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Divergent selection of mice for high and low swim stress-induced analgesia alters polymorphism at microsatellite *loci**, **

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The objective of this study was to determine microsatellite polymorphism in mice lines, divergently selected over 60 generations for high (HA) or low (LA) swim stress-induced analgesia. The polymorphism analysis covered 40 microsatellite markers within two lines (20 and 19 individuals for HA and LA line, respectively. The selection breeding strategy was based on a heterogeneous, outbred population of Swiss-Webster mice. The lines were earlier found to differ in brain opioid receptor density and in the expression of opioid-mediated phenomena, such as analgesic sensitivity to opiates and reversibility of swim stress-induced analgesia (SSIA) by naloxone. Apart from nociception-related traits, the HA mice displayed, as compared to the LA animals, higher emotionality in various behavioural tests, and higher degree of hypothermia when subjected to a hypothermic challenge. The present study showed that selection for HA and LA phenotypes affects the frequency of microsatellite alleles. The number of alleles per *locus* varied from 1 to 6 with a mean value of 2.9 for HA and 2.7 for LA line. Thirty-seven alleles were identified as specific to HA and 30 as specific to LA line. The expected heterozygosity ranged from 0.324 to 0.797 (mean 0.618). Of the 40 examined markers *loci* five had relatively high PIC value (> 0.7). It is concluded that HA

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and LA mice constitute a valuable source for identification of genes determining the magnitude of pain sensitivity.

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Propensity to stress and stress-related disorders is known to exhibit large inter-individual differences, caused by environmental and genetic factors and their interactions [Overstreet *et al.* 1997, Cooper 2001, Tiret 2002, Jacobs *et al.* 2006]. Despite the considerable progress in the fields of molecular biology and stress research during the last decade, the mechanisms of stress susceptibility and post-stress illness are still enigmatic. This arises principally from problems with stress measurement, as perception of stress is subjective and very complex. However, it is well known that humans and animals, exposed to stressful stimuli, e.g. swimming, display decreased sensitivity to pain [Vendruscolo *et al*. 2004, Hohmann *et al*. 2005, Blustein *et al.* 2006, Kenunen *et al.* 2006]. This phenomenon was used to create mice lines displaying high (HA) or low (LA) genetically determined swim stress-induced analgesia (SSIA). The selection breeding strategy was based on a heterogeneous, outbreed population of Swiss-Webster mice [Panocka *et al.* 1986ab].

Apart from nociception-related traits, the HA mice display, as compared to the LA animals, higher emotionality in various behavioural tests, and higher degree of hypothermia when subjected to a hypothermic challenge [Konarzewski *et al.* 1997, Lapo *et al.* 2003ab]. Moreover, the HA mice appeared to be more susceptible to the mutagenic effect of whole body γ-radiation and mitomycin C injection and lower nucleolar organizer regions activity [Sacharczuk *et al.*, 2003ab]. Especially interesting is that the lines were earlier found to differ in brain opioid receptor density and in the expression of opioid-mediated phenomena, as analgesic sensitivity to opiates and reversibility of SSIA by naloxone [Kest *et al.* 1993, Mogil *et al.* 1994, Kest *et al.* 1999].

In the earlier study [Sacharczuk *et al*. 2005] we applied multilocus DNA fingerprinting method (DFP) to demonstrate that selection for magnitude of SSIA had differentiated the parental outbred population into two distinct genotypes characterized by specific minisatellite sequences for each line that may be genetic markers for particular physiological and neuro-behavioural traits. It was concluded that the selection altered the frequencies of minisatellites which are linked to genes determining susceptibility to stress, resulting in differentiation of stress-related traits. Multilocus minisatellite banding patterns have a Mendelian basis, yet specific bands cannot be associated with specific *loci* and therefore their applicability in gene mapping is low [Sacharczuk *et al.* 2005]. For such studies microsatellites are the best known markers, widely dispersed throughout animal genomes [Tauz 1989]. Microsatellite *loci* are simple sequence repeats (STRs) of mono-, di-, tri-, tetra- or penta-nucleotide units, and are uniformly distributed at approximately 100-kbp intervals on all chromosomes except the chromosome Y. Presently, genetic information about more than 7000 *loci* and high throughput polymorphism analysis are available [Lyons 2001, Sakai *et al.*

2004]. STRs are primary genetic markers; they are highly polymorphic *loci* that have been used to map quantitative trait loci (QTL), to estimate genetic variation, to determine parentage, and to determine the phylogeny of organisms [Ashley and Dow 1994, Orti *et al.* 1997].

Highly polymorphic microsatellite markers have been used in an efficient method known as 'DNA pooling' for the identification of complex disease *loci*. DNA pooling relies on differences observed in the allelic distribution between pools from affected and unaffected individuals. One of the differences is in a reduced number of alleles in the affected pool indicating the sharing of a chromosomal region. Application of that strategy led to the identification of several linked disease *loci* in the human genome [Daniels *et al.* 1998].

In the present study the genetic diversity was investigated at microsatellite *loci* in mouse HA and LA lines. For each line, estimation was made of the allelic composition and frequencies as well as the degree of polymorphism (number of alleles, heterozygosity, and polymorphic information content). It was expected that the genetic data obtained in this study would provide valuable information to be used in the search for genes determining post-stress analgesia and correlated traits.

Materials and methods

Animals

The analysis of polymorphism of 40 microsatellite DNA markers was performed on the six-week-old Swiss-Webster mice of both sexes, weighing 27-30 g at the start of the experiment (20 and 19 individuals for HA and LA line, respectively). They were obtained from the colony of mice born and reared at the Institute of Genetics and Animal Breeding of Polish Academy of Sciences, Jastrzebiec, which had been selectively bred over 60 generations for high (the HA line) and low (the LA line) swim stress-induced analgesia. The selection protocol for HA and LA was given in a paper by Panocka *et al.* [1986a]. Briefly, outbred Swiss-Webster mice, 2 min after completion of 3-min swimming in 20°C water, were screened for the latency of a nociceptive reflex on a hot plate at 56°C. Animals were tested at week 6 of age and in each generation of selective breeding, mice with postswim latencies of $\leq$ 10s and $\geq$ 50s were mated to form or continue the HA and LA lines, respectively. A similar procedure was repeated in each offspring generation, but only subjects displaying the longest and the shortest post-swim hot plate latencies were mated to maintain the survival of the lines. The mice were housed 4-5 siblings to a cage, at ambient temperature of 22 ± 2°C and 55 ± 5% relative humidity on a 12-h light/dark cycle (lights on at 07:00 a.m.), given free access to tap water and pelleted feed (rodent block chow).

The protocol for the experiments on live mice was approved by the State Ethics Commission, in conformity with the Polish law. All the procedures are commonly used and considered ethically acceptable in all the European Union countries and North America. They conform to the NIH Guide for the Care and Use of Laboratory Animals.

DNA Samples

About 2 ml of fresh peripheral blood was collected after decapitation to a sterile S-Monovette 2,7 ml EDTA (as a anticoagulant) coated syringe (SARSTEDT AG, Germany). DNA was extracted from blood using DNA Blood Isolation Spin-Kit (AppliChem, Germany) according to the manufacturers' protocol and its concentration was determined spectrophotometrically. Next, the DNA was diluted to a final concentration of 0.1 μg/μl.

Microsatellite markers

Microsatellite *loci* distributed across five (1-5) autosomes were typed using a polymerase chain reaction (PCR) protocol optimized in our laboratory for each microsatellite. A total of 40 primer pairs that selectively amplify microsatellite *loci* were purchased from the Polish Academy of Sciences Institute of Biochemistry and Biophysics. All primer sets were originally designed by Whitehead Institute/ MIT Center for Genome Research (Cambridge, USA), based on their screens of polymorphic microsatellite *loci* in mice. A complete list of markers and their physical positions is given in Table 1.

DNA genotyping

The PCR was carried out in a volume of 8.0 μl comprising 100 ng of template DNA, 2.5 pmol of each primer, 100 μM of each dNTP, 0.5 units of DNA Taq polymerase, 10 mM Tris-HCl (pH 8.8), 1.5 mM MgCl2, 50 mM KCl, and 0.1% Tryton X-100. One primer for each *locus* was labelled with fluorescein (indodicarbo-cyanine-Cy5). The PCR reaction was carried out in a thermal cycler (MJ RESEARCH PTC-200, Watertown, Mass.) as follows: 5 min. of denaturation at 94°C, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 48-68°C, and a final elongation cycle at 72°C for 10 min. The fluorescent PCR products were separated on 6% denaturing polyacrylamide gels, using an Automated Laser Fluorescent (ALFexpress) DNA Sequencer. The PCR products were analysed after 5 min. of denaturation in a 50% formamide solution containing blue dextran. In each lane, 1 to 3 (multiplex) PCR products, differing in size range, were loaded together with a standard size marker. The results were visualized and the genotyping was completed with the Allele Links 1.01 software. After automated allele calling and binning within Allele Links 1.01, individual genotypes were manually inspected before exporting the genotypes database to Excel.

Heterozygosity and polymorphic information content

Two genetic parametres were estimated from marker allelic frequencies: the probability of heterozygosity (HET) for a marker *locus* in the mouse lines [Weir 1990] and polymorphic information content (PIC) – Bostein *et al.* [1980], Anderson *et al.* [1993].

HET ranges from 0 to 1. The heterozygosity in one *locus* describes the equation:

$$H = \frac{2N(-\sum q_i^2)}{2N-1}$$

where:

N – number of individuals in population;

q_1 – the frequency of i-th allele at a *locus*.

To determine whether the microsatellite markers chosen would be informative for genome-wide scans in crosses of HA and LA lines, the PIC was calculated the PIC according to the formula:

$$PIC = 1 - \left(\sum_{i=1}^{n} p_i^2\right) - \left(\sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2p_i^2 p_j^2\right)$$

where:

p_i and p_j – frequencies of i-th and j-th allele at a *locus*

Genetic distance

Genetic distance (D_s) was measured according to the formula developed by Nei [1972]:

$$D_s = -\ln \frac{G_{XY}}{\sqrt{G_X G_Y}}$$

where:

$$G_X = (2n_x \sum x_i^2 - 1)/(2n_x - 1)$$

$$G_Y = (2n_y \sum y_i^2 - 1)/(2n_y - 1)$$

$$G_{XY} = \sum x_i y_i$$

x_{ij} and y_{ij} – frequencies of the i-th allele at the j-th *locus* in population X and Y, respectively.

Statistical

To compare differences between HA and LA lines in genetic parametres of diversity, *i.e.* the number of alleles per *locus*, the HET for a marker *locus* in the mouse lines and the PIC) the data were evaluated with *t*-test. Criterion for significance was set at $P<0.05$. Spearman correlations were used to assess the relationship between these parametres at probability level of $P<0.05$.

Results and discussion

Variant alleles between HA and LA mouse lines were analysed with a set of 40 microsatellite primers along mouse chromosomes 1-5. The average spacing between microsatellite markers along each chromosome was 11.7 cM. At the 40 microsatellite *loci* examined, the total number of alleles amounted to 115 and 110 for HA and LA mice, respectively. The number of alleles at a single *locus* ranged from 1 to 6. Mouse line-specific alleles were observed for 33 (82.5%) of the 40 microsatellite markers. The frequencies of alleles for HA and LA mice are presented in Table 2. For HA line, 37 specific alleles were found at 24 *loci*, while for LA line 30 specific alleles in the same number of *loci* were found (Tab. 1).

Table 3 presents the number of alleles *per locus*, HET and PIC indexes. The mean number of alleles at a single *locus* was 2.9 for HA line and 2.7 for LA. On the basis of the microsatellite polymorphism the mean genetic distance estimated between lines amounted to 0.485. The mean number of alleles at a single *locus*, as well as HET and PIC index for 40 analysed markers were nearly the same for HA and LA lines (Tab. 3) and differences were not found significant (*t*-test; $p > 0.05$).

Heterozygosity refers to the fraction of *loci* within an individual that are heterozygous. HET ranges from 0 to 1. The average HET values found for microsatellites selected were 0.538 for HA and 0.501 for LA mice. The highest HETs (>0.7) were estimated for D1Mit180 (0.787), D1Mit42 (0.753), D2Mit325 (0.706), D2Mit425 (0.759), D3Mit137 (0.741), D3Mit163 (0.756), D3Mit200 (0.707), D4Mit176 (0.755), D4Mit19 (0.718), D5Mit157 (0.728), D5Mit20 (0.837), D5Mit351 (0.758), and D5 Mit430 (0.743), whereas the lowest HET was identified for D5Mit77 (0.319). Generally, above 61.84% of tested markers were highly informative in respect to heterozygosity level.

PIC is a probability of determining dams' and sires' alleles in a genotype of the progeny. The higher heterozygosity of a population determines the higher PIC value. PIC ranges from 0 to 1. The most useful in genome mapping and parentage control are markers with PIC > 0.7. Microsatellite markers, which were selected in this study had an average PIC of 0.447 and 0.415 for HA and LA line, respectively. Markers were either highly informative (12.5 % with PIC >0.7) or informative (52.5 % with 0.5<PIC<0.7). The highest PIC value was estimated for *loci* D1Mit180 (0.741), D2Mit425 (0.704), D3Mit163 (0.704), D5Mit20 (0.737) and D5Mit351 (0.708). The same marker – D5Mit77 (0.273) – has lowest level of PIC and HET.

The analysis of the relationship between parametres of genetic diversity – the number of alleles per *locus*, HET for a marker *locus* in the mouse lines, and PIC – showed the significant correlation between them (Tab. 4).

The availability of high resolution genetic map of the mouse based on microsatellite markers makes the mouse a useful model for studying multigenic or quantitative trait human disease phenotypes [Frankel 1995].

The mouse lines divergently selected for high (HA) or low (LA) swim stress-induced analgesia, are widely used in behavioural and physiological studies. However,

Table 1. Characteristics of 40 microsatellite *loci* used in this study. Underlined are allele sizes absent from the Jackson Laboratory database. [A]Alleles specific for HA line. [B]Alleles specific for LA line

Microsatellite *locus*	Chromosome	Position (cM)	Primer sequence(5'→3')	Observed allele size (bp)
D1Mit64	1	5.0	AGTGCATTATGAAGCCCCAC TCAAATTTTAAAACAACCCATTTG	126, **128,**
D1Mit330	1	35.8	TCTGGTAAAAGCAGAAAATCTGG CTGTCTGTGTGCATACATGATATAGG	**88**[B], **90, 100,**
D1Mit180	1	41.0	TCTCTAAGACTAGTAACTTGCCACTCC GTCCTGTAGAGACTGTGGGTCC	**136**[B], **138, 154**[A], **156**[A], **158**[A]
D1Mit101	1	73.0	TTGGCTAATTTTTACTGCATGC CACAGGAGACAGGTATATCAGGG	**166**, 168, 170
D1Mit42	1	78.0	CTCAGGCACCATTCTAAACATG ATAGGGCAAAAAACATTCTTGC	236, **238**[B], 254
D1Mit273	1	102.5	CAGTAGCCCATGCAGACAGA CCCAGTGTGGTCTCCTCAGT	**144**[B], **146, 152**, 154[B]
D2Mit425	2	1.0	CTTCATAGCACAAGATAAGGGTAGC CATAAAAGCATGCACATGCC	**92**, 94, **116, 118, 166**[B]
D2Mit79	2	10.0	TAGAGGAAGCAAGCCACACA GACATGTGACATGAATGCTGC	**206**[B], **210**
D2Mit181	2	33.0	GGTGGCTGGAATTCTGAAAA CTATAAAAGATTGAAATCAAAGCGC	**138, 140**
D2Mit325	2	38.3	GGAAAAATTGGAAGCATGGA GATGACAAATAATATTGAATGTGTGTG	**156**[A], **168**[B], 170, 172[A], 174
D2Mit418	2	43.5	TTAATCTGACTTCAGAAAACATACACA GTAAACACTGAAGGACACCGTG	174, 176, 178[B]
D2Mit104	2	66.0	GTGACTGGACACCTTTCTTGG CCCTGAGTTCCATTCCTAATACC	**144**[A], **146**, 148, 150
D2Mit309	2	71.0	ACAAATGCCACTCTCACATCC TATTTCTCAGAGTCACTAGGAGTGATG	**116**, 118
D2Mit279	2	76.1	GGGAAAAGAAACTCCGCTTT CTGAGTTTACTGCTTAACACAACATA	**148**[B], **150**[B], **152, 154**[A]
D2Mit199	2	104.0	GGATTGAGGAAGACGTCCAA CCAAGTGAGCAGCCTTTAGG	**300, 302**, 308
D3Mit60	3	0.0	GACATCCTGGGCAACATTG GGTGTTGTTTGCTGTTGCTG	**154**[A], **158**
D3Mit130	3	3.9	AACACATGAAACGTGTGCGT TGATAGGCATGCTTAAGCCC	**122**, 136
D3Mit183	3	25.0	ATTTTCCCCAATCCAAGACC AGAATGTCTATGAATACTCCTTTCTCC	**136**[B], **138, 140**
D3Mit137	3	35.2	CTGGTATGTGCATGTAACCTTAGC ATGTAAAAGTGCTTTATCATTATCACG	**156, 174, 176**, 178[A], 180, **182**[A]
D3Mit106	3	55.0	ACTTGTGCATGGTGTGTATGC TGTGATGGCACCTTTGGTAA	**142**, **162**[A], 164[A], **166**[A]
D3Mit43	3	58.8	TGACCTCCAGAGAGTCTTCCA CTGTGCATGAGACCACTACCA	**118**[A], **120**, **122**, 124[B], **126**[B]
D3Mit200	3	77.3	CAACTTCAGTTTCTCATTTGAATTG GCAAATGGAAGAGGTTTCTCC	**105**[A], 107[A], 127, 129, 131

Table 1. Continued

Microsatellite *locus*	Chromosome	Position (cM)	Primer sequence(5'→3')	Observed allele size (bp)
D3Mit114	3	82.3	CAGGTGAAAAATCTCAGAAGGG ACTCTCATACATACATACACACCTTCA	**278**, 280
D3Mit163	3	87.6	TGGATACATACATATACATGGAAATGC TTTCTCCAGACCCATGAACC	141^{A}, 143^{A}, **145**^{A}, 147, 149^{A}, 151^{B}
D4Mit19	4	5.0	ACAGATGTGCATGATATCATTTCC GCAGGCTTCATTCCTAGCC	**204**, 206, **214**, 216, **218**^{B}
D4Mit41	4	10.5	GAAGGAGCAGACCAACTTGC TTATTTTATGTTTTGGTGTGTGCC	**106**^{A}, 112, **114**
D4Mit241	4	24.5	ATCAAAGGCTGCAGCACC TCAGGTCCTCACCCCCTC	**92**^{A}, **94**, **96**, **98**^{B}
D4Mit132	4	35.6	ACAATATTGACAGGTTCAATCAATT TCCACCTCCATATGTGTCACA	**122**^{B}, **124**, **126**^{A}, **128**^{A}
D4Mit176	4	46.5	AGATAATCCTCCAGACAGACATCC GTAAGGATATACCTATGAAGGGTTCG	**134**, **136**, 140^{B}, **142**^{B}
D4Mit57	4	56.0	ACCCTGTCTCAAAAATAACTCTGG CATCTGTCCAGTCCCCATG	**124**^{A}, **126**, 128^{B}
D4Mit127	4	77.5	GTGTGCTGATGCAGGCAC GAGAGGAATGCTGGTAGGCA	**168**^{B}, 170, **172**^{A}
D5Mit49	5	0.0	TTGTGGGACCTGCACATG CCTTATGCAAACTTAATTCAATGG	**128**^{A}, 130, 132
D5Mit180	5	10.0	TGTTTGTTTGCTCATATTTGCC CACACCGCCTGCTACTGTAA	142^{A}, **144**
D5Mit351	5	20.0	TATGTGTGTATACATTTGTGTCTGTGT GGAAGGCATCCAACATCG	**103**^{B}, **105**, 107, **109**^{A}, 111^{A}
D5Mit77	5	24.0	GCTCAGACCAAAGGCTGAAC TTCTTTACAAATTATCCAGCCTCC	**106**, 108, **112**^{A}
D5Mit233	5	29.0	TCCCCTCTGATCTCCTCAGA CCTCCTAGAATACAATTCAATGTGG	**144**^{B}, **146**, **160**^{A}, **162**, 176
D5Mit20	5	52.0	TGAATCTGTGGCCAAATGAA CTTTGCCAGAGCAGCCAT	128^{B}, **140**^{A}, **142**, 146^{B}, 148^{B}
D5Mit157	5	57.0	TAGGTATGTGGGCTTGCACA TGGCTGCTGAATTTTAGCG	**107**^{B}, **109**, 111^{A}, 121^{B}, 123^{B}
D5Mit430	5	75.0	TTCCACGTGATCATCTCTAAACA ATCCATACACAGACACACAGGC	**132**^{A}, **134**^{A}, 140, 142^{B}
D5Mit144		86.0	GAATGGCCCCATAGGTTCTT CCAGTGACACACTTCCTCCA	**150**^{B}, **152**, **154**, **156**

the utility of HA and LA mice for genetic mapping is limited by the lack of information regarding DNA allele variants between these lines. The positive response to long-term selection implies that the level of post-stress analgesia is determined by several genes. In this study it was assumed that because of inbreeding and selection, the experimental material became fixed as regards SSIA allele at all major *loci*.

Using DNA fingerprinting method, variations between HA and LA mice in minisatellite sequences were recently reported by Sacharczuk *et al.* [2005]. An analysis

Table 2. Frequency of alleles for HA and LA mouse lines and genetic position of microsatellite markers on the chromosomes

Locus	Chromo-some	Position (cM)	Allele (bp)	Frequency of allele		
				HA	LA	HA + LA
D1Mit64	1	5.0	126	0.550	0.947	0.744
			128	0.450	0.053	0.256
D1Mit330	1	35.8	88	0.000	0.447	0.218
			90	0.825	0.368	0.603
			100	0.175	0.184	0.179
D1Mit180	1	41.0	136	0.000	0.500	0.244
			138	0.125	0.500	0.308
			154	0.350	0.000	0.179
			156	0.225	0.000	0.115
			158	0.300	0.000	0.154
D1Mit101	1	73.0	166	0.500	0.158	0.329
			168	0.132	0.132	0.132
			170	0.368	0.711	0.539
D1Mit42	1	78.0	236	0.184	0.553	0.368
			238	0.000	0.447	0.244
			254	0.816	0.000	0.408
D1Mit273	1	102.5	144	0.000	0.342	0.167
			146	0.600	0.368	0.487
			152	0.400	0.132	0.269
			154	0.000	0.158	0.077
D2Mit425	2	1.0	92	0.275	0.211	0.244
			94	0.425	0.184	0.308
			116	0.175	0.316	0.244
			118	0.125	0.263	0192
			166	0.000	0.026	0.013
D2Mit79	2	10.0	206	0.000	0.553	0.269
			210	1.000	0.447	0.731
D2Mit181	2	33.0	138	0.500	0.389	0.397
			140	0.500	0.528	0.603
D2Mit325	2	38.3	156	0.225	0.000	0.115
			168	0.000	0.237	0.115
			170	0.425	0.474	0.462
			172	0.125	0.000	0.064
			174	0.225	0.263	0.244
D2Mit418	2	43.5	174	0.575	0.132	0.359
			176	0.425	0.474	0.449
			178	0.000	0.395	0.192
D2Mit104	2	66.0	144	0.050	0.000	0.026
			146	0.250	0.474	0.359
			148	0.275	0.368	0.321
			150	0.425	0.158	0.295
D2Mit309	2	71.0	116	0.325	0.211	0.269
			118	0.675	0.789	0.731

Table 2 continued.

Locus	Chromo-some	Position (cM)	Allele (bp)	Frequency of allele		
				HA	LA	HA + LA
D2Mit279	2	76.1	148	0.000	0.389	0.189
			150	0.000	0.500	0.243
			152	0.500	0.111	0.311
			154	0.500	0.000	0.257
D2Mit199	2	104.0	300	0.225	0.237	0.231
			302	0.375	0.184	0.282
			308	0.400	0.579	0.487
D3Mit60	3	0.0	154	0.425	0.000	0.218
			158	0.575	1.000	0.782
D3Mit130	3	3.9	122	0.900	0.158	0.538
			136	0.100	0.842	0.462
D3Mit183	3	25.0	136	0.000	0.211	0.103
			138	0.500	0.500	0500
			140	0.500	0.289	0.397
D3Mit137	3	35.2	156	0.100	0.105	0.077
			174	0.425	0.184	0.141
			176	0.275	0.447	0.436
			178	0.100	0.000	0.141
			180	0.050	0.263	0.179
			182	0.100	0.000	0.026
D3Mit106	3	55.0	142	0.300	1.000	0.641
			162	0.100	0.000	0.051
			164	0.325	0.000	0.167
			166	0.275	0.000	0.141
D3Mit43	3	58.8	118	0.100	0.000	0.051
			120	0.225	0.237	0.231
			122	0.675	0.526	0.603
			124	0.000	0.053	0.026
			126	0.000	0.184	0.090
D3Mit200	3	77.3	105	0.450	0.000	0.231
			107	0.025	0.000	0.013
			127	0.050	0.263	0.154
			129	0.350	0.553	0.449
			131	0.125	0.184	0.154
D3Mit114	3	82.3	278	0.175	0.421	0.295
			280	0.825	0.579	0.705
D3Mit163	3	87.6	141	0.300	0.000	0.154
			143	0.450	0.000	0.231
			145	0.050	0.000	0.026
			147	0.175	0.553	0.359
			149	0.025	0.000	0.013
			151	0.000	0.447	0.218
D4Mit19	4	5.0	204	0.125	0.158	0.141
			206	0.175	0.132	0.154
			214	0.325	0.237	0.282
			216	0.375	0.447	0.410
			218	0.000	0.026	0.013

Table 2. Continued.

Locus	Chromo-some	Position (cM)	Allele (bp)	Frequency of allele		
				HA	LA	HA + LA
D4Mit41	4	10.5	106	0.450	0.000	0.231
			112	0.150	0.474	0.308
			114	0.400	0.526	0.462
D4Mit241	4	24.5	92	0.150	0.000	0.077
			94	0.500	0.474	0.487
			96	0.350	0.447	0.397
			98	0.000	0.079	0.038
D4Mit132	4	35.6	122	0.000	0.447	0.218
			124	0.375	0.553	0.462
			126	0.175	0.000	0.090
			128	0.450	0.000	0.231
D4Mit176	4	46.5	134	0.500	0.053	0.282
			136	0.500	0.053	0.282
			140	0.000	0.421	0.205
			142	0.000	0.474	0.231
D4Mit57	4	56.0	124	0.425	0.000	0.218
			126	0.575	0.500	0.538
			128	0.000	0.500	0.244
D4Mit127	4	77.5	168	0.000	0.368	0.179
			170	0.450	0.632	0.538
			172	0.550	0.000	0.282
D5Mit49	5	0.0	128	0.175	0.000	0.090
			130	0.375	0.500	0.436
			132	0.450	0.500	0.474
D5Mit180	5	10.0	142	0.450	0.000	0.231
			144	0.550	1.000	0.769
D5Mit351	5	20.0	103	0.000	0.368	0.179
			105	0.250	0.500	0.372
			107	0.275	0.132	0.205
			109	0.375	0.000	0.192
			111	0.100	0.000	0.051
D5Mit77	5	24.0	106	0.225	0.132	0.179
			108	0.750	0.868	0.808
			112	0.025	0.000	0.013
D5Mit233	5	29.0	144	0.000	0.026	0.013
			146	0.275	0.842	0.551
			160	0.025	0.000	0.013
			162	0.675	0.105	0.397
			176	0.025	0.026	0.026
D5Mit20	5	52.0	128	0.000	0.211	0.103
			140	0.475	0.000	0.244
			142	0.525	0.053	0.295
			146	0.000	0.263	0.128
			148	0.000	0.474	0.231
D5Mit157	5	57.0	107	0.000	0.026	0.013
			109	0.375	0.289	0.333
			111	0.625	0.000	0.321
			121	0.000	0.500	0.244
			123	0.000	0.184	0.090

Table 2. Continued.

Locus	Chromo-some	Position (cM)	Allele (bp)	Frequency of allele		
				HA	LA	HA + LA
D5Mit430	5	75,0	132	0,559	0,000	0,264
			134	0,324	0,000	0,153
			140	0,118	0,526	0,333
			142	0,000	0,474	0,250
D5Mit144	5	86,0	150	0,000	0,105	0,051
			152	0,225	0,632	0,423
			154	0,175	0,105	0,141
			156	0,600	0,158	0,385

Table 3. Number of alleles, heterozygosity (HET), and polymorphic information contents (PIC) index as per *locus* for 40 microsatellite *loci* for HA and LA lines

Microsa-tellite *locus*	Alleles/ *locus* (n)		Heterozygosity expected			Heterozygosity observed			PIC		
	HA	LA	HA	LA	HA + LA	HA	LA	HA + LA	HA	LA	HA + LA
D1Mit101	3	3	0.630	0.478	0.599	0.421	0.474	0.447	0.516	0.409	0.507
D1Mit180	4	2	0.759	0.528	0.797	0.950	1.000	0.974	0.669	0.375	0.741
D1Mit273	2	4	0.505	0.744	0.674	0.500	0.947	0.718	0.365	0.651	0.601
D1Mit330	2	3	0.304	0.665	0.572	0.350	0.842	0.590	0.247	0.553	0.496
D1Mit42	2	2	0.317	0.522	0.665	0.368	0.579	0.474	0.255	0.372	0.572
D1Mit64	2	2	0.521	0.105	0.391	0.900	0.105	0.513	0.372	0.095	0.309
D2Mit104	4	3	0.714	0.649	0.699	0.800	0.632	0.718	0.618	0.536	0.614
D2Mit181	2	2	0.442	0.528	0.492	0.600	1.000	0.795	0.332	0.375	0.364
D2Mit199	3	3	0.683	0.607	0.646	0.800	0.737	0.769	0.573	0.511	0.558
D2Mit279	2	3	0.528	0.621	0.763	1.000	1.000	1.000	0.375	0.501	0.695
D2Mit309	2	2	0.462	0.351	0.404	0.650	0.421	0.538	0.342	0.277	0.316
D2Mit325	4	3	0.739	0.686	0.715	0.900	0.895	0.897	0.652	0.586	0.654
D2Mit418	2	3	0.514	0.636	0.649	0.850	0.947	0.897	0.369	0.519	0.556
D2Mit425	4	5	0.734	0.794	0.769	0.600	0.895	0.744	0.646	0.708	0.704
D2Mit79	1	2	0.000	0.522	0.404	0.000	0.684	0.333	0.000	0.372	0.316
D3Mit106	4	1	0.757	0.000	0.553	0.950	0.000	0.487	0.665	0.000	0.496
D3Mit114	2	2	0.304	0.513	0.427	0.350	0.421	0.385	0.247	0.369	0.329
D3Mit130	2	2	0.189	0.280	0.510	0.200	0.211	0.205	0.164	0.231	0.374
D3Mit137	6	4	0.757	0.722	0.751	0.950	0.947	0.949	0.682	0.633	0.697
D3Mit163	5	2	0.709	0.520	0.766	1.000	0.789	0.897	0.617	0.372	0.704
D3Mit183	2	3	0.526	0.655	0.597	1.000	1.000	1.000	0.375	0.550	0.494
D3Mit200	5	3	0.691	0.623	0.716	0.650	0.895	0.769	0.594	0.524	0.651
D3Mit43	3	4	0.509	0.663	0.587	0.650	0.842	0.744	0.427	0.574	0.524
D3Mit60	2	1	0.514	0.000	0.350	0.850	0.000	0.436	0.369	0.000	0.283
D4Mit127	2	2	0.521	0.491	0.614	0.200	0.632	0.410	0.372	0.357	0.528
D4Mit132	3	2	0.659	0.522	0.696	0.950	0.895	0.923	0.548	0.372	0.625
D4Mit176	2	4	0.526	0.626	0.765	1.000	0.947	0.974	0.375	0.509	0.698
D4Mit19	4	5	0.745	0.740	0.727	0.900	0.947	0.923	0.654	0.655	0.659
D4Mit241	3	3	0.637	0.601	0.613	1.000	0.895	0.949	0.527	0.474	0.516
D4Mit41	3	2	0.647	0.526	0.656	0.700	0.947	0.821	0.534	0.374	0.566
D4Mit57	2	2	0.514	0.528	0.619	0.850	1.000	0.923	0.369	0.375	0.536

Table 3. Continued.

Microsatellite *locus*	Alleles/ *locus* (n)		Heterozygosity expected			Heterozygosity observed			PIC		
	HA	LA	HA	LA	HA + LA	HA	LA	HA + LA	HA	LA	HA + LA
D5Mit144	3	4	0.588	0.585	0.668	0.400	0.737	0.564	0.497	0.515	0.583
D5Mit157	2	4	0.493	0.667	0.738	0.750	0.632	0.692	0.359	0.567	0.666
D5Mit180	2	1	0.521	0.000	0.364	0.900	0.000	0.462	0.372	0.000	0.292
D5Mit20	2	4	0.525	0.696	0.794	0.950	1.000	0.974	0.374	0.600	0.737
D5Mit233	4	4	0.492	0.294	0.551	0.550	0.316	0.436	0.397	0.261	0.440
D5Mit351	4	3	0.749	0.630	0.768	0.850	1.000	0.923	0.657	0.516	0.708
D5Mit430	3	2	0.605	0.526	0.754	0.529	0.947	0.750	0.492	0.374	0.684
D5Mit49	3	2	0.659	0.528	0.592	0.950	1.000	0.974	0.548	0.375	0.485
D5Mit77	3	2	0.407	0.241	0.324	0.500	0.158	0.333	0.329	0.202	0.273
Mean	2,875	2,750	0.552	0.515	0.618	0.707	0.708	0.708	0.447	0.415	0.539

Table 4. Correlations between number of alleles per *locus*, PIC and HET for HA line (a), LA line (b) and HA and LA lines pooled (c)

a)

Trait	LA PIC	LA Heterozygosity (observed)	LA Heterozygosity (expected)
HA Alleles/*locus* (n)	0,75***	0,33***	0,66***
HA Heterozygosity (expected)	0,98***	0,63***	
HA Heterozygosity (observed)	0,58***		

b)

Trait	LA PIC	LA Heterozygosity (observed)	LA Heterozygosity (expected)
LA Alleles/*locus* (n)	0,75***	0,33***	0,66***
LA Heterozygosity (expected)	0,98***	0,63***	
LA Heterozygosity (observed)	0,58***		

c)

Trait		HA + LA PIC	HA + LA Heterozygosity (observed)
HA + LA Heterozygosity (expected)		0,99***	0,46***
HA + LA Heterozygosity (observed)		0,44***	

***P<0.001, n=43.

of the representative DNA fingerprinting patterns led to the identification of specific bands for mouse lines with a high or low sensitivity to stress. The *Hinf*I enzyme /33.6 and 33.15 probe combinations produced bands with different frequencies in each line. The average level of HET calculated on the basis of DFP patterns generated by two probes was 0.295 in HA and 0.267 in LA line. The mean genetic distance between HA and LA lines was 0.088 [Sacharczuk *et al*. 2005].

In this study, 40 microsatellite *loci* located on chromosomes 1, 2, 3, 4 and 5 were selected from The Jackson Laboratory database (http://www.informatics.jax.org/) on the basis of their utility and widespread location (potentially linked to genes affecting the response to stress). As a result of the analysis of polymorphism at these *loci*, 37 specific alleles were identified for HA and 30 for LA line. Similarly to the results obtained with DFP method, the mean level of HET was slightly higher in the HA line. The microsatellite markers shoved a mean HET higher for two lines than that calculated on the basis of DFP. The mean HET values calculated for microsatellites selected for this study amounted to 0.538 for HA and 0.501 for LA mice, while those calculated on the basis of DFP patterns generated by two probes appeared nearly two-fold lower – 0.295 in HA and 0.267 in LA line. Larger difference between these methods was observed for genetic distance. On the basis of microsatellite polymorphism the mean genetic distance was 0.485, whereas according to DFP method only 0.088. The lower diversity within lines and higher similarity between them as calculated with DFP method may be caused by higher linkage of microsatellites with genes affecting selected trait when compared to minisatellites. It indicates, that although results obtained based on minisatellite and microsatellite polymorphism analysis display the same tendency in genetic parametres of diversity, their values may differ significantly.

The selection for a high or low level of SSIA had different effects on the frequencies of alleles of the analysed microsatellite *loci*. A further analysis is needed to check whether this response reflects a linkage to neighbouring genes. Because of similarities in the expression of this trait in mice and humans, an investigation of the *loci* in mice may provide useful insights into locating genes affecting response to stress in the latter. Mouse lines selected for high or low SSIA constitute a valuable source enabling the identification of real genes causing the effect of interest.

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Rozbieżna selekcja myszy na wysoką i niską analgezję postresową wywołaną pływaniem zmienia polimorfizm ich sekwencji mikrosatelitarnych

S t r e s z c z e n i e

Celem badań było określenie polimorfizmu sekwencji mikrosatelitarnych w liniach myszy selekcjonowanych rozbieżnie przez ponad 60 pokoleń na wysoką (HA – *high analgesia*) i niską (LA – *low analgesia*) analgezję postresową wywołaną pływaniem. Selekcja oparta była na outbredowej populacji myszy Swiss-Webster. Analiza molekularna dotyczyła polimorfizmu 40 markerów mikrosatelitarnych w obrębie wymienionych dwóch linii, które – jak wykazano we wcześniejszych badaniach – różnią się gęstością receptorów opioidowych i cechami związanymi z aktywnością układu opioidowego, tj. wrażliwością analgetyczną na opiaty i blokowaniem przez nalokson analgezji wywołanej stresem pływania. Oprócz cech związanych z nocycepcją, myszy linii HA w porównaniu z LA wykazywały wyższy poziom zachowań emocjonalnych w różnych testach behawioralnych i wyższy poziom hipotermii po umieszczeniu ich w środowisku hipotermicznym. Prezentowane badania wykazały, że selekcja w kierunku fenotypów HA i LA spowodowała zmiany we frekwencji alleli mikrosatelitarnych. Liczba alleli w *locus* wahała się od 1 do 6 i wyniosła średnio 2.9 w linii HA i 2.7 w linii LA. Zidentyfikowano 37 alleli specyficznych dla myszy linii HA i 30 alleli specyficznych dla myszy linii LA. Przewidywana heterozygotyczność mikrosatelitów wynosiła od 0.325 do 0.797 (średnio 0.618). Spośród zbadanych 40 markerów mikrosatelitarnych pięć wykazywało relatywnie wysoką wartość PIC (> 0.7). Uzyskane wyniki wskazują, że myszy linii HA i LA stanowią bardzo dobry model do identyfikacji genów warunkujących odczuwanie bólu.