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Reproduction and genetic structure of a freshwater crustacean *Lepidurus arcticus* from Spitsbergen

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Abstract: Genetic variability of two populations of Arctic freshwater crustacean *Lepidurus arcticus* (Crustacea, Notostraca) occuring in small tundra reservoirs near the Polish Polar Station in Hornsund (Spitsbergen) was studied. The allozyme polimorphism analysis of 11 enzyme systems indicates a considerable distinctness and genetically heterogeneous character of the populations of *L. arcticus* inhabiting freshwater reservoirs of similar hydrological conditions situated close to each other (2 km). Our research revealed a complicated and genetically heterogeneous character of the populations. Three hypotheses about genetic structure and type of reproduction were tested: hypothesis M – the free transfer without assigning a clone for particular reservoir and the lack of double mutations; hypothesis I – separation of individuals between reservoirs and the possibility of double mutations; hypothesis S – presence of partial sexual reproduction in the population, probably with males. In conclusion participation of males in reproduction is probable, despite their presence was not recorded in our study. Males usually occur in low numbers or not every year. The populations' clonal structure as well as the genetic diversity typical of species reproducing sexually was observed. The Hardy-Weinberg genetic equilibrium is maintained as new clonal lines appear due to the genetic diversity increasing incidentally as a result of sexual reproduction.

Key words: Arctic, Svalbard, Crustacea, *Lepidurus arcticus*, parthenogenesis, males, allozymes.

Introduction

Lepidurus arcticus (Pallas, 1793) (Crustacea, Notostraca) occurs in shallow freshwater reservoirs of various areas of the Arctic. Its presence has been recorded in Spitsbergen, Bear Island, Iceland, Siberia, Novaya Zemlya, Franz-Josef Land, Greenland, Canadian Arctic (Olofsson 1918; Janiec 1996; Beaton and Hebert 1988; Vekhof 1997; Eriksson *et al.* 1999; Christofersen 2001; Jeppesen 2001; Hessen *et al.* 2004; Wiśniewska-Wojtasik and Walczak 2005). It has also been recorded outside the Arctic in the lakes in Southern Norway (Fjellheim *et al.* 2001, 2007). The mode of reproduction of this predatory crustacean is not clear and was described as

parthenogenetic in high latitudes with unisexual populations (Hessen *et al.* 2004) as well as hermaphroditic mode of reproduction (Sassaman 1991). Occasionally males were also recorded (Beaton and Hebert 1988). The role of males in reproduction and evolution remains unclear (Beaton and Hebert 1988; Dodson and Frey 1991; Green and Noakes 1995). The data concerning occurrence of males in *L. arcticus* populations vary. Although, Beaton and Hebert (1988) reported the occurrence of one male per 100 females, observations made in the region of Iceland reveal the absence of males in the analysed reservoirs (Eriksson *et al.* 1999). *L. arcticus* has a single-season life cycle which begins when lake bottom sediments defrost (Eriksson *et al.* 1999), usually it terminates in August or September with laying eggs which hibernate. The species is widespread in Spitsbergen (Olofsson 1918), still it seems to be fastidious about selecting its living environment (Wiśniewska-Wojtasik and Walczak 2005). Morphological parameters (carapax length, carapax width, telson length) of *L. arcticus* from Svalbard were rather variable, with large differences between minimum and maximum size (Hessen *et al.* 2004).

In branchiopod crustaceans the reproduction process varies. Androdioecy has been described from two orders. In spinicaudatan clam shrimp of the genus *Eulimnadia* and in notostracan tadpole shrimp *Triops newberryi* one can find both hermaphrodites and males. The hermaphrodites can either self-fertilize or can mate with males but they cannot cross with other hermaphrodites (Weeks *et al.* 2006). In notostracans a few population types were observed: unisexual (a complete absence of males in a large sample >50 individuals), bisexual, unisexual + bisexual coexisting, and with different mode of reproduction: hermaphroditic, outcrossing, parthenogenetic and outcrossing + hermaphroditic coexisting (Sassaman 1991). The self-fertilization of hermaphrodites observed in tadpole shrimp *Triops* is considered as a degenerated sexuality. The hermaphroditic populations of *Triops* sp. tend towards a complete individual homozygosity, but frequently with high levels of polymorphism in the population. Their existence demonstrates that these genomes have evolved by tolerating high levels of homozygosity (Garcia-Velazco *et al.* 2009). Correlation of morphological and genetic differentiation of notostracans (*Triops* species) were not observed (Sassaman *et al.* 1997).

The aim of our research was to analyse the genetic structure of the populations inhabiting freshwater reservoirs of similar hydrological conditions: temperature, oxygenation, electrolytic conductivity, pH (Nowiński and Wiśniewska-Wojtasik 2006) and separated by a distance of about 2 km. The knowledge of hydrological parameters in reservoirs studied is necessary for analysis of the genetic structure of populations by allozyme polymorphism, because of reported influence of environmental conditions on some selected alleles or their frequency (Zamer *et al.* 1989; DiMichele and Powers 1991; Shikano *et al.* 2000; Virgilio and Abbiati 2004; Yap *et al.* 2004).

We tested three hypotheses on genetic structure and type of reproduction. The hypothesis M assumes: (a) the free transfer of individuals without assigning a clone to the particular reservoir, (b) only single mutation. The hypothesis I as-

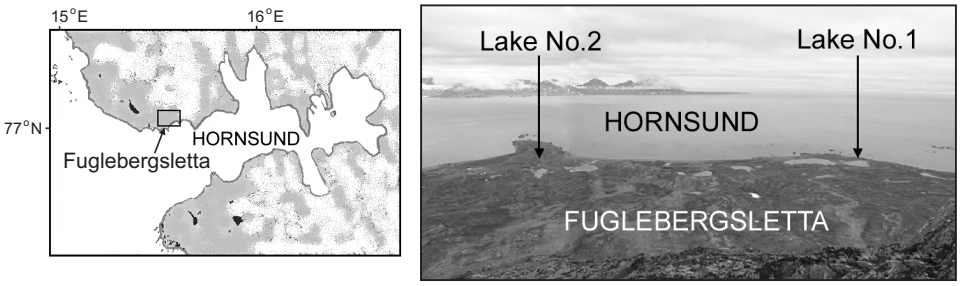

Fig. 1. Research area and sampling stations: Lake No. 1 and Lake No. 2.

sumes: (a) transfer of individuals between reservoirs forbidden, (b) the possibility of double mutations. The hypothesis S assumes partial sexual reproduction in population, probably with presence of males.

Study area

The area of Hornsund fiord has a typical, periglacial landscape. In the annual hydrological cycle streams and ponds of Hornsund area have two basic phases: a passive one, taking place in the period of temperatures below 0°C when accumulation and retention of water occurs and and active one (in summer) when the hydrological network is functioning. In the ionic structure of the reservoirs studied, the domination of bicarbonates was observed, yet considerable proportion of chlorides was also noted. This indicates the influence of land factors as well as sea factors (Krawczyk *et al.* 1990). Physico-chemical properties of shallow water reservoirs in the Hornsund region are rather variable (Nowiński and Wisniewska-Wojtasik 2006). Out of 50 reservoirs examined only in 13 ponds *L. arcticus* has been recorded. To collect the material for genetic studies we have chosen two reservoirs, where comparatively rich populations of this species occurred (Fig. 1). These reservoirs are situated in the vicinity of Polish Polar Station in Hornsund, in Fuglebergsletta area, separated by a distance of about 2 km. Their hydrological parameters (temperature, electrolytic conductivity, pH, oxygen saturation) during summer season 2001 were very similar (Fig. 2).

Material and methods

Material was collected at the end of July 2001. A hundred of mature specimens (females) from each population (called P1 and P2) were sampled and frozen in liquid nitrogen for transportation, then stored at -80°C prior to analyses. Before conducting allozyme polimorphism analysis the individuals were measured (without furca). The mean value of the length of *L. arcticus* was calculated.

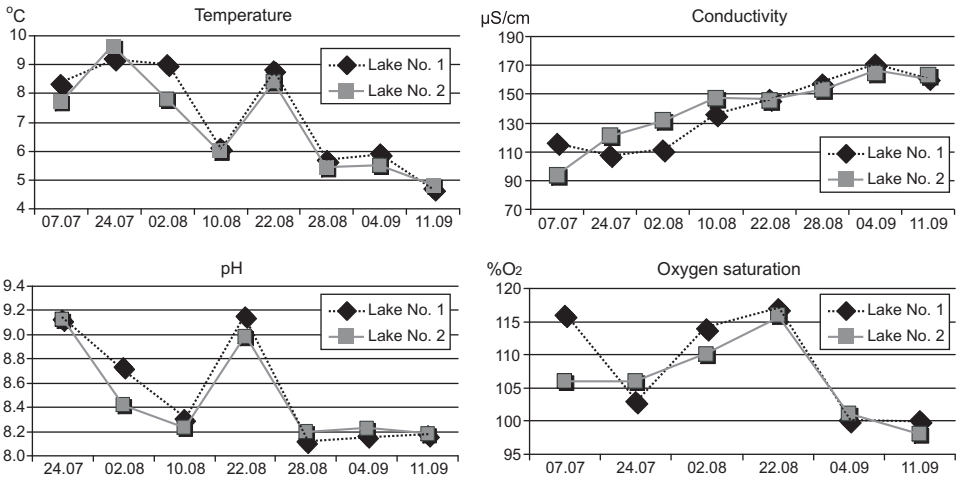

Fig. 2. Seasonal changeability of temperature, conductivity, pH and oxygen saturation in studied lakes, summer 2001 (according to Nowiński and Wiśniewska-Wojtasik 2006).

The allozyme polymorphism analysis were conducted in concordance with standard procedures (Richardson *et al.* 1986; Hebert and Beaton 1989). The 11 enzyme systems analysed were: alcohol dehydrogenase (ADH, E.C.1.1.1.1), alkaline phosphatase (ALP, E.C.3.1.3.1), esterases (EST, E.C.3.1.1.1), glucose-phosphate isomerase (PGI, E.C.5.3.1.9), hexokinase (HEX, E.C.2.7.1.1), isocitrate dehydrogenase (IDH, E.C.1.1.1.42), lactate dehydrogenase (LDH, E.C.1.1.1.27), malate dehydrogenase (MDH, E.C.1.1.1.37), malic enzyme (ME, E.C.1.1.1.40), 6-phosphogluconate dehydrogenase (6PGDH, E.C.1.1.1.44), phosphoglucomutase (PGM, E.C.2.7.5.1). The results of electrophoretical pictures were analysed with respect to genotypes of all individuals.

Results

Only females were found in the investigated populations in all checked reservoirs and no males were recorded among specimens collected for the genetic analysis. The length variable in populations P1 and P2 was low and similar to each other. The mean length of female bodies from P1 population was 6.9 mm (standard deviation +/-1.3 mm), and from population P2 7.0 mm (standard deviation +/-1.5 mm). The analysis of 11 enzyme systems for the population from lake 1 (population 1) and lake 2 (population 2) revealed the presence of 16 genetic loci, of which 7 loci were monomorphic (Tables 1, 2). The other loci were polymorphic but the clonal structure analysis based on 4 polymorphic loci: IDH-1, LDH, MDH and ME was possible only for 49 specimens of each population (Table 3). Good electrophoretic pictures for all studied loci in individuals were collected only for this number of specimens.

The Hardy-Weinberg equilibrium was calculated for IDH-1 and ME on account of the observed presence of heterozygotes and two alternative homozygotes (Table 2).

The lack of observed males but also the presence of heterozygotes only for ALP-1, EST-1 (population P2), PGI-1 and HEX-1 (population P1) shows clonal structure of populations. According to Hardy-Weinberg principle, homozygotes and heterozygotes should occur if males take part in reproduction. If we take into account only the mentioned enzymes of the investigated populations, it should be stated that two clones occur (No. X1 and No. X2), characteristic of the populations from reservoirs 1 and 2 respectively. Heterozygosity for one of populations is 1 (H = 1). Clone No. X1: aa [ALP-1], aa [EST-1], ab [PGI-1], ab [HEX-1]; Clone No. X2: ab [ALP-1], ab [EST-1], bb [PGI-1], bb [HEX-1]. The conducted analyses allowed the determination of the clonal structure of the investigated populations which is a distinctive feature in the case of parthenogenetic organisms.

Table 1

Allelic frequencies observed at the 16 loci in the studied populations of *L. arcticus* (H – heterozygosity, N – number of individuals), grey cells – monomorphic loci

Locus	Lake 1 Population P1	Lake 2 Population P2	Locus	Lake 1 Population P1	Lake 2 Population P2
ADH			IDH-2		
a	1.0	1.0	a	1.0	1.0
H (N)	0.0 (70)	0.0 (58)	H (N)	0.0 (68)	0.0 (40)
ALP-1			LDH		
a	1.0	0.5	a	0.006	0.0
b	0.0	0.5	b	0.994	1.0
H (N)	0.0 (44)	1.0 (16)	H (N)	0.012 (84)	0.0 (95)
ALP-2			MDH		
a	1.0	1.0	a	0.011	0.0
H (N)	0.0 (48)	0.0 (16)	b	0.989	1.0
			H (N)	0.022 (93)	0.0 (99)
EST-1			ME		
a	1.0	0.5	a	0.121	0.011
b	0.0	0.5	b	0.879	0.989
H (N)	0.0 (33)	1.0 (32)	H (N)	0.212 (66)	0.023 (87)
EST-2			PGI-1		
a	1.0	1.0	a	0.5	0.0
H (N)	0.0 (70)	0.0 (29)	b	0.5	1.0
			H (N)	1.0 (52)	0.0 (17)
HEX-1			PGI-2		
a	0.5	0.0	a	1.0	1.0
b	0.5	1.0	H (N)	0.0 (96)	0.0 (81)
H (N)	1.0 (48)	0.0 (51)			
HEX-2			PGM		
a	1.0	1.0	a	1.0	1.0
H (N)	0.0 (61)	0.0 (48)	H (N)	0.0 (87)	0.0 (33)
IDH-1			6PGDH		
a	0.167	0.0	a	0.0	0.009
b	0.833	1.0	b	1.0	0.991
H (N)	0.303 (66)	0.0 (49)	H (N)	0.0 (48)	0.019 (54)

Table 2

Polymorphic loci and genotypes in the studied populations of *L. arcticus*

Locus genotype	Population P1 Number of individuals	Population P2 Number of individuals	Locus genotype	Population P1 Number of individuals	Population P2 Number of individuals
ALP-1			LDH		
aa	44	0	ab	1	0
ab	0	16	bb	83	95
EST-1			MDH		
aa	33	0	ab	2	0
ab	0	32	bb	91	99
HEX-1			ME		
ab	48	0	aa	1	0
bb	0	51	ab	14	2
			bb	51	85
			χ^2	0.001 $p > 0.99$	0.017 $p > 0.99$
IDH-1					
aa	1	0	PGI-1		
ab	20	0	ab	52	0
bb	45	49	bb	0	17
χ^2	0.552 $p > 0.90$				
			6PGDH		
			ab	0	1
			bb	48	53

The variants observed for IDH-1, LDH, MDH, ME and 6PGDH may indicate either new mutations or male participation in reproduction. The value χ^2 calculated for IDH-1 and ME indicates the genetic equilibrium in the investigated populations (Table 2). However, assuming that we deal with a clonal structure only on the grounds of the analysis of 4 polymorphic genetic loci, the occurrence of 7 clones can be stated (Figs 3 and 4). The same clone had the highest frequency both in lake 1 and lake 2 (clone No. 1). Another clone (clone No. 2) was recorded in reservoir 2, and five other clones (clones 3–7) co-occured with clone No. 1 in reservoir 1. The recorded clones are even 5 mutations apart (Table 3), being a significantly high value and may suggest migration and population mixing, which is contrary to the data of clones No. X1 and No. X2.

Two hypotheses considering the origin of clones can explain our results. The first, hypothesis M, assumes migration whereas the second, hypothesis I, assumes isolation. For both hypotheses we regarded the clone No. 1 as ancestral because of its highest frequency. The hypothesis M – assumes: (a) the free migration of a clone to particular reservoir, (b) the lack of double mutations, only single mutation (Fig. 3). As far as the seventh clone is to be concerned there are at least two mutations that differentiate it from other clones. Thus it can be supposed that clone No. 7 was transferred from the other reservoir or clones between No. 3 and No. 7

Table 3

Genotypes, clones number and frequency in populations P1 and P2

Locus	Genotype	Clone No.	Population P1 (Lake 1)		Population P2 (Lake 2)	
			Number of individuals	Frequency	Number of individuals	Frequency
IDH-1 LDH MDH ME	bb bb bb bb	1	31	0.64	47	0.96
IDH-1 LDH MDH ME	bb bb bb ab	2	0	0.0	2	0.04
IDH-1 LDH MDH ME	ab bb bb bb	3	8	0.16	0	0.0
IDH-1 LDH MDH ME	ab bb bb ab	4	7	0.14	0	0.0
IDH-1 LDH MDH ME	ab ab bb ab	5	1	0.02	0	0.0
IDH-1 LDH MDH ME	bb bb bb aa	6	1	0.02	0	0.0
IDH-1 LDH MDH ME	aa bb ab bb	7	1	0.02	0	0.0

were not observed. This hypothesis does not explain the isolated character of clones No. X1 and No. X2.

The hypothesis I assumes: (a) Isolation of individuals between reservoirs, (b) the possibility of double mutations (Fig. 4), and very well describes the isolated character of clones No. X1 and No. X2. Genetic structure of polymorphic loci (IDH-1, LDH, MDH, ME) of populations P1 and P2 evolved in different ways and gave 7 clones (with maximum distance 5 mutations) and 2 clones (only one mutation) respectively (Tables 1, 2). This result is difficult to explain because hydrological conditions in both reservoirs were very similar.

In conclusion it is reasonable to claim that there exists only incidental participation of males in *L. articus* reproduction, hypothesis S (Fig. 5). Number of mutations between clones 1–7 in both, parthenogenesis and sex reproduction, was lower (Fig. 5) than only in parthenogenetic reproduction (Figs 3 and 4, Table 4). It

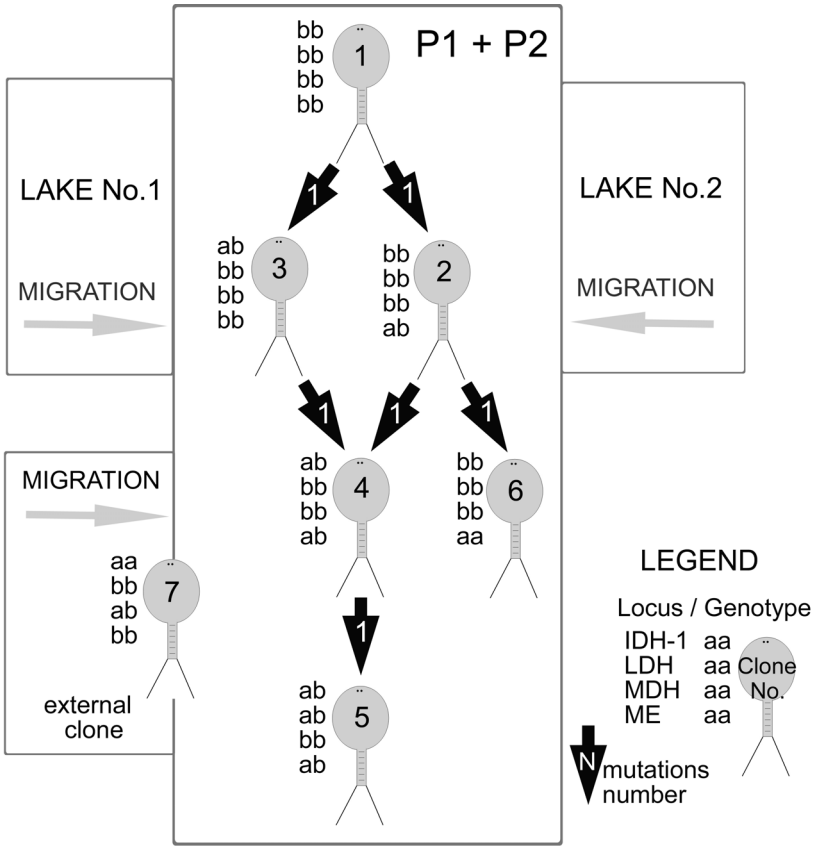

Fig. 3. Hypothesis M, migration between populations P1 (Lake No. 1) and P2 (Lake No. 2).

Table 4

Number of mutations between clones: white cells – number of mutations between clones 1–7 in parthenogenetic reproduction only, (N*) – number of double mutations, grey cells – number of mutations between clones 1–7 in two ways of reproduction: parthenogenesis and sex reproduction (Hypothesis S, Fig. 5); before/after sex reproduction, a minus "-" without mutations

		Clone number						
		1	2	3	4	5	6	7
Clone number	1		-/-	1/-	2/-	-/-	-/-	-/-
	2	1		-/-	-/-	-/-	-/-	-/-
	3	1	2		1/-	-/-	-/-	-/-
	4	2	1	1		-/1	-/-	-/1
	5	3	2	2	1		-/-	-/-
	6	2 (1*)	1	3 (1*)	2	3		-/-
	7	3 (1*)	4 (1*)	2	3	4	5 (2*)	

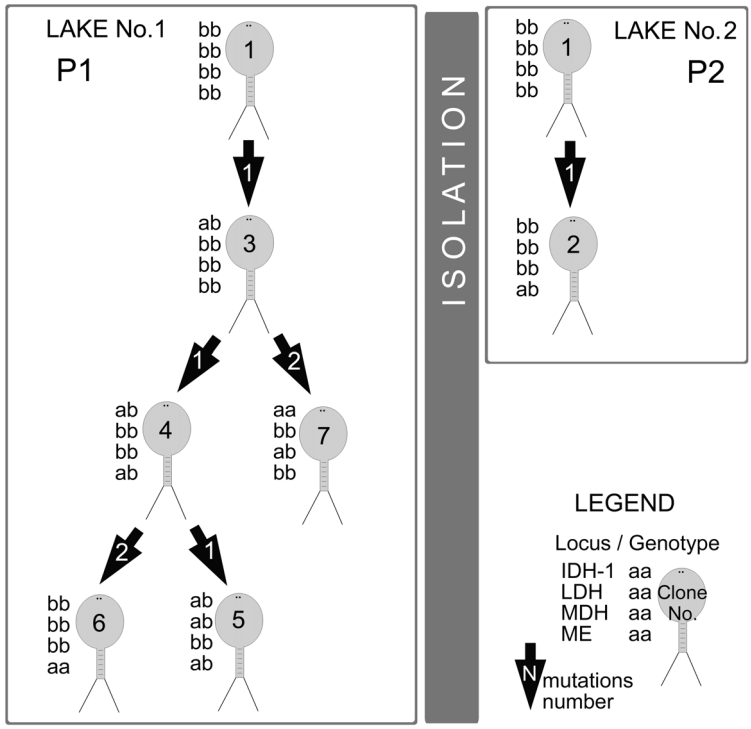

Fig. 4. Hypothesis I, populations P1 (Lake No. 1) and P2 (Lake No. 2) were isolated.

is the most probable way to explain differences between populations P1 and P2 from very close lakes.

Discussion

The results obtained are surprising when compared with results of the earlier studies upon 13 populations coming from the Melville Peninsula in the Canadian Arctic (Beaton and Hebert 1988), where the presence of homozygotes only and homogeneity of the investigated populations was observed. The above authors examined 22 enzyme systems, 8 of which were the same as in the presented results (HEX, IDH, LDH, MDH, ME, PGI, PGM, 6PGDH). The presence of two loci was observed for HEX and IDH, whereas only one was present for PGI (there are two loci in the results of our study). Four loci were recorded for AMY and PEP(A), while there was one locus for other examined enzymes. Beaton and Hebert 1988 used different numbers of specimens and populations for their studies. The results obtained for ME and MDH deserve special attention: they investigated 13 populations and 369 and 437 specimens respectively). Only homozygotes were recorded for both enzymes. The picture obtained for ME and MDH in the present work indicates higher genetic diversity, especially in the case of ME. One of the conclusions

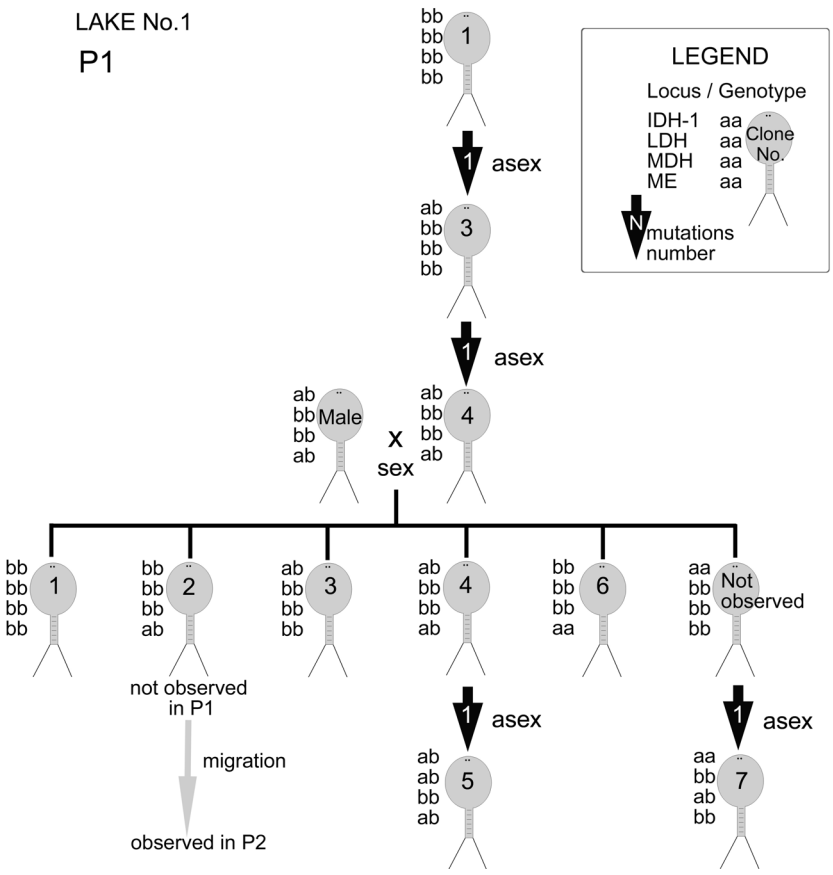

Fig. 5. Hypothesis S, the probable role of males in genetic structure of *L. arcticus* in the population P1.

of the study of *L. arcticus* populations from the Melville Peninsula was an unclear role of males in the reproduction process because of the lack of genetic diversity – only homozygotes were observed. However, the picture obtained in the case of sexual reproduction and the occurrence of homozygotic females and males with respect to the examined enzymes would be consistent with the one acquired in the cited work (Beaton and Hebert 1988).

The study by Hessen *et al.* (2004) of a 484bp segment of 12S rDNA from 48 populations of *L. arcticus* from different Arctic regions revealed a few haplotypes but only one in Svalbard (from where specimens from 7 localities were analysed). The haplotype recorded in Svalbard occurred also in other regions (Russia, Bear Island), but scarcely. The cited results show the lack of genetic diversity among populations in Svalbard. The authors concluded that *Lepidurus* can also reproduce parthenogenetically in high Arctic latitudes. Asexual reproduction is still under investigation and asexuality promotes dispersal abilities. Single individuals or eggs may be sufficient to start a new populations.

Our study reveals a complicated and genetically heterogeneous character of the population of *L. arcticus* inhabiting the two reservoirs in Hornsund area. The genetic distance between the analysed populations proved to be astonishingly great with respect to the close vicinity of the reservoirs (2 km) and the potential possibility of *L. arcticus* being spread by birds that are very common in that region. In the case of the hypothesis M doubts appear because of a relatively high clonal diversity between reservoirs 1 and 2. It is almost impossible that there is no migration in case of the hypothesis I, because of the birds migrating between these reservoirs (Sywula *et al.* 1994). Male participation in reproduction is probable, although presence of males was not recorded, perhaps because of the low number of males in populations or the possibility that they do not occur every year. Therefore further studies of the clonal structure of these populations are necessary as well as studies of the genetic diversity typical for species reproducing sexually and remaining in the Hardy-Weinberg genetic equilibrium. Following Hessen *et al.* (2004) our results show a parthenogenetic mode of reproduction of *L. arcticus*, but coexisting with sex process in the south Spitsbergen freshwater reservoirs.

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