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Department of Environmental and Health Studies

Master thesis

Extra pair copulation in the Eurasian beaver *(Castor fiber)?*

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University College of Southeast Norway
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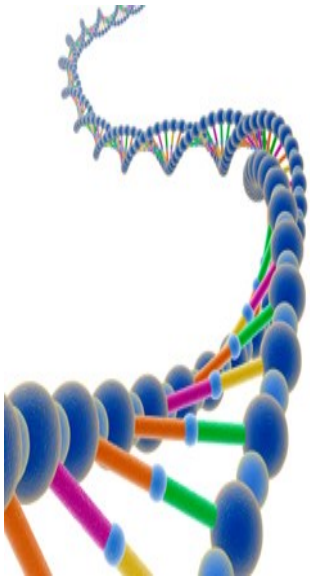

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Preface

This Master thesis is a part of my Master degree program from 2014-2016 at the Department of Environmental and Health Studies at University College of Southeast Norway (USN).

I would like to thank my supervisors, Professor Frank Rosell and Associate Professor Mona Sæbø for including me in this project. They always supported and gave me an excellent guidance. I would like also to say thanks to PhD candidate Priyank Sharad Nimje, for helping me in the genetic lab and for supported me with all kind of information. I would like to say thank as well to the PhD coordinator Helga Veronica Tinnesand for helping me in some part of statistical analysis and to understand the trapping file. Thanks also the beaver team for all kind of information that they gave me during this project. Thanks Martin Mayer for the picture of beaver that I used on my master thesis cover page. I would also like to say thanks to the staff at the library and at the IT department for their invaluable assistance. I would like to say thank you to manager of exchange programs (between Georgia and Norway) Tim Teemuraz Abesadze that gave me biggest opportunity to study in Norway. Thank you to my lovely family and the best friends for the emotional and the spiritual support. In the end, I would give the biggest thanks to the University College in Southeast Norway (USN) for providing me with financial support for my master.

15. May 2016 Bø i Telemark, Norway

Inga Shavadze

Extra pair copulation in the Eurasian beaver (*Castor fiber*)?

Abstract

In mammalian species, primarily in rodents, primates and canids, social monogamy is found in only 3-5% species and genetic monogamy appears to be rare. There is compelling evidence that the beavers (genus *Castor*) are a monogamous species.

In this study we examined the genetic mating system of social monogamous Eurasian beaver (*Castor fiber*) and tested for extra-pair copulation (EPC) in a free-ranging population in Norway. In this region beavers have been captured and monitored since 1998. We used 30 Single Nucleotide Polymorphism (SNPs) to test for EPC in 100 beavers (44 offspring and 56 dominant individual of beavers in 10 family groups). For all the 100 samples all putative parents were known.

Of the 30 SNPs used in this study we got reliable results for 27. We did not find any evidence for EPC in this population. Based on the genetic data it appears that the Eurasian beaver is a strict genetically monogamous species. These results are in concordance with the observational data.

This is the first genetic study on EPC in Eurasian beavers by using SNPs.

Key words: *Castor fiber*, beaver, extra-pair copulation, SNPs, genetic, monogamy, social monogamy, parentage.

1. Introduction

In mammalian species, primarily in rodents, primates and canids, social monogamy has been detected in only 3-5% of species (Kleiman 1977, Haimoff 1986). Social monogamy implies a close association between males and females and collaboration between them in breeding activities. They persist to be together, at least for one reproductive season or in some cases whole lifespan (Lack 1968, Kleiman 1977, Gowaty 1996). Social monogamy has been described in different vertebrates such as birds, mammals, reptiles and teleost fish (Lack 1968, Bull, et al. 1998, Taylor, et al. 2003). In birds, social monogamy is a common mating system and it has been documented in more than 90% of the species (Yezerinac, et al. 1995).

Social monogamy does not necessarily imply genetic monogamy. The first time social monogamy was differentiated from genetic monogamy by Wickler and Siebt (Wickler and Siebt, 1983). In socially monogamous species, all offspring are not necessarily from the pair living together. In genetic monogamy all offspring are exclusively from the pair that live together and this cohabitation is accompanied by exclusive parentage that have high degree of bi-parental care, where both of parent care of their offspring together (Westneat et al. 1990, Møller and Ninni 1998). Genetic studies using molecular methods have shown that a strict genetic monogamy in species that are socially monogamous in nature appears to be rare (Girman et al. 1997, Masello et al. 2002). The strict genetic monogamy has been reported for only five mammalian species: California mouse (*Peromyscus californicus*) (Ribble 1991), Kirk's dik-dik (*Madoqua kirkii)* (Brotherton et al. 1997), Malagasy giant rat (*Hypogeomys antimena*) (Sommer and Tichy 1999), Coyote (*Canis latrans*) (Hennessy et al. 2012), and Azara's night monkey (*Aotus azarae*) (Huck et al. 2014, Syrůčková et al. 2015). The studies of genetic monogamy in other social monogamous species, have shown a high degree of extra-pair copulations (EPC) like in gibbon (*Hylobatidae*) (Reichard, 1995) alpine marmot (*Marmota marmot*) (Goossens, 1998) and in dwarf lemur (*Cheirogaleus andysabini*) (Fietz, 2000).

Various types of molecular markers can be used for parentage analysis (Jones et al. 2010). The most common types of markers are microsatellites, also known as simple sequence repeats (SSR), and Single Nucleotide Polymorphism (SNPs) (Tautz 1989, Nathan el al. 2008). Genotyping error rates tend to be low for SNPs (Kennedy et al. 2003). They are one of the most powerful molecular markers to use for parentage analyses in mammal species despite the fact that SNPs occur at a frequency of approximately 0.3-1 SNP/kb (Marth et al. 2001).

The Eurasian beaver (*Castor fiber*) and North American beaver (*Castor canadensis*) are the only surviving member of the once large family of *Castoridae*. Beavers are the second largest species of rodent in the world (Collen, 2000). These species are similar, both morphologically and behaviorally, and were originally classified as one species. They are considered "ecosystems engineer organisms" because they are able to create and maintain habitats for themselves and for other species by water logging and building dams (Rosell, Bozser et al. 2005). Beavers have a powerful effect on basins as well as on stream communities' structure and are able to modify the nutrient cycling and decomposition dynamics, which ultimately effect on animal community composition (Jones et al. 1994). Beavers are well suspected to be the causes of habitat heterogeneity and species richness (Wright et al. 2002).

Beavers are strongly territorial and considered a social monogamous species (Herr, 2004). Their mating system provides an ideal approach to investigate the evolution of mating system and test for genetic monogamy (Busher et al. 2007). The first study to apply molecular methods to test for EPC in the North American beaver reported a wide range of genetic relationships among colony members and the presence of EPC in 56% of the litter (Crawford et al. 2008). On the other hand, a recent study of EPC in Russia has not shown any conclusive evidence of EPC in Eurasian beaver. They found that the beavers in their study area are genetically monogamous (Syrůčková et al. 2015).

The main aim of our study was to examine the Eurasian beavers for genetic monogamy by using SNPs as the molecular marker. Molecular analysis was combined with long-term observational data. This is the first genetic study on EPC in Eurasian beavers using SNPs as molecular markers.

2. Materials and methods

2.1 Study site, animal hair sample data collection and study area

Hair samples from beaver have been collected by the researchers at University College of Southeast Norway (USN). The study population comprises several beaver colonies in the rivers Straumen (59°29´ N, 09°153´ E), Gvarv (59°386´ N, 09°179´ E) and Sauar (59°444´ N, 09°307´ E) (See figure a.b.). They are located in the County of Telemark, in southern Norway, and form part of the catchment of Lake Nordsjø (Campbell, et al. 2013)

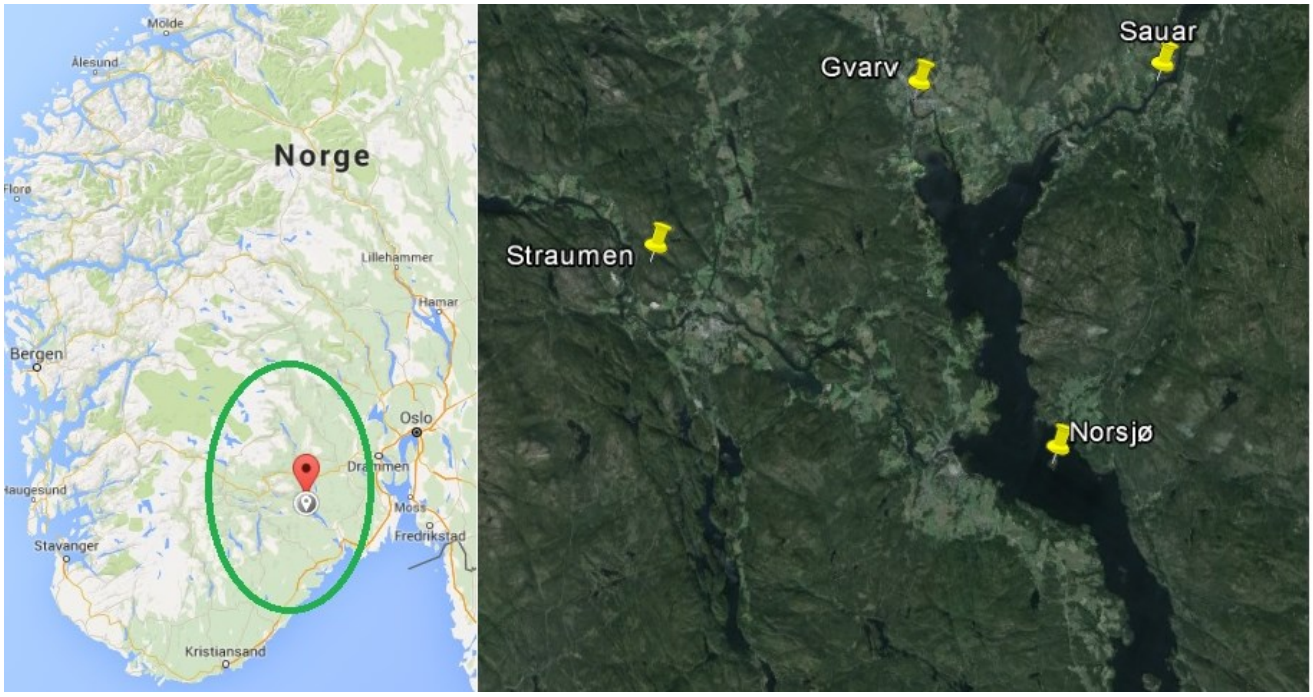

Figure a b. Study area in the south east Norway in the county of Telemark, the three rivers (Straumen, Sauar and Gvarv) part of the catchment of Lake Nordsjø, where beaver samples have been collected since 1998.

The beavers in the study area have been monitored since 1998. They are captured by landing-nets between March and November every year (Rosell and Hovde 2001, Campbell et al. 2005). The captured animals are immobilized in sacks while samples collected and scientific observations are taken. All trapped beaver are assigned to an age-class depending on their body weight. Sex determination is based on the color and viscosity of their anal gland secretion (AGS) (Rosell and Sun 1999, Campbell et al. 2005). All individuals have been marked with ear-tag combination for recognition with unique color-plastic (Dalton) and metal tags (National Band and Tag Co) and tagged with microchip (Campbell et al. 2010).

Guard hairs were plucked and stored in paper envelopes at room temperature at USN. All procedures including the trapping and handing processes were approved by the Norwegian Experimental Animal Board and the Norwegian Directorate for Natural Management.

2.2 Observational data to determining parent-offspring relationship

We used observational data and trapping documents that contain information about, territory borders, family composition, age, gender, trapping year, weight, the length of pair bonds, breeding and dispersal events etc. (Campbell et. al. 2012, 2013). All the beavers in our study area were given names along with ID for easy identification during field observations. We defined dominant pair-offspring relationship based on the long-term observational data. Dominance status was determined by previous trapping and sightings, body mass and incidences of lactation in females (Campbell et al. 2010). For the parentage study, mostly we used families where both individuals in the dominant pair were identified and the period of the pair-bond was clear (see Table 1). To avoid false exclusions, we used only those putative offspring, which were trapped for the first time as young. If individuals were trapped the first time as yearlings, they were used in analysis only if these beavers were also trapped or seen in the territory in the following years. The animals are from 9 family groups and contain 56 dominant individuals and 44 offspring from 9 different territories. Three individuals in this study were used both as offspring and as parents.

Table 1. Completed family groups parents and their offspring from different territories. Year of birth and ID number was determined during trapping period.

Colony	Parents	Offspring	Year of Birth	ID #
Lunde 4a	Jørn			72
	Hanne			80
		Bram	1998	81
		Celine	1999	113
		Bruno	2000	137
		Johanne	2001	156
		HannaChristi	2003	170

Lunde 4b	Lasse			189
	Gyda			247
		Iain	2009	301
		Roisin	2009	300
		Clara	2009	299
		Montana	2010	325
		Darwin	2010	338
		Luna	2011	363
		Eirik	2012	354
		Arvid	2012	355
		Ellie	2012	351
		Joe	2012	356
		Leaf	2013	390
Lunde 5c	Lasse			189
	Female			247
	is unknown	Kyrgyz boy	2006	235
		Alfhild	2006	234
		Minigreen	2007	296
		Eilidh	2007	248
		Paula	2007	250
		Carry	2008	279
		Martin	2008	264
Lunde 6a	Bram			81
	Maud			256
		Todd	2010	343
		Yasmin	2012	352
Patmos 5	Dino			204
	Rosa			202
		Kolbjørn	2005	203
		Rocco	2005	220
Patmos 6	Ludwin			266
	Karin			225

		Hygrid	2008	316
		Mini Bjørnar	2009	303
		Simon	2009	317
		Flint	2013	381
		Keiko	2013	383
Evjutunet	Greg Burly			38
	Demi			22
		Gerard	2009	294
		Volker	2010	335
Norsjø 1	Male is unknown Jodie			41
		Alasdair	2008	269
		Angus	2008	267
		Eoghann	2008	270
		James	2008	268
		Tina	2012	366
Bråfjorden a	Laurits			226
	Leslie			263
		Pablo	2012	360
		Mathis	2013	372
		Benjamin	2014	398
		Claudia	2014	397
		Maja	2014	404

2.3 *DNA extraction*

DNA was extracted from the 119 beaver samples. The 5-10 hairs of samples were extracted in isolated and sterilized laboratory in order to avoid DNA contamination. Each hair was examined for hair follicle by eye-sight. Hair samples were cut individually 0.5 cm above the hair root (hair follicle). DNA extraction from beaver hair samples was performed using the modified (Qiagen blood and tissue kit) protocol. 5 µl DTT was added while incubating samples at 56^{0}C for complete hair strand degradation. 200 µl ATL Buffer was added instead of 180 µl. Finally, DNA was eluted in 100 µl AE buffer.

The purity and concentration of DNA were checked by Picodrop Microlitre Spectrophotometer version 3.1 (Picodrop Ltd). The accepted purity ratio for A260/280 was 1.8 and the concentration approximately 10 ng/ µl. Some of the beaver samples DNA were extracted more than once due to non-sufficient concentration. Finally, all the samples were diluted to final concentration of 10ng/ µl for further SNP genotyping.

2.4 DNA amplification by Real-Time PCR

SNPs for Eurasian beavers were developed by Senn et al (2013) based on a RAD-sequencing of the Eurasian beaver. We selected 30 SNPs based on their heterogeneity. The primers and probes were ordered and created by the Applied Biosystems, Warrington, UK. (See table.2).

The real-time PCR program: initial denaturation at 95^0 C for 15s, annealing at 57^0C for 30s and extension at 72^0C for 45s. We used master mix (TaqMan ^{R} GTX press ^{TM} Applied Biosystems). Total volume of each reaction was 10 µl, containing 2 µl DNA sample (diluted to 10 ng/µl) 3.9 µl GTX press master mix, 0.2 µl probe - primer mix and finally 3.9 µl dH_2O instead to complete volume to 10 µl.

For all PCR reactions (48 plates) we added one negative control in order to avoid inaccurate positive amplification. 10% of the samples were run twice for calculating genotyping error.

Table 2. List of primers for 30 SNPs used in this study.

Primer Name	Forward Primer Seq. 5`-3	Reverse Primer Seq. 5`-3
BEVcfSNP102408	CTGGGAAAAATTCAACACACCTTGT	AGGAGACAGGTGACCAAGGT
BEVcfSNP102426	AGTGGCCTCAAACAGTGATTCTC	TGGCTCACACTTGTCATCACA
BEVcfSNP105238	ACACAATGGTGCTGTGAAGTGT	GGTCTGTACTGATATTTCTTTTTTGAGTCACT
BEVcfSNP108377	CCAGACTGGGCTTAGAAACCA	TGTGCTCCTGTTTATCCAACTCTTG
BEVcfSNP109525	AGCTCAAGCTGTGCAGCAT	GTCACAGGTATTGTTGCTGCTTTT
BEVcfSNP112139	GCCCTTCTCAATAACCCCACTTAC	TCCCGTAGTACATAGCACAAAATTAATGG

BEVcfSNP11809	TCTCGAAGACTACAAGCCTCTCAT	GCTGCAGAAAAACCCAGTGT
BEVcfSNP16114	TGCTCCAACTCCCAGTATTTTTCC	CAGAAAGTATTTGAAAGCACATTGAACTGT
BEVcfSNP30128	AGTCCTAGGATTTGATCTTCAGTGAAATTC	CCTAGACCTGGACTATCTTCTTAGAATGA
BEVcfSNP34297	TGGCTTCAAATTGGAGTGAGGAA	CTGACAAGTGCAGGGATTTCTG
BEVcfSNP34680	TTGTCATCCTCCCTCCCAGAT	CCAGGGCATCCAAGAAACACTT
BEVcfSNP40318	GTCACATAGACCCTGCTCCTTATTT	GTGCTGGCCAGCAATCC
BEVcfSNP44292	GAGTCCACTGGACCTGGTTTT	GCAGTTGAACTTGCACACAGT
BEVcfSNP45990	TGGCCAGGCTTTTCTCAAGAG	GGACCTGGATGAATCATGAAACCTT
BEVcfSNP50941	CCTAGCAAGAAGGCAAATTAGAGTCA	GCCCCAGCATCAGGTCTAAATG
BEVcfSNP55280	CACGTGGCCCTCAGTGA	GGCTGCTTAGAAACACAAAGTCTTT
BEVcfSNP56140	GTCTGGATGATAGACTGCATCAAATGA	CCAACACAGACTTCCTAAACTGGAA
BEVcfSNP57669	GTGTTCCTCAGCTGGTGTCT	GAAAGAAGGCGAAAAGCAGACT
BEVcfSNP58111	CAATCAAATTAAATTTTGAGAGAAACATTGTACCTTTC	GGTTATGAACTAGGTGAAGGGCAAT
BEVcfSNP61846	ATTATGCTGATGTCTTTTTGTCTTAAAACATGT	AAATGAAAGAAATTGTCCATAAGCCCTTTTT
BEVcfSNP63983	TGTAACAGTGGAAATGAGAGAGAACTTG	CATCATTCTTGTTTCTTTCTTCGGTTTGA
BEVcfSNP67449	ATCGACACTGTCAGCTGATTTAACT	CATTCACTTGACCAAGGCTTTCTG
BEVcfSNP7071	GGAGTACATATACTAATTTGTTCATTCACTCTGC	GCAAAGAGTAGGTTCTCCATGAGT
BEVcfSNP73032	CCCAGAAGAAAATCAGGATGACTCT	CACTCTATCCACAAACCATCCATCA
BEVcfSNP77200	GCCAGCCTTCTTTGGTGTACTTT	TTTTCCAGAAGGCTCTTTGAGTCA
BEVcfSNP79605	CCATACCAAACGAAGCCTGAAGTAA	CTTCCCCTCACACTGTCTTGAAAA
BEVcfSNP81918	CAGGAGTTAGAAGCCTTCAGTACAT	CACCAATGAGGGCTGATTCTAATGA
BEVcfSNP95943	CTCTGTGAATGTCAAGTCTGAAGCT	CCCCACTCTCGTTTGGATTATCAG
BEVcfSNP96886	TTTGTTAATGCAAAGCAAAGTGGAAGT	GAGCCTGCCTGCTGTCT

Table 3. List of probes for 30 SNPs used in this study

Reporter Name	Reporter 1 Sequence (VIC)	Reporter 2 Sequence (FAM)
BEVcfSNP102408	CTTTGTCTCAGTACAGTTT	TTTGTCTCAGTGCAGTTT
BEVcfSNP102426	CCTCTGAGAATACTCTGC	CCTCTGAGAATTCTCTGC
BEVcfSNP105238	ACATTTACACGTTTTCTG	CATTTACACATTTTCTG
BEVcfSNP108377	CATTCCTGTTGGGTACAAT	CATTCCTGTTGAGTACAAT
BEVcfSNP109525	ATGGTGGACTATAGTCC	TGGTGGACTGTAGTCC
BEVcfSNP112139	ACAGGTCTAGCATCTGAT	CAGGTCTAGCGTCTGAT
BEVcfSNP11809	ACAGCTCTACCTTATTCTA	AGCTCTACCTCATTCTA
BEVcfSNP16114	TTCTATGGTCGTTGCCTAA	ATTCTATGGTCATTGCCTAA
BEVcfSNP30128	AAGAAAGTCAGCTGGTTAAG	AAGAAAGTCAGCTAGTTAAG
BEVcfSNP34297	CATAACAAAGAAAATGC	ATAACAAAGGAAATGC
BEVcfSNP34680	CACCAACACTAGAGGTCAG	CCAACACTAGAAGTCAG
BEVcfSNP40318	ACGTATGTTCCGTGAACAG	ACGTATGTTCCATGAACAG
BEVcfSNP44292	AAACCTGTTAAAAGATGAGTG	AAACCTGTTAAAAGTTGAGTG
BEVcfSNP45990	ACTTCTCTCACTTTGAGTTC	TTCTCTCACTCTGAGTTC
BEVcfSNP50941	TGGGTCCGTGTGGCT	CTGGGTCCATGTGGCT
BEVcfSNP55280	ATTCTCCTCAGGATCTC	TCCTCGGGATCTC
BEVcfSNP56140	TTCATGGGAAAAATC	TTCATGGAAAAAATC
BEVcfSNP57669	CCATCCTACCTAGTCTCC	CATCCTACCTGGTCTCC
BEVcfSNP58111	CCAAATCATAACACGCCCCT	CCAAATCATAACATGCCCCT
BEVcfSNP61846	TTCCCTCAGGTCTCCCT	TCCCTCAGATCTCCCT
BEVcfSNP63983	AATGGTAGAGCAACAATA	ATGGTAGAGCGACAATA
BEVcfSNP67449	CAAGCTGCTAATAAAAGA	CAAGCTGCTAATGAAAGA
BEVcfSNP7071	CGCTCACCCATCATC	TCGCTCACCTATCATC
BEVcfSNP73032	AAACAGGGAGAGAACT	AACAGGGAAAGAACT
BEVcfSNP77200	CACCCTTCTCATATAGGAAA	CCCTTCTCATACAGGAAA

BEVcfSNP79605	CAATAAACCCCAGTAAGCA	AATAAACCCCAATAAGCA
BEVcfSNP81918	AGCAGAGTCAGTGTTCAA	AAGCAGAGTCAATGTTCAA
BEVcfSNP95943	TGCTAGGGATCCTACTCCT	CTAGGGATCCCACTCCT
BEVcfSNP96886	CACAAGAGTAAACGGTCACT	CACAAGAGTAAACAGTCACT

2.5 *Exclusion method for parentage analysis*

The exclusion method is a simple method to examine parent-offspring relationships (Jones et al. 2010). Given the rules of Mendelian heritage for diploid organisms, a parent will have at least one common allele per locus within offspring (Jones, et al. 2010). The most meaningful is a loci with an important deviation from Hardy-Weinberg Equilibrium (HWE) since null alleles are a common cause of such modification and a pattern of repeated homozygote-homozygote mismatches in known parent-offspring pairs is typical for a locus with a huge null allele density (Pemberton, Slate et al. 1995). We did therefore only accept exclusion based on mismatches at two loci, or where the mismatch included at least one heterozygote individual. Mismatches between offspring and both of putative parents were not accepted as true, but rather interpreted as a result of either observational mistake.

2.6 *Data analysis*

We used the computer software Cervus 3.07 (Kalinowski, Taper et al. 2007) and GenAlex, Genetic Analysis in Excel 6.5 (Peakall and Smouse 2006) for the parentage analysis. We assigned parents to their offspring by calculating allele frequencies from HWE, and the frequencies of null alleles for each locus. Based on the allele frequency data and null allele estimates for all polymorphic loci, we calculated the probability of false exclusion.

$$P = \sum_{i=1}^{k} P\, iPk\, (1 - Pi)$$

Equation.1. The equation used for calculating the probability of false exclusion of a true parent (Dakin and Avise 2004) for a population with k-1 visible alleles with population frequencies p_i (i=1 to k-1) and a null allele with frequency p_k.

3. Results

3.1 DNA extraction

We were able to successfully extract DNA from 110 samples out of 119. The average concentration of DNA sample was10ng/ µl with the purity 1.8. Some of beaver samples were extracted several times, but in the most cases (99%) this was not effective.

3.2 DNA amplification by Real-Time PCR

All the 110 samples were used for SNP genotyping by Real-Time PCR for 30 SNPs. Only 27 out of the 30 SNPs yielded reliable results. For 3 of the SNPs we got different results for the same samples when we retested them (BEVcfSNP58111, BEVcfSNP77200, BEcfSNP108377). For this reason they have not been used for further analysis. For 80 out of 110 samples we had results for all 27 SNPs. For two samples minimum amplified SNPs were 23 and for remaining samples we had 26 SNPs.

3.3 Allele frequency analysis

Out of the 110 extracted DNA samples, only 100 were used for parentage analysis. This was based on the available observational data for specific family groups, which included data regarding putative parents and their offspring. The estimated null allele frequency was low for most SNPs. Only one SNP (BEVcfSNP102408) had a null allele frequency higher or equal to 0.1229 (see table 3.) The mean of polymorphic Information Content (PIC) was 0. 33. The mean proportion of SNPs amplified was 99.15 %. The mean of expected heterozygosity was 42 % (see Table 4).

Table 4. Characteristics of the 27 SNPs used in this study.

SNPs	N	H_O	H_e	PIC	HWE	*F* (Null)
BEVcfSNP102408	99	0.384	0.494	0.371	NS	0.1229
BEVcfSNP102426	99	0.434	0.501	0.374	NS	0.0684
BEVcfSNP105238	100	0.350	0.339	0.281	***	-0.0181
BEVcfSNP109525	100	0.460	0.482	0.365	NS	0.0213

BEVcfSNP112139	100	0.380	0.405	0.322	NS	0.0296
BEVcfSNP11809	96	0.440	0.490	0.369	NS	0.0509
BEVcfSNP16114	100	0.448	0.463	0.354	NS	0.0138
BEVcfSNP30128	100	0.410	0.418	0.329	NS	0.02
BEVcfSNP34297	96	0.340	0.422	0.332	NS	0.1053
BEVcfSNP34680	100	0.424	0.491	0.369	NS	0.0704
BEVcfSNP40318	100	0.418	0.501	0.374	NS	0.0876
BEVcfSNP44292	99	0.388	0.456	0.355	NS	0.0808
BEVcfSNP45990	98	0.192	0.174	0.158	***	-0.0436
BEVcfSNP50941	98	0.500	0.502	0.375	NS	-0.0005
BEVcfSNP55280	99	0.540	0.478	0.363	NS	-0.0632
BEVcfSNP56140	98	0.410	0.351	0.288	NS	-0.0807
BEVcfSNP57669	100	0.495	0.486	0.367	NS	-0.0117
BEVcfSNP61846	98	0.398	0.452	0.349	NS	0.0613
BEVcfSNP63983	100	0.330	0.351	0.288	NS	0.0276
BEVcfSNP67449	98	0.414	0.420	0.331	NS	0.0051
BEVcfSNP7071	100	0.320	0.356	0.291	NS	0.0507
BEVcfSNP73032	100	0.280	0.297	0.252	***	0.0264
BEVcfSNP79605	100	0.374	0.456	0.351	NS	0.0971
BEVcfSNP81918	99	0.444	0.432	0.338	NS	-0.0163
BEVcfSNP95943	99	0.455	0.462	0.354	NS	0.0060
BEVcfSNP96886	99	0.495	0.502	0.375	NS	0.0043
BEVcFSNP9667	99	0.394	0.384	0.309	NS	-0.0148

N: number of individuals with successfully amplification for each SNP_s, H_0: observed heterozygosity, H_e: expected heterozygosity, PIC: polymorphic information content, HWE: Hardy Weinberg equilibrium, NS: not significant, ***: significant at the level p<0, 01, *F* (Null): estimated null allele frequency.*(The PIC the 33, 18% indicates intermediate level of locus diversity according to the Botstein 1980).

Table 5. Characteristic of 27 loci used in this study for 100 individuals and the mean of Allele frequency data.

Number of individuals:	100
Number of loci:	27
Mean number of alleles per locus:	2.037
Mean proportion of loci typed:	0.9915
Mean expected heterozygosity:	0.4284
Mean polymorphic information content (PIC):	0.3327
Combined non-exclusion probability (parent pair):	0.00034110
Combined non-exclusion probability (sib identity):	0.00000632

3.4 EPC analysis

It was possible to compare the genotype of all putative parents and offspring for all the 100 animals for a total of 27 SNPs. For 40 individuals, out of 44 (90.9 %), observational and genetic data was in concordance. For two beaver colonies from Lunde 4 and Lunde 5C, 11 out of 18 offspring's matched both the putative parents, while the 7 offspring of this colony did not match putative mother. Genetic analysis and observational data reveal that dominant male used to live in Lunde 5c before (2008) with another family. In 2009 he moved in Lunde 4b and got a new mate.

The four offspring out of the 44 samples (9.1%), did not match both putative parents and this can be due to observational mistake.

4. Discussion

The main result of this study is that Eurasian beaver is a strict monogamous species. We have not found any evidence for EPC in our study area.

4.1 Parentage analyses

This genetic study suggests that Eurasian beaver and North American beaver differ in genetic mating system. Crawford et al. (2008) found more than half of the litter (5 of 9 litters) of the North American beaver was product of EPC, while our genetic study has not found any clear evidence of EPC in Eurasian beaver. The recent study by Syrůčková et al. (2015) of EPC in Eurasian beaver is concordance with our study.

There could be many reasons for the differences between results of these two studies (EPC in Eurasian and North American beaver). The beaver colonies in our study have been observed for more than 17 years. This includes observational data of parent-offspring relationship (Tinnesand et al. 2013). In the study of EPC in North American Beaver, trappers attempted to collect samples for over two weeks with no observational data to correlate (Crawford, et al. 2008). Without good observational data it may be a challenge to do unbiased parentage analysis only based on genetic data. A good example of this can be found in our study in beaver colonies (the 18 offspring) from Lunde 4b and Lunde 5c. The dominant male in Lunde 4b beaver colony had different family in 2008 and he also lived in a different place. In 2009 he moved to Lunde 4b from Lunde 5c where he got a new mate and they had offspring together. Without observational data it would have been easy to consider these offspring as Extra Pair Young (EPY) based only on the genetic analysis. The dominant female, which lived in Lunde 5c before 2009, most likely died or found a different partner. In monogamous mammals "divorce" to change mate hypothesis already have been documented in Alpine marmot (*Marmota marmota*) (Cohas, et al. 2006, Lardy et al. 2011).

There is however also a possibility that the frequency of EPC can be influenced by high density of population (Lott 1984, Bryja et al. 2008). The study of North American beaver was conducted on two populations (central and southern Illinois). In central Illinois colony density was estimated at 0. 40 colonies/km^2 of stream, in Southern Illinois colony density was estimated at 3. 3 colonies/km^2 (Crawford et al. 2008). The beaver families within our study were smaller. While Crawford et al. (2008) reported an average of 3.8 and 9.0 beavers per colony while for our study average colony size was 3.7. Beavers in the North American

study were trapped inside the border of a known territory at a given time as one family colony and this may overestimate of the proportion of EPC.

Molecular markers may also explain the differences in these studies. In the study by Syrůčková el al. (2015) 26 microsatellites were used which were designed for Eurasian beaver (Syrůčková et al. 2015). In the study by Crawford el al. (2008), 7 microsatellites designed for North American beavers were used (Crawford, et al. 2008). There is a possibility that low level of variation of microsatellite markers may overestimate the proportion of EPC in monogamous species (Pemberton, 1995). In our study we used SNPs as molecular markers. SNPs have greater advantage as compared to microsatellites e.g. they are easier to analyze, are in greater abundance (Heaton, Harhay et al. 2002) and have more genetic stability in mammals (Thomson et al. 2000, Lindblad-Toh et al. 2000). According to one study, 25 SNPs give similar results as 11-12 microsatellites (Fernández et al. 2013). In *Angus cattle* population for the kinship analysis researchers achieved the same results in two different molecular markers (SNPs and microsatellites) which stated that 24-31 SNPs was equivalent to the 12-18 microsatellites. (Fernández et al. 2013).

Study of EPC using molecular markers is becoming more common in many mammals (Garnier, 2001; Csilléry, 2006; Lawson and Handley, 2007; Lukas, 2013; Forstmeier, 2014). Researchers have found different proportion of EPC in different mammals e.g. in California mouse (*Peromyscus californikus*), red fox (*Vulpes vulpes*) and dwarf lemur (*Cheirogaleus andysabini*) extra-pair young (EPY) comprises 88%, 92% and 44% of litter respectively (Ribble 1991, Fietz et al. 2000, Baker et al. 2004).

There is a possibility that some environmental and behavioral factors may limit Eurasian beavers to get EPC. The breeding period of beaver is in the winter, when ponds are very frozen in high latitude areas. This type of environmental condition limits beaver movement in the breeding season (Ulevičius and Janulaitis 2007). Hence, it follows that without a stable residential environment for beaver it is absolutely big risk to seek a new mate (Herr and Rosell 2004). Beaver needs to cross territory lines to find extra pair mates that include

competition between two beaver, high opportunity of hazard being detected and attacked by other territory owners (Busher et al. 2007). Moreover, seeking EPC is a big risk for female, as there's high chance to lose the parental care provided by her social partner (Muller-Schwarze, 2011). Bi-parental care is extensive in beaver and some in mammalian species like in California mouse (*Peromyscus californicus*) (Gubernick, 1987). Bi-parental care is beneficial for beaver especially in winter, when beaver kits are completely dependent on their parents (Sum 2003) losing even one parent may influence kit's survival.

However, there is a possibility that low number of SNPs (n=27) may have limitation in estimating proportion of EPC, as Weinman et al (2015) have suggested, to have ~ 60 SNPs for similar analysis. The biggest advantage of our study is the observational data. All families were been monitored for more than 17 years. Good observation of Eurasian beaver (Tinnesand et al. 2013) and genetic analysis together may help prevent bias.

5. Conclusion

In conclusion, we did not find any evidence for EPC in Eurasian beavers by using the molecular marker SNPs. This suggests that Eurasian beaver is strict genetically monogamous.

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1. Appendix

Table 1. Additional candidate mothers (the 6) for the 44 offspring from nearest territory that was used for parentage analysis. Birth of years and ID number has been given during trapping period

Colony	Candidate mother	Year of Birth	ID #
Lunde 3	Randi	1996	60
Lunde 6	Sonja	1996	65
Patmos 4	Tanja	2004	219
Norsjø 1	Sofie	1996	126
Gvarvbrua	Fatima	1995	30
	Teresa	2006	243

Table 2. Additional candidate fathers (the 33) from nearest territory area from the 44 offspring for the parentage analysis. Birth of years and ID number has been given during trapping period.

Colony	Candidate father	Year of Birth	ID #
Lunde	Grønn	1996	112
Lunde 1	Jon	1996	63
Lunde 2a	Ørjan	1996	57
	Frode	1996	68
Lunde 2b	Frode	1996	68
	Loran	1996	121
Lunde 4	Bram	1998	81
	Rory	2008	340
Lunde 5	Carl	1996	71
	Easy	1999	114
	Chris	1999	111
	Sander	2004	190

Lunda 6	Harald	1996	70
Patmos 0	Stuart	2003	211
Patmos 2	Ola By	1998	102
	Tommy	1999	159
Patmos 3	Erlend	1999	157
Patmos 4	Horst	2004	245
	Ivo	2010	2010
Patmos 6	Ludwin	2005	266
Lille Patmos/Bråfjorden	Edwin	2006	286
Bråfjorden b	Moritz	2005	253
Lile patmos	Kjartan	2002	205
	Elliott	2010	347
Gvarvbrua	Klumpen	2000	163
	Paddy	2008	274
	Franky	1995	49
	Harrison	2009	336
	Franky	1995	49
Norsjø 1	Jobu	1995	54
	Alasdair	2008	269
	Terje	1998	106
Norsjø 2	Hr. Nilsson	1998	44

Table 3. Beaver samples (the 9) that we did not get good DNA concentration and purity

Beaver name	ID number	DNA
Frouke	**79**	***
Rambo	**118**	***
Mærta	**134**	***
Mett-Marit	**191**	***
Ida	**214**	***
Anne Line	**283**	***
Anna	**361**	***

Forsberg	**385**	***
Harald	**70**	***

*** DNA quality was not sufficient for further analysis.

Table 4. The Beaver colony from Norsjø 1 that we did not used for parentage analysis, because we did not have hair of father sample in this family group.

Colony	Parents	Offspring	Year of Birth	ID #
	Tåkehode		1995	46
	Sofie		1996	126
Norsjø 1		Birken	1997	45
		Gunnar	1997	42
		Terje	1998	106
		Andrine	1998	39
		Jodie	1998	41
		Bjørnar	1999	105
		Rambo	1999	118
		Jojannes	2004	185
		Øystine	2004	186