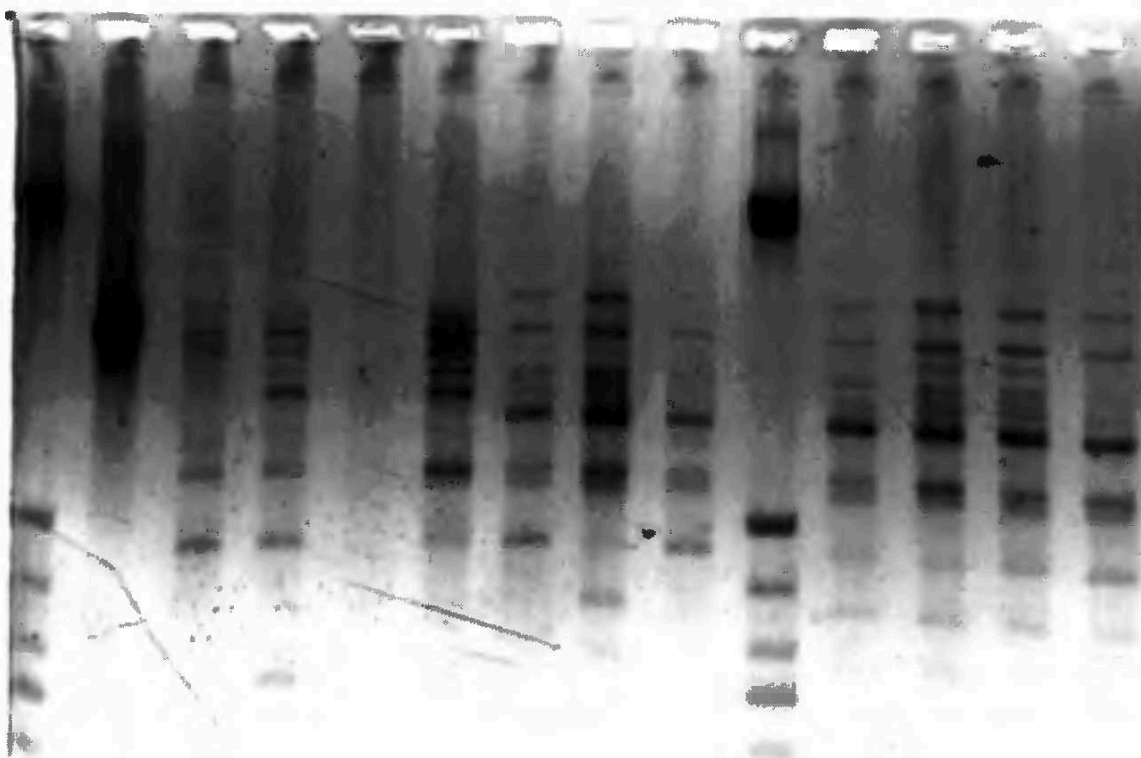
To study the molecular-genetic structure of Gentoo penguins' populations 3 primers (OPA-10, OPP-12, OPM-02) were chosen, which allowed revealing the most polymorphic and reproducible profiles and detecting from 3 to 13 RAPD-markers (300–1100 bp at the average).

DNA polymorphism was observed not only in the presence or absence of RAPD-patterns fragments with certain size but also in intensity changes of amplification fragments of the same size in different individuals. But this parameter concerning low reproducibility level wasn't taken into account. A DNA amplification with OPM-02 primer revealed 13 polymorphic RAPD-patterns (polymorphism  $D=23\%$ ), and another 10 amplification products present in all individuals of a given group, that is they were monomorphic. The same result was achieved with primers OPA-10 and OPP-12. Thus 11 marker bands were revealed using primer OPA-10: 4 of them were polymorphic ( $D=36,3\%$ ). The use of OPP-12 primer allowed to detect 9 RAPD-bands and 3 of them were polymorphic ( $P=33,3\%$ ).

To investigate further the molecular-genetic structure in Gentoo penguin's populations, a RAPD-PCR was carried out with 2 groups of birds: <sup>1</sup> 1 - 20 specimens from Livingston Island and <sup>1</sup> 2 - 18 penguins from Petermann Island.

Similarly to previous RAPD-analyses of Gentoo penguins' DNA, which suggested an approximately small variability, the DNA of related species was investigated: Adelie penguins (3 individuals) and Chinstrep penguins (4 individuals).

1 2 3 4 5 6 7 8 9 10 11 12 13 14



Picture 1.

Picture 1 represents one of the genomic profiles, which was obtained by means of a RAPD-analysis using a OPM-02 primer. 3 of 9 bands of Chinstrap penguins were polymorphic, 4-5 of 13 bands of Gentoo penguins' No 1, and 3 of 13 for group No 2 of Gentoo penguins.

An electrophoregram of RAPD-PCR: DNA samples of different penguin species (primer OPM – 02).

1, 10 - molecular weight marker -  $\delta$ UN19 plasmid, digested by MvaI restriction endonuclease and  $\delta$ UN19 plasmid digested by MspI restriction endonuclease;

2 - positive control (890 bp PCR fragment);

3 - DNA of Adelie penguin;

4,6 - DNA of Chinstrap penguins;

5 - negative control of PCR;

7 - 9 - DNA of Gentoo penguins (group 1 2);

11 - 14 - DNA of Gentoo penguins (group 1 1).

**Table 1.** Comparative analysis of the intra-specific variability using RAPD markers ( $\hat{D}\hat{I}$  – 02 primer)

Sample №	Number of individuals	Mean number of amplified fragment per individual ( $\pm$ SD)	Mean value $S_w$ and $S_b$ ( $\pm$ SD)	95% Confidence interval $S_w$	Percentage of polymorphic loci ( $\pm$ SD)
Pygoscelis papua (Gentoo) 1. Sample №1 (Livingston Island)	20	11,33 $\pm$ 0,685	0,886 $\pm$ 0,041	(0,682-1,144)	28,4 $\pm$ 0,585
2. Sample №2 (Petermann Island)	18	12,25 $\pm$ 0,622	0,918 $\pm$ 0,046	(0,708-1,129)	24,50 $\pm$ 0,521
3. №1+№2	38	11,79 $\pm$ 0,453	0,906 $\pm$ 0,042	(0,755-1,165)	25,45 $\pm$ 0,348

The mean number of amplified fragment per individual (N), the percentage of polymorphic loci (P) and similarity indexes ( $S_w$ ) didn't differ significantly in groups of Gentoo penguins (primer  $\hat{D}\hat{I}$ -02) (Table 1). The variability range was 11,33 – 12,25 (N), 28,4 – 24,5 (P) and 0,886 – 0,918 ( $S_w$ ). Thus we suggest a relatively low polymorphism level between individuals in each group and among groups  $F_{st} =$

7,89% (Kulikova et al. 2002, Morozova et al. 2002). A relatively lower  $S_w$  value and a higher  $D$  value in Group 1 was observed, that may be due to the higher nesting area of the particular group.

**Table 2.** Comparison of the inter-specific similarity

Primers	Species, $S_w$ ( $\pm$ SD)	Adelie	Chinstrap
OPM-02	Gentoo, sample №1 (Petermann Island)	0,323 $\pm$ 0,081	0,344 $\pm$ 0,047
OPA-10	Gentoo, sample №1 (Petermann Island)	0,482 $\pm$ 0,073	0,493 $\pm$ 0,069
OPM-02	Gentoo, sample №2 (Livingston Island)	0,354 $\pm$ 0,038	0,373 $\pm$ 0,013
OPA-10	Gentoo, sample №2 (Livingston Island)	0,508 $\pm$ 0,067	0,507 $\pm$ 0,054
OPM-02	Adelie	--	0,429 $\pm$ 0,07
OPA-10	Chinstrap	0,509 $\pm$ 0,158	--

The  $S_w$  comparison of  $\hat{I}\hat{D}\hat{A}$ -10 and  $\hat{I}\hat{D}\hat{I}$ -02 primers at the inter-specific level has shown a higher variability rate of the  $\hat{I}\hat{D}\hat{I}$ -02 primer (table 2). The variability range of the  $\hat{I}\hat{D}\hat{I}$ -02 primer amounted to 0,323 - 0,429, and was higher than that of  $\hat{I}\hat{D}\hat{A}$ -10 (0,482 - 0,509) (Morozova et al. 2002; Semyenova et al. 2002).

## CONCLUSIONS

Thus the self-descriptiveness of the RAPD markers for the molecular-genetic analysis of penguins' populations was determined. Accordingly to the obtained results there were no significant differences between two groups of Gentoo penguins

at all indices. Variability rate with  $S_w \cong 0,9$ , are typical mostly for intra-population variability  $F_{st} = 7,89\%$ . Therefore, high rate of relationship between specimens of two Antarctic islands could be suggested. Besides, primer ÎÐÏ-02 in comparison to primer ÎÐÀ-10 revealed a higher rate of polymorphism upon further analysis of individuals in three species ( $0,2 < S_w < 0,6$ ), this rate are mostly typical of the inter-specific variability rate.

## ACKNOWLEDGMENTS

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## REFERENCES

- CABOT J., CARBONERAS C., A. E., et al. (1992). Handbook of the birds of the world. Barcelona, Spain: Lynx Editions. 696 p.
- DE WOLF H., BLUST R., BACKELJAU T. (2004) The use of RAPD in ecotoxicology. - *Mutat Res*, 566(3): 249-262.
- KULIKOVA I.V., CHELOMANA G.N., GURAVLEV U.N. (2002) RAPD – PCR analysis of genetic variability in *Phasianus colchicus*. - *Genetic (Moscow)*, 38(6): 836 – 841. (In Russian, Abstract in English)
- MALIUTA S.S., DYBKOV M.V., TELEGEEV G.D. (1996) Study of DNA polymorphism in Ukrainian human population detected by M13 phage as a probe. - *Cytology and genetic (Kiev)*, 33(1): 31-35. (In Russian, Abstract in English)
- MISHTA M., DUBEY N., TOTTEY S.M. (2002) Phylogenetic relationships and genetic polymorphisms in wild Indian mice. - *Biomolecular Engineering*, 18: 281 – 288.
- MOROZOVA E.V., RYSKOV A.P., SEMYENOVA S.K. (2002) RAPD Variation in two trematode species (*Fasciola hepatica* and *Dicrocoelium dendriticum*) from a single cattle populations. - *Genetic*, 38(8): 1155 – 1162. (In Russian, Abstract in English)
- SEMYENOVA S.K., VASILYEV V.A., MOROZOVA E.V., et al. (2002) Fingerprinting and genetic diversity of American bison (*Bison bison*), Aurochs (*Bison bonasus*), and Gray Ukrainian cattle (*Bos Taurus*). - *Genetic*, 36(11): 1535–1545. (In Russian, Abstract in English)
- WILLIAMS T.D. (1995). *The Penguins*. Oxford University Press, Oxford. 295 p.