Identifying QTL for fur quality traits in mink (*Neovison vison*)

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Abstract

Mapping of quantitative trait loci (QTL) affecting fur quality traits (guard hair length, guard hair thickness and density of wool) was performed in a 3-generation population (F_2-design). In the parental generation, Nordic wild mink were crossed reciprocally with American short nap mink. Twenty one wild mink were mated to 25 short nap mink. In the F_1-generation, 103 mink were mated. 934 mink were obtained in the F_2-generation. The mink were genotyped using 104 microsatellites covering all 14 autosomal chromosomes. The marker spacing was approximately 11 cM. Recordings of fur quality traits were made on all genotyped mink by Kopenhagen Fur. The QTL analyses were performed by least square regression implemented in the software GridQTL. Evidence was found for QTL for the fur quality traits on eight autosomal chromosomes (LOD score >3.0). QTL were detected for guard hair thickness on chromosomes 1, 2, 3, 4, 6, 11 and 13, for guard hair length on chromosomes 2, 3 and 6 and for wool density on chromosome 6, 7 and 13. Similar locations of QTL for guard hair length and guard hair thickness on chromosomes 2, 3 and 6 suggest that these traits are in part under the influence of the same genes.

Keywords: genetics, F_2-design, microsatellites, guard hair, wool.

Introduction

Fur quality is important in mink production. Fur quality is a composite trait, including e.g. skin length, color and purity as well as the structure of guard hair and density of wool. The identification of genes influencing fur quality traits and knowledge of the action of the genes in question provide a useful tool for improving fur quality. In mink, most of the work so far has been focused on identifying coat color genes (Anistoroaei and Christensen 2007, Anistoroaei *et al.,* 2008; Benkel *et al.,* 2009).

The mapping of quantitative trait loci (QTL) is the first step towards the identification of genes and causal mutations that affect traits of importance in agricultural production (Goddard and Hayes 2009). The purpose of QTL-analyses is to identify chromosomal areas, where loci affecting quantitative traits are harbored and provide a solid background for further analysis narrowing the chromosomal regions (Anderson 2001). It also provides the basis for understanding the genetic architecture underlying the characters (Anderson 2001) and for identifying chromosomal variability associated with a particular trait in a population in order to know if it would be possible to exploit this variability in breeding programs (Alfonso and Haley 1998).

A QTL project with the aim of identifying genomic regions harboring genes with effect on fur quality traits is being conducted. Here we present results of a search for QTL affecting guard hair length, guard hair thickness and wool density.

Materials and methods

Animal material

A reciprocal F_2-design between divergent lines was used. The lines were Nordic wild mink and American short nap mink. Mink from the Nordic line were large with coarse and long guard hair. They were brown with reddish wool of poor quality. The line has been selected for large size for several generations. American short nap animals were small with silky and short guard hair and dense wool of good quality. The mink was black, with short head and blunt snouts. The American line was established in 1998 from Patrick × Nova Scotia.

Forty-six mink from the two lines, five males from each line and 16 females from the Nordic line and 20 females from the American line, were selected for the parental generation. To establish the F_1-generation two 'blocks' were made. Short nap males were mated to Nordic wild females (block A) and Nordic wild males were mated to short nap females (block B). Five males were each mated to maximum five females. The F_2-generation was established by mating both within and between blocks. This mating was performed using 31 males and 108 females. To ensure sufficient power for statistical test (Weller *et al.,* 1990) the mink were mated in two succeeding years. It was attempted to minimize relationship between parents. Because of increased litter size in two year old females compared to one year old females, two year old females were selected in the parental generation. Mothers of the males were avoided and neither of the female mink were sibs.

Genotyping

In total 1,083 mink (parental, F_1 and F_2) were genotyped. DNA was extracted from spleens sampled at pelting from all breeding animals in the parental and the F_1-generation and from all animals in the F_2-generation.

Genomic DNA was extracted by the phenol-chloroform method. One hundred and four microsatellite markers positioned with a distance of approximately 11 cM were used for the study. Fluorescence labeling of the PCR amplicon was achieved by labeling the forward primer with FAM, HEX, or TAMRA. The 3-primer methodology (Schuelke *et al.,* 2000) has been employed as a cost-effective and efficient means of fluorescently labeling PCR fragments. Typing was produced on an ABI 3130xl sequencer (Applied Biosystems®). Scoring was conducted automatically using genotyper v. 2.5 (Perkin Elmer®).

Genetic linkage map

The published linkage map (Anistoroaei *et al.,* 2009) was improved with newly developed microsatellite markers using the pedigree information and genotypes from F_1- and F_2-generations. The software Crimap 2.4 (Green *et al.,* 1990) was applied for the analysis. A two-point analysis,

with a cut-off threshold of log of odds (LOD) 3, was performed to identify linkage groups. A pair wise recombination fractions and LOD ratios were computed. The FLIPS and BUILD options were employed for assigning the final order of the marker. A total of 104 markers were mapped to 14 linkage groups corresponding to the mink autosomes. The average linkage map spans 1,457.7 centiMorgan (cM) with an average intermarker distance of 11 cM.

Phenotyping

Phenotypes were collected for all genotyped animals. The phenotypic assessments were made at Kopenhagen Fur. All mink from the three generations were phenotyped simultaneously. Twelve phenotypic traits were evaluated. Here, results for guard hair thickness, guard hair length and wool density are presented. Guard hair thickness was measured in four categories from finest (1) to thickest (4). The guard hair length is measured as the hair protruding from the wool. The guard hair length is measured in 7 categories from very short (1), which is also called velvet 3 and is shorter than the wool, to very long (7). The wool is evaluated in 6 categories from best (1), which has the highest wool density to the poorest (6), which has the lowest density of wool. Table 1 shows mean and standard errors for the different traits.

Data-analysis

The QTL analyses were performed by a least square regression method developed by Haley *et al.* (1994) for crosses between outbreed lines, and implemented in the software program GridQTL (Seaton, 2006). The option of fitting a single QTL was used. A significance level of 0.01 was set because of a risk for marginal significance by chance due to the large datasets (Weller *et al.,* 1990). Permutation tests with 1000 permutations were used for estimating the significance level (Churchill and Doerge, 1994). Log of odds threshold (LOD score) was set to 3.7 and suggestive QTL significant at 0.05 level was set to LOD score threshold of 3 based on the aforementioned significance level. A model including adjustments of the effects of sex and year was used. Confidence intervals were estimated using bootstrap analyses with 10,000 iterations (Visscher *et al.,* 1996).

A positive additive effect indicates that alleles from the parental Nordic wild mink increase the trait value on the categorical scale. A positive dominance effect of the same order of magnitude as the additive effect indicates that a heterozygote phenotypic is similar to the wild mink,

Table1. Mean and standard errors (SE) of guard hair thickness, guard hair length and wool density of Nordic wild mink, short nap mink, and F_1- and F_2-mink born in 2006 and 2007, respectively.

	Nordic wild mink	Short nap	F_1	F_2 (2006)	F_2 (2007)
Guard hair thickness	4	1	2.78 (0.06)	2.45 (0.04)	2.29 (0.04)
Guard hair length	6.3 (0.18)	1.43 (0.13)	4.44 (0.06)	4.03 (0.04)	3.85 (0.05)
Density of wool	4.24 (0.13)	2.43 (0.34)	3.05 (0.07)	3.12 (0.05)	3.25 (0.05)

indicating that the alleles originating from the wild mink is dominant to the alleles originating from short nap. A negative dominance effect but of the same order of magnitude as the additive effect indicates that a heterozygote phenotypic is similar to the short nap, indicating that the alleles originating from short nap is dominant to alleles originating from the wild mink. The phenotypic value of the heterozygote depends on the degree of dominance relative to the additive effect. Dominance effect around zero indicates that a heterozygote is phenotypic intermediate between to the parental lines. If the dominant effect is greater or less than the additive there is overdominance. This indicates that the heterozygote has better or worse trait values than either of the parental lines. Modes of gene action are assessed from additive and dominance effects.

Results

We found evidence for QTL affecting guard hair thickness on seven chromosomes, guard hair length on three chromosomes and wool density on three chromosomes. In all, QTL affecting the traits were found on eight out of the 14 mink autosomes.

QTL affecting guard hair thickness were found on chromosome 1, 2, 3, 4, 6, 11 and 13 (Table 2). QTL on chromosome 1, 4, 11 and 13 did not reach significance level of 0.01, corresponding to a LOD score >3.7, but are suggestive at significance level of 0.05, corresponding to a LOD score >3. Alleles originating from the wild mink increase guard hair thickness (shown by positive additive effect). On chromosomes 1, 2, 3, 4 and 11 there is no dominance effect and the alleles act additively. The additive alleles from the wild mink increase the trait by a factor 0.14 (0.08) – 0.30 (0.05) on the scale. On chromosome 6, the allele from the Nordic wild mink acts dominantly to the short nap allele (shown by a dominant effect in the order of magnitude as the additive effect). On chromosome 13, the results show overdominance (shown by a larger dominance effect relative to the additive effect). Thus the heterozygote has thicker guard hair.

Table 2. Chromosome number, chromosomal location with 95% confidence intervals (CI), Lod score, additive and dominance effect with standard errors (SE) for guard hair thickness.

Chromosome	Location cM (95% CI)	Lod-score[1]	Additive effect (SE)	Dominance effect (SE)
1	0 (0.0-100.0)	3.85**	0.22 (0.05)	0.0 (0.09)
2	58 (10.0-120.0)	6.48**	0.20 (0.05)	-0.06 (0.08)
3	9 (2.0-79.0)	7.29**	0.30 (0.05)	-0.03 (0.08)
4	49 (0.0-86.0)	3.12*	0.17 (0.05)	0.08 (0.08)
6	36 (19.0-53.0)	6.55**	0.27 (0.06)	0.21 (0.08)
11	78 (0.0-81.0)	3.48*	0.19 (0.05)	-0.08 (0.07)
13	27 (0.0-63.0)	3.68*	0.14 (0.08)	0.33 (0.09)

[1] * Significant at 0.05 level; ** Significant at 0.01 level.

QTL affecting guard hair length were detected on chromosome 2, 3 and 6 (Table 3). Alleles from the Nordic wild mink add with additive effect in the range of 0.22 (0.06) – 0.31 (0.05). On chromosomes 2 and 3 there is incomplete dominance where heterozygote are phenotypic more alike short nap. On chromosome 6, the alleles originating from the wild mink are dominant to the short nap alleles.

QTL affecting wool density were found on chromosomes 6, 7 and 13 (Table 4). QTL on chromosome 7 does not reach significance level of 0.01, but is suggestive at 0.05 level. Alleles from the wild mink contribute with additive effect in the range of 0.05 (0.06) – 0.28 (0.09). Alleles from the wild mink on chromosomes 7 and 13 are dominant to short nap alleles. On chromosome 6 there is overdominance, where the heterozygotes have much lower density of wool. There is practically no additive effect (0.05 (0.06)) of this allele on chromosome 6.

The QTL on chromosomes 2, 3 and 6 affecting guard hair length and thickness were detected at the same location or in very close proximity. There are more QTL affecting guard hair thickness compared to guard hair length. Guard hair thickness is also influenced by QTL on chromosome 1, 4, 11 and 13. An analysis of correlation between the traits (Spearmans correlation test) showed very high phenotypic correlation between guard hair length and thickness (r=0.69, p=7.5 e^{-31}).

Table 3. Chromosome number, chromosomal location with 95% confidence intervals (CI), Lod score, additive and dominance effect with standard errors (SE) for guard hair length.

Chromosome	Location cM (95% CI)	Lod-score[1]	Additive effect (SE)	Dominance effect (SE)
2	58 (11.0-60.0)	9.26**	0.31 (0.05)	-0.11 (0.09)
3	9 (0.0-79.0)	4.23**	0.22 (0.06)	-0.14 (0.09)
6	36 (19.0-53.0)	7.38**	0.24 (0.06)	0.27 (0.08)

[1] ** Significant at 0.01 level.

Table 4. Chromosome number, chromosomal location with 95% confidence intervals (CI), Lod score, additive and dominance effect with standard errors (SE) for wool density.

Chromosome	Location cM (95% CI)	Lod-score[1]	Additive effect (SE)	Dominance effect (SE)
6	23 (11.0-40.0)	5.34**	0.05 (0.06)	0.31 (0.08)
7	46 (36.0-78.0)	3.18*	0.26 (0.09)	0.32 (0.13)
13	83 (0.0-83.0)	3.97**	0.28 (0.09)	0.27 (0.12)

[1] ** Significant at 0.01 level.

Discussion

We found QTL affecting guard hair length, guard hair thickness and wool density on 8 of the 14 mink autosomes. QTL affecting guard hair thickness were detected on chromosomes 1, 2, 3, 4, 6, 11 and 13. QTL affecting guard length were found on chromosomes 2, 3 and 6. QTL affecting wool density were found on chromosomes 6, 7 and 13. As expected, the alleles that increase the phenotypic values came from the Nordic wild mink. Alleles originating from the Nordic wild mink provide longer and thicker guard hair and lower density of wool. All of the QTL affecting guard hair length are located at the same positions or in very close proximity to QTL effecting thickness. These findings suggest that the traits are under influence of the same genes or that some of the genes affecting the traits might be linked. An analysis of correlation between the traits showed very high phenotypic correlation between guard hair length and thickness. This might have implications on selection programs, as it does not seem possible to select for long guard hair without also selecting for thick guard hair.

Cadieu *et al.* (2009) found that very few genes control guard hair length, coarseness and curly hair in dogs. We found that several QTL affect guard hair length and guard hair thickness. It is believed that in dogs short hair is dominant to long hair and that long hair is caused by mutations in the FGF5 gene (e.g. Housley and Venta 2006). The present study shows that the heterozygote are more alike the short nap mink, indicating that the alleles providing short hair are partly dominant to long hair. The FGF5 gene in dogs is located on chromosome 32, homolog to chromosome 11 in mink. We did not find a QTL at 5% significant level on chromosome 11 but a suggestive QTL (LOD = 2.58). In dogs RSPO2 on chromosome 13 is responsible for coarseness of the fur (Cadieu *et al.,* 2009). The position of this gene on chromosome 13 is homolog to the p arm of chromosome 4 in mink. We detected similarly a QTL affecting guard hair thickness on chromosome 4 (LOD = 3.48). In our analysis we found that that some of the QTL affecting guard hair length and guard hair thickness are located close to each other, or might even be the same genes. In the study of Cadieu *et al.* (2009) they found the genes affecting guard hair length and coarseness on different chromosomes. Thus our findings suggest a different genetic background for guard hair in mink and dogs.

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