

;hjbkh\Z bHp_gdZ \ebygby]_h]jZnbq_kdhc baheypbb gZ kl_i_gv]_g_lbq_kdhc

;hjbkh\Z.¹, F_s_jkdB^{e2}, RiZdH<², =eZahF.², Eblh\c.B³, Jh`ghA<²

Hp_gdZ \ebygby]_h]jZnbq_kdhc baheypbb gZ kl_i.
kdhc h[hkh[e_gghklb \ ihimeypbDelphinapterosb

leucas jhkkbckdh]h >Zevg_]h <hklhdZ

Fhkdh\kdbc]hkm^Zjkl_gguc mgb_jklb_l bf_gb F < Ehfghghkh\Z Fh
Bgklblml ijh[e_f wdheh]bb b w\hexpbb bf : G K_jph\Z J:G Fhkdl
QmdhILBGJH :gZ^ujv Jhkkby

BorisovaE.A.¹, MescherskyI.G.², ShpakO.V.², GlazovD.M.², Litovka D. I.³, RozhnoV.V.²

Evaluation of effect of geographical isolation on level of genetic distinctness in beluga whale (*Delphinapterus leucas*) populations in Russian Far East

1. Lomonosov Moscow State University, Moscow, Russia
2. A.N. Severtsov Institute of Ecology and Evolution, RAS, Moscow, Russia
3. Chukotka branch of Pacific Research Fisheries Center, Anadyr, Russia

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gbdh]Azn \hklhqgh]h qlh k\b^
hij_ ^_ezgghc]_h]jZnbq_kdhc
fhjkdbo [_emo hl [_emo ;_jbg]h
db F_ev gbdh\ H^gZgdklujZ
^_e_gby hl^_evguo ihimeypbc
fhjy ^h kbo ihj hklZ\ZeZkv kikhj
ij_ ^_e_gby Zee_e_c fbdjhkZI_
ihdZaZe qlh [_em-Zf rkjZ d Z fh gb
lZjkdh]h e_lgbo kdhie_gbc ij_ ^
ihimeypbx Yau^jh\Zg Bkl k[hjg
[hevrbc bgl_j_k ij_ ^klZ\ey_l bh
jh\Zgghklb `b\hlguo wlbo kdhi
e_lm xsbo m [_j_]h\ aZiZ^ghc
^Z wlbo jZchgZ b jZa^_e_gu
q_f \ df abfh\dZ ©kZoZe
k_jgujZchgZo fhjy j_amevlZ
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ij_ ^iheZ]Zlv \hafh`ghklv bo \kZ
gby k ©dZfqZlkdbfb^a\k_ g b b c_i_j
>ey hp_gdb kl_i_gb]_g_lbq_kd
[_emo Hohlkdh]h fhjy dhZd lfZ d^
hlghr_gbx d [_emoZf ;_jbg]hVZ
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fb fbdjhkZI_eeblguo ehdmkh\
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b

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_ m aZiZ^gh]h ih[_j_`vy DZfq

The beluga whale is a species commonly occurring in the Sea of Okhotsk and the Bering Sea (De_c_g_g[_j] b ^j 1964). But beluga whales are rarely seen off southwest coast of the Kamchatka Peninsula and are almost never off the eastern coast of Kamchatka, which indicates that the Sea of Okhotsk beluga whale stock is geographically separated from the Bering Sea stock and the Arctic stock (F_evg b2001). However, the possibility of existence of separate stocks within the Sea of Okhotsk has been denied. Analysis of allelic distributions of microsatellite loci has shown that beluga whales that form the Sakhalin summer aggregation and the Shantar Islands summer aggregation belong to the same population (A_u d h b Z j, this book). Of even greater interest is the issue whether beluga whales that aggregate in the above-mentioned sites are isolated from the stock summering off the D P F K D W N I coast. Although the Sakhalin summer aggregation is separated from the D P F K D W N D V s u z h e r w g g f aggregation by a distance of over 1000 km, the fact that Sakhalin Amur stock spends the winter in the northern part of the Sea of Okhotsk (as was indicated by satellite tracking data RiZ d ^j 2010) leaves open a possibility of the encounters and mating between the Sakhalin Amur stock and Kamchatka D V Z H stock during the winter and spring months.

To assess the degree of genetic isolation both between different groups of the Sea of Okhotsk population and between the Sea of Okhotsk and the Bering Sea populations of beluga whales, we estimated allele frequencies at eight nuclear DNA microsatellite loci: DlrFCB4, DlrFCB5 and DlrFCB17, EV94Mn, 415/416, 417/418, 464/465 and 468/469.

kdhf ebfZg_ ;hevrbgkl\h ijh[f_lh^hf ^bklZgpbhgghc [bhikb qZklwlfzj\uo `b\hlguo MgbDZ jZaph\ \ayluo f_lh^hf [bhikbb ijhp_kk_]_ghlbbibjh\ZgbiybgiZeb ghklk ihfhsvx lPbZ]ghklbbd ih Jhma Rosel Dhebq_kl\h hkf k[hjZ Z lZd`_ iheh\hc khklZ\ gu \ lZ[e Z ihdZaZI_eb jZa ^hfm ba e h d m k f e

Samples were collected in the Sakhalin Gulf, on Khar-FKDWND, V, and in the Anadyr Estuary. Most of the samples were collected by the method of remote biopsy sampling. Some samples were collected from dead animals. Uniqueness of biopsy samples was identified by genotyping. Gender verification was performed by PCR, using the method described in (Rosel 2003). The number of individuals, the years the samples were collected, and gender identity are shown in Table 1. Diversity indices for all loci are shown in Table 2.

JZchg k[Locality	= h^u years	f	,	g h n/r	bcero total
KZoZebgkdb/Sakhalin Bay	2009-2011	29	24	2	55
AZiZ^gZy DZfZqZldZ W, Kamchatka	2010-2011	25	7	-	32
:gZ^ujkdbc ebfZg Anadyr Estuary	2010-2011	57	15	2	74

LZ[e JZchgub]h^u k[hjZdhebq_kl\h ij ZgZebabh\Zgguo hkh[_c
Table 1. Localities, years and samples sizes.

LZ[e JZaghh[jZ f b d Z k e Z e e b l g u o e h d m k h \ \ \ 0 j i z d Z g a l s e g m k o e l b a h f_q_gguo Z e b g Z d e x ^ Z _ f u c m j h \ _ g v] _ l h e a b] b ^ z h k l u c m j h \ _ g b] _ l _ j h a] h l g h k b m j h \ _ g v a g Z q b f h k l b i j b h p _ g d _ i g k h b l g b k l C a z j g o k _ j j Z l k e _ ^ g y y k l j h d Z ^ e y q b k e Z Z e e _ e _ c i j b \ h ^ b l k y b o k m f f Z ^ e y i h d Z a Z I _ e _ c k j _ ^ g b _ a g Z q _ g b y

Table 2. Allelic diversity of eight microsatellite loci in samples of beluga whale from three localities. Als – number of alleles; Ho – observed heterozygosity; He – expected heterozygosity; P – statistical significance level for escape from Hardy-Weinberg equilibrium. Bottom line: a sum for number of alleles and means for heterozygosity indices.

E h d m Locus	KZoZebgkdbc aZ Sakhalin Bay, n=55				AZi DZfZqZldZ W, Kamchatka, n=32				:gZ^ujkdbc ebfZg Anadyr' Estuary, n=74			
	Als	Ho	He	P	Als	Ho	He	P	Als	Ho	He	P
Cb4	10	0,80	0,80	0,66	7	0,68	0,78	0,08	8	0,81	0,76	0,18
Cb5	5	0,71	0,66	0,58	4	0,66	0,58	0,95	4	0,56	0,6	0,67
Cb17	8	0,72	0,78	0,76	7	0,81	0,77	0,98	11	0,72	0,84	0,18
Ev94	4	0,65	0,68	0,24	4	0,68	0,69	0,40	6	0,81	0,79	0,71
415	4	0,74	0,69	0,69	4	0,75	0,74	0,97	4	0,62	0,72	0,17
417	8	0,78	0,75	0,17	8	0,78	0,81	0,05	9	0,79	0,81	0,25
464	3	0,41	0,50	0,04	3	0,39	0,63	0,01	5	0,4	0,49	0,14
468	5	0,53	0,69	0,04	4	0,55	0,66	0,10	7	0,64	0,75	0,11
	47	0,67	0,69		41	0,66	0,71		54	0,67	0,72	

M ©ZgZ^ujkdbo^a `b\hlguo ih kj Z fhjkd b f b ^ a h l f _ q _ g h [h e v r _ _ d h e b q [h e v r b g k l \ Z e h o m k k h e b v d h [h e _ _ y u g b _ i h d Z a Z I _ e b j Z a g h h j j Z a b y < f h j y [h e v r _ _ q b k e h Z e e _ e _ c [u e i Z o Z e b g k d b o ^ a [_ e m o h ^ g Z d h k j _ ^ g b a b] h l g h k l b m ^ \ m o h o h l h f h j k d b o h ^ b g Z d h \ u F _ ^ m \ k _ f b l j [f h j d Z u f b h l f _ q _ g u y u k l h \ _ j g u _ h l e b q b y \ q Z k l h I Z o \ k l j _ l Z [e q l h k \ b ^ _ l _ e v k l \ m _ l h [b j Z a g u f j _ i j h ^ m d l b \ g u f j j m i i z f i h i m

Beluga whales from the Anadyr Estuary population had a greater number of alleles at most loci; somewhat higher mean diversity index values than beluga whales from the Sea of Okhotsk population. Comparison of different groups of the Sea of Okhotsk population showed a greater number of alleles in Sakhalin stock, but mean heterozygosity values both Sea of Okhotsk stocks were almost the same. The highly significant differences in allele frequencies between three sample sets tested (Table 3) show that three stocks belong to different reproductive groups.

ljb wlfh mjh_gv]_g_lbq_kdbo hZebgkdh]h aZeb\Z hl`b\ghghuoihaZjDZfqZldb hdZau\Z_lky ^Z`_g_kdm_gv hlebqbc ©dZfqZlkdbo^a [_emob]h ebfZgZ

Moreover, the genetic differences between the Sakhalin VWRFN DQG WKH .DPFKDWND¶V even somewhat greater than the genetic differences between the Anadyr Estuary stock and the Kamchatka ND¶V ZHVW FRDVW VWRFN

:gZeba f_lh^hf deZkl_jbaZp Structure 2.3.3, Pritchard et al. 2000) went to confirm the existence of obvious heterogeneity of the entire universal set: there was a higher probability that the samples being analysed were divided into three groups (the log probability LnP = -537.2) rather than two (LnP = -600.3). The heterogeneity directly correlated with the sample collection locations. All the three sets of samples (samples from the Anadyr Estuary, Kamchatka, Sakhalin) had a very high probability of belonging to their own stock (Fig. 1).

Using a clustering method (the software Structure 2.3.3, Pritchard et al. 2000), went to confirm the existence of obvious heterogeneity of the entire universal set: there was a higher probability that the samples being analysed were divided into three groups (the log probability LnP = -537.2) rather than two (LnP = -600.3). The heterogeneity directly correlated with the sample collection locations. All the three sets of samples (samples from the Anadyr Estuary, Kamchatka, Sakhalin) had a very high probability of belonging to their own stock (Fig. 1).

Table 3. Samples pairwise FSTs (above diagonal) and statistical significance level (below diagonal) for the samples comparison by frequencies of eight microsatellite loci alleles.

Locality	Sakhalin Bay	W. Kamchatka	Anadyr' Estuary
Sakhalin Bay	***	0,06768	0,07422
W. Kamchatka	0,00000	***	0,06556
Anadyr' Estuary	0,00000	0,00000	***

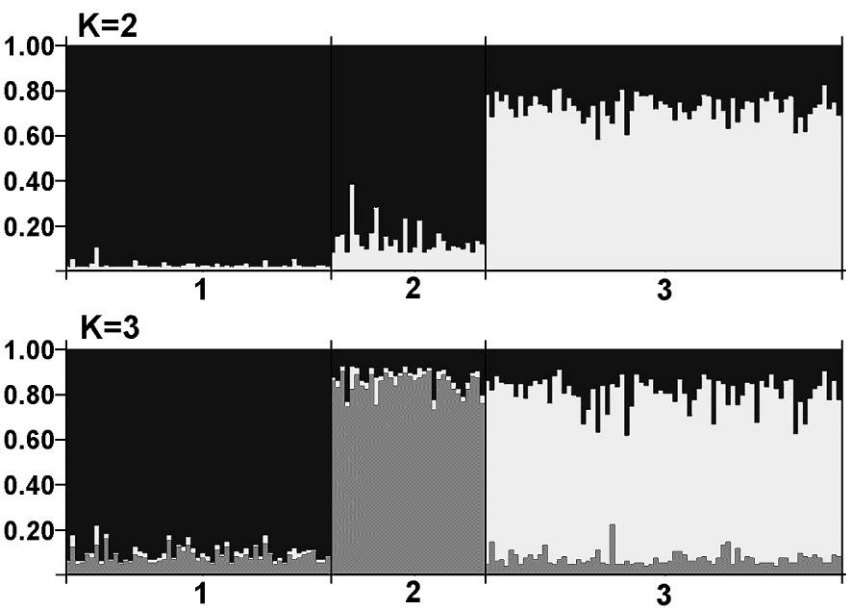


Fig. 1. Probability of individuals of total sample (n=161) to belong to one of two (K=2) or three (K=3) hypothetic genetic clusters. Admixture-LOCPRIOR model, 500000 replications. Sampling localities: 1 – Sakhalin Bay, 2 – Western Kamchatka, 3 – Anadyr' Estuary.

LZdbf h[jZahf ijb hlkmlkl\bb \uz nbq_kdbo ij\]jZ^_eZo _^bg hc Z\hafh`ghc [ebahklb jZchgh\ abth

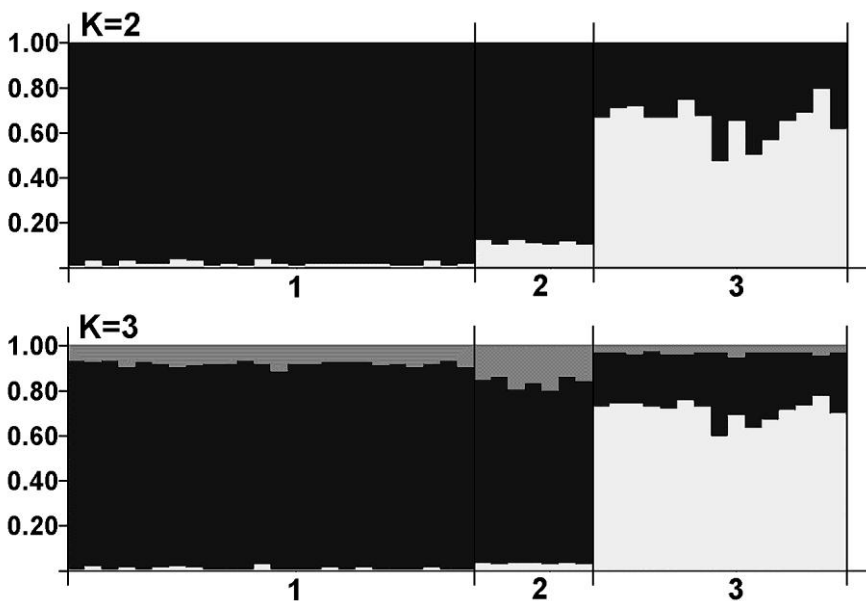
Thus, despite the absence of obvious geographical obstacles between two stocks inhabiting the same Sakhalin and despite the possible nearness of their

]jZpbc f_`^m ^\mfy]jmiiZfb [_
 fhjy ih^^_j`b\Z_lky g_ f_gvrbc m
 kdhc h[hkh[e_gghklb q_f f_`^r
 jZa^_e_ggufb ihimeypbyfb HohM
 fhj_c
 <Z`gh hlf_lblv qlh ijb jZa^_evgh
 b kZfhd klZlkbq_kdb ^hklh_jg
 lj_fy jZchgZfb khojZgyxlky ^ey Zh
 dh mjh_gv hlebqbc kZfph\ DZfd
 (Fst= hdZau\Z_lky \ ^\Z jZa^_evgh
 fhdba wlbo jZfgh_ ljb wlhf
 aZiZa^_gZfqZlkdhc \uf[h^h kZfpZfb
 dZfb lZd`_ h[gZjm`b\Zxlky kms_k
 \ qZklhlZo \klj_qZ_fhklFst=0.0356
 P=0,018 \ lh \j_fy dZd \u[hjdb kZf
 KZoZebgkdh]h ba Zeb\gZ^ujkdh]h_e
 lbq_kdb h^ghjh^gu
 ljb bkihevah\Zgbb f_lh^Z dheZklh
 e_gghklv kZfhd dZfqZlkdhc]jmZ
 oZebgkdh]h aZeb\Z g_ \uy\ey_lZ
 aZgh djbFst=0.0356 jZaebqby
 dZd klZlkbq_kdb ^hklh_jgZbf_gvr_Z
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]jmiiZf @ZgZ^ujkdhc^ b @hLmP=±
 ijb D LnP= ± ijb D jbk
 \j_fy dZd ijb ZgZeba_ kZfph\ ijb
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tering grounds and migration routes. These two stocks have a level of genetic isolation that is as high as the level of genetic isolation between the geographically separated the Sea of Okhotsk stock and the Bering Sea stock.

It is of importance that separate analyses for males and females still showed statistically significant differences between the three areas tested. Although the level of genetic isolation between the Kamchatka and Sakhalin females (Fst=0.0372). Moreover, there was a significant difference in allele frequencies (Fst=0.0356 P=0.018) between males and females of the Sakhalin Gulf stock or between males and females of the Anadyr Estuary stock.

The clustering method did not reveal any genetic isolation between the Kamchatka and Sakhalin stocks and females of the Sakhalin Gulf stock, though we have already mentioned that there were statistically significant differences in the Fst values between two groups (the Anadyr and the Sea of Okhotsk ones) had the minimum value of the log probability: LnP= -1059.9, D=2 and LnP= -1062.2, D=3, Fig. 2. However, the analysis of males by the clustering method showed isolation of all the three groups: LnP= -2449.6, D=3 and LnP= -2485.4, D=2, Fig.3.



Jbk <_jhylghklv
 e_`ghklb hkh[_c \
 kZfhd Q d h^g
 ^\mo D beblj_c
]bihl_lbq_kdbo_]_c
 kdbo deZkl_jh\bFf
 jZchg[hjZ[jZa^_evgh
 jbk1.

Fig. 2. Probability of individuals of sample of females (n=46) to belong to one of two (K=2) or three (K=3) hypothetical genetic clusters. Model and localities – see fig.1.

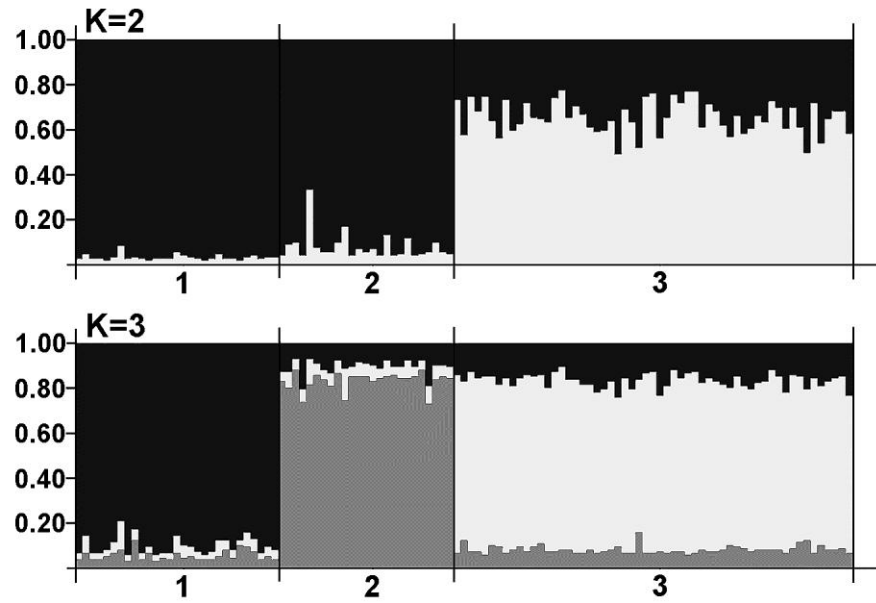
>ey ij_^klZ\e_gby h lhf k q_f fh

To understand the possible causes of the isolation,

;hjbkh\Z b H p _gdZ \ebygby]_h]jZnbq_kdhc baheypbb gZ kl_i_gv]_g_lbq_kdhc

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bkke_^h\Zgby b m_ebq_gb_ h[t_
JZ[hIZ \uiheg_jZdZo Ijh]jZffu ©
;_euc dbI^a BIWW J:G Ihklhygg h
wdki^bpb J:G ih ba mlgugb xDjZ
dgb]b Jhkkbckdhc N_^_jZpbb b
guo `b\hlguo nZmgu Jhkkbb ijtk
kdh]h]_h]jZnbq_kdh]h h[s_kl\Z

ther research and large sample sizes are required.
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Society. The program is part of the Permanent exped
ition of Russian Academy of Sciences studying Red Book
animals and other focus species of Russia



Jbk <_jhyIghklv ij
e_`ghklb hkh[_c \ \u[
kZfph\ Q d h^ghf
^mo D beblj_o D
]bihl_lbq_kdbo_]_g_lb
kdbo deZkl_jh\bFh^_c
jZchgu[hjZ[jZch\k f
j b k 1.

Fig. 3. Probability of individuals of sample of males (n=111) to belong to one of two (K=2) or three (K=3) hypothetic genetic clusters. Model and localities – see fig.1.

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De_cg_g[_j] K ? Y[e h d h \ : < ;_evdh\bq < F LZjZk_\bq F G
>.OHLQH QE H U J 6 (< D E O R N R Y \$ 9 % H O B N U g a (D e l p h i n a p t e r u s F e d c a s) M V H Y
investigation of the species. Academy of Sciences of the USSR, Moscow. Translated by Israel Program for Scientific Translations. 455 pp.]
F_evgbckh\2001.;_emoZ Hohlkdh]h-fhjy JKamevIZlu bkke_^h\Zgbc fhjkdb
sbo >Zevg_]h <hklhdZ]] <G,BFJHk d [Melnikov V.V. 2001. Beluga Whale of the Sea of
Okhotsk. Pp. 5-58 in Research work on marine mammals in the North Pacific in-2000. VNIRO, Moscow]
RiZdH<, Wg^jxk>, =eZa>hF., Eblh\c.Z, Oh[[kD, FmoZf_EhF 2010. K_ahggu_ fb]jZp
hohlhfjhkdhc [_emob 'HOSK L Q D S W H U X V Z O n h j * B D M k d l r g i e] h g b k y Z o Z l e b e k h
36(1): 5662 [Shpak O.V., Andrews R.D., Glazov D.M., Litovka D.I., Hobbs R.C., Mukhametov L.M. 2010.
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56-62]
Ya ud h F. Z., F_s_jkd B e., RiZ d H <, = e Z a > h F., E b l h \ c . Z , ; h j b k h ? Z., J h ` g h < 2012.
F h e _ d m e y j g h l b q _ k d b c Z g Z e Z a m j k d z l p b o h r Z g l Z j k d h] h e _ l g b o D e l p h i n e _ g b
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Litovka D.I., Borisova E.A., Rozhnov V.V. 2012. Molecular genetic analysis of Sakhalin and Shantar bel
J D ¶ ' H O S K L Q D S W H U X V O n h j * B D M k d l r g i e] h g b k y Z o Z l e b e k h
Pritchard J.K., Stephens M., Donnelly P. 2000. Inference of population structure using multilocus genotype da
Genetics, 155(2): 945-959.
Rosel P.E. 2003. PCR-based sex determination in Odontocete cetaceans.. Genetics 4: 647-649.