Animal Science Papers and Reports vol. 23 (2005) no. 2, 129-138
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DNA fingerprinting patterns in two lines of mice divergently selected for high and low swim stress-induced analgesia*

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(Received February 3, 2005; accepted May 25, 2005)

DNA fingerprinting patterns (DFPs) obtained by multi-*locus* probes have been shown to be useful in assessing genetic variation and genetic distances in laboratory animals. Using this method DFP profiles were analysed of mice belonging to lines divergently selected over 56 generations for high (HA) and low (LA) swim stress-induced analgesia. 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. In earlier study the authors showed that HA mice appeared to be more susceptible to the mutagenic effect of whole-body ă-radiation and mitomycin C injection. Lower nucleolar organizer regions (NOR) activity was also observed. In the present study the use of DFP method demonstrated that selection for magnitude of swim stress-induced analgesia 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 is concluded that the selection altered the

*Supported by the EU Framework 6 NEWMOOD Integrated Project, Polish Academy of Sciences grants No. CPBP-04.01/6.13 and No. CPBP-05.05/5.1 and the Polish Committee for Scientific Research (KBN) grant No. 6 PO4 018 18. The selective breeding programme has been financed by the Polish Academy of Sciences Institute of Genetics and Animal Breeding, Projects No. S.5.5., No. S.2.9. and No. S.6.4.

frequencies of minisatellites which are linked to genes determining susceptibility to stress, resulting in differentiation of stress-related traits.

KEY WORDS: DNA fingerprinting / minisatellites / mouse / selection / stress

Since the formulation of stress theory by Selye [1946] many studies have been conducted in this area. 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, display decreased sensitivity to pain [Moskowitz *et al*. 1985, Mogil 1999]. This phenomenon was used to create mouse lines displaying high (HA) or low (LA) swim stress-induced analgesia. The selection and breeding strategies were based on a heterogeneous, outbred population of Swiss-Webster mice [Panocka *et al*. 1986b]. Expression of behavioural traits of the HA and LA mice is not affected by conditions in a number of independent laboratories in which experiments using these lines were carried out, since the results showed high repeatability. For this reason, outbred animals seem to be more valuable as experimental material than inbred strains, which are very sensitive to the environmental and experimental conditions [Abramovici and Wolman 1995, Crabbe *et al*. 1999].

The selected mouse lines differ not only in the expression of stress-related phenomena, such as the magnitude of analgesia elicited by swimming or electric footshock, but also display different emotionality in a variety of behavioural tests. Compared with the LA line, the HA mice are less active in open field, manifest an enhanced startle response to acoustic stimuli [Błaszczyk *et al*. 2000], and when tested in the forced swim and tail suspension tests, they adopt passive behaviour assumed to reflect a depressive-like state. A similar phenomenon occurs in humans as a consequence of severe psychological load [Panocka *et al.* 2001]. These behavioural traits suggest, that the selected lines may constitute a model for studying the genetic background of stress-induced behavioural and physiological responses and post-stress disorders.

A major effect of selection was achieved already in the first generation, which argues strongly that susceptibility to stress is highly heritable. However, the HA line may not have reached asymptotic response until the 21st selected generation [Panocka *et al*. 1986a]. Moreover, HA selection appeared to progress faster than that of the LA selection. Beginning with the 21st generation, the HA mice have displayed maximum hot-plate latencies after swim stress, while in the LA line stress-induced analgesia has been undetectable Mogil *et al*. 1995a,b]. It is worth noting that the HA mice still displayed a high variation in hot plate latencies (selected trait), as compared to the LA line [Sacharczuk *et al*. 2003a]. Thus, the lines differed not only in the mean, but also in variation of the trait.

To monitor asymmetrical selection progress on the molecular level, screening of HA and LA genomes by multi-*locus* DNA fingerprinting patterns (DFP) analysis was performed. Multi-*locus* probes using DNA fingerprinting method detect minisatellites,

which are segments of DNA typically of several kb in length. They are composed of tandem repeats of short motifs, generally 15-100 bp in length [Jeffreys *et al*. 1985]. Alleles at a specific *locus* may vary in length because of variation in the number of repeat units [Jeffreys *et al*. 1991]. Multiple *loci* may be detected using a probe containing multiple copies of a "core" sequence common to the repeat units at many different *loci* [Jeffreys *et al*. 1985]. The high degree of variation at minisatellite *loci* has been a key factor in incorporating these markers into studies on laboratory animals [Russell *et al*. 1993, Benavides *et al*. 1998]. Multi-*locus* minisatellite banding patterns have a Mendelian basis, yet specific bands cannot in general be associated with specific *loci.* However, in the absence of detailed knowledge, multi-*locus* probes provide an interim technique for assessment of the genome, until more detailed knowledge of the composition and architecture of the polygenes involved is obtained.

Material and methods

Animals

Used were Swiss-Webster mice males, six weeks old with mean body weight of 30 g (no differences were not found in DNA fingerprinting pattern between males and females), from lines divergently selected for 49 generations towards high (HA) and low (LA) swim stress-induced analgesia. The selection was carried out at the Polish Academy of Sciences Institute for Genetics and Animal Breeding, Jastrzębiec, Poland as described by Panocka *et al*. [1986b]. Briefly, outbred mice were exposed to 3 min swimming in 20°C water. Two minutes after completion of the swim the animals were measured for the latency of a nociceptive reflex on a hot plate at 56°C. Males and females displaying the longest (50-60 s) and the shortest (<10 s) post-swim latencies of the hind paw flick or lick response were chosen as progenitors of the high analgesia (HA) and the low analgesia (LA) line, respectively. A similar procedure was repeated in each offspring generation, and only individuals displaying the longest and the shortest post-swim hot plate latencies were mated to maintain, respectively, the HA and the LA line.

The animals were housed in same-sex and same-family groups of four-five per cage (290 × 210 × 100 mm) at 23°C. In all experiments mice were maintained on 12:12 light-dark cycle and had unlimited access to murine feed and water.

The protocol for the experiment was approved by the Third Local Ethics Commission on Animal Experimentation. All the procedures followed in this study are commonly used and considered ethically acceptable in all the European Union countries and Norh America [Smith and Jennings 1998, Stafleu *et al*. 1999, Hawkins 2002].

DNA fingerprinting

For the present study DNA was extracted from the tail tissue of 36 individuals in total, half from the HA line and half from the LA line. Eight mice from each line (HA and LA) were used to analyse individual patterns and 10 from each line for preparing pooled DNA. Extraction and probing protocols were according to Bruford *et al*.

[1992]. Genomic DNA from each sample (individual or pooled) was digested overnight (at 37°C) using the restriction enzyme *Hinf*I. Digested samples were purified by phenol/chloroform/isoamyl alcohol (25:24:1) extraction followed by chloroform/isoamyl alcohol (49:1) and ethanol precipitation.

DNA concentrations were determined fluorimetrically at 270, 320 and 360 nm. To determine DNA pattern 8 μg of digested DNA of each sample were used. Samples were run side by side on a 20 × 20 cm 0.8% agarose gels, with Tris-borate (0.089 M Tris, 0.089 M borate, 2 mM EDTA, pH 8.3) tank and gel buffers. An additional molecular-weight marker (*Hind*III-digested lambda DNA) was added in the outside lane and gel running times were set so that fragments >1.0 kb were retained on the gels. The gels were blotted onto nylon membrane (Hybond-Nfp; AMERSHAM) using basic capillary techniques [Sambrook and Russell 2001], air-dried, and fixed using ultraviolet irradiation (320 nm). A filter previously dampened in 1×SSC was placed in a glass tube. The filter was pre-hybridized for 40 min at 50°C in a buffer composed of: 0.495 M Na_2HPO_4 (pH 7,2); 0.1% SDS (100 μl of buffer per 1cm^2 of filter). Next, the buffer was changed for hybridization by adding 1% casein as a blocking factor and 8 μl of probe 33.6 (NICE CELLMARK DIAGNOSTIC). The filter was hybridized for 30 minutes in constant temperature (50°C). The chemiluminescent signal was detected using Lumi-Phos® 530 solution (CELLMARK DIAGNOSTIC) and transferred to Kodak X-ray film. Finally the probe was removed from the filters and rehybridized with probe 33.15.

Computational analysis

The DNA-ProScan software was used to analyse the band patterns, being a method more efficient than visual inspection. The results were derived from the analysis performed by two researchers (inter-researcher results correlation was close to 1). The DNA fingerprinting (DFP) analysis included only bands representing fragments larger than 2 kb. Banding patterns were compared between the lanes to identify shared and non-shared bands. Bands were regarded as non-shared if they differed in their position by more than half of band width and if intensity ratio was less than 1:2.

Statistical

For computing genetic parametres within and between lines two types of DNA fingerprints were prepared: individual DNA samples and mixtures of equal DNA amounts taken from animals within line (pooled samples). DNA fingerprints of the former were used to determine the band sharing degree and other genetic parametres within lines. The pooled DNA from lines was used to produce DNA fingerprinting patterns, which are representative of the lines.

Based on the results of patterns generated by DNA fingerprinting mathematical calculations were done to compare analysed individuals and to assess genetic distance between lines. Main statistical parametre of band patterns, *i.e*. band sharing (BS), based on the number of common bands between two individual samples, was used to describe the similarity between profiles of DFP. On the basis of BS parametres the probability

of identity – P [Wetton *et al*. 1987], the total number of distinct and recombinationally separable hypervariable *loci* – L [Lynch 1990], average genetic variation between individuals – AVB [Kuhnlein *et al*. 1989], heterozygosity – H [Stephens *et al.* 1992], and genetic distance between lines – DL [Lynch 1990] were determined to compare analysed individuals within and between lines. All means were compared using the General Linear Models procedure [SAS Institute 1990]. A detailed description of mathematical calculations is quoted by Sacharczuk *et al*. [2005].

Results and discussion

The analyses with the use of 33.6 and 33.15 multi-*locus* probes identified 29-38 storable bands a 2.0-20.0 kb in size per gel (Photos 1, 2 and 3). Resolved were 17 to 22 bands per individual. Measures of band sharing among individuals within and between lines were moderately high (0.765 and 0.917, respectively) and approximately normally distributed. Thus, band sharing values in the lower range of the distribution were more commonly observed in comparisons of individuals within the same line, while higher values were more commonly observed in interline comparisons. Generally, analysis of DFP showed higher variation within lines than between the lines. It is commonly observed both in animal and in humans, that differences in subtle traits do not affect overall differences between groups.

According to phenotypic variation also DFP parametres indicate the higher variation in HA than in LA line (Tab. 1). Major part of genetic variation is generated by the bands of high molecular level, which is accounted for by the higher probability of differences to appear in the length of minisatellites represented by those bands.

Analysis of DNA fingerprinting patterns for individual and pooled DNA fingerprints led to an identification of bands differentiating the

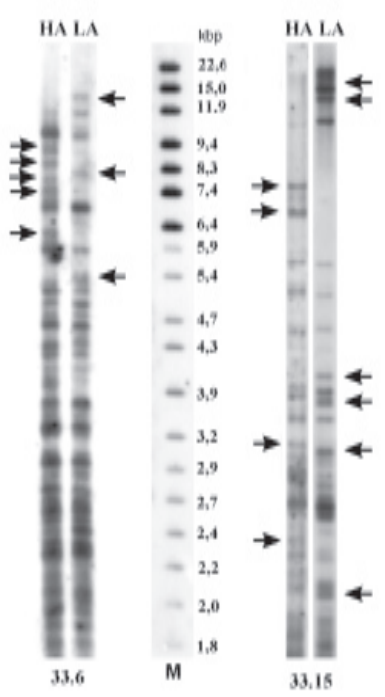

Photo 1. DNA fingerprinting of representative patterns for the HA and LA lines as revealed by two probes (33.6 and 33.15) hybridised to the same filter. Arrows indicate specific bands for each line.

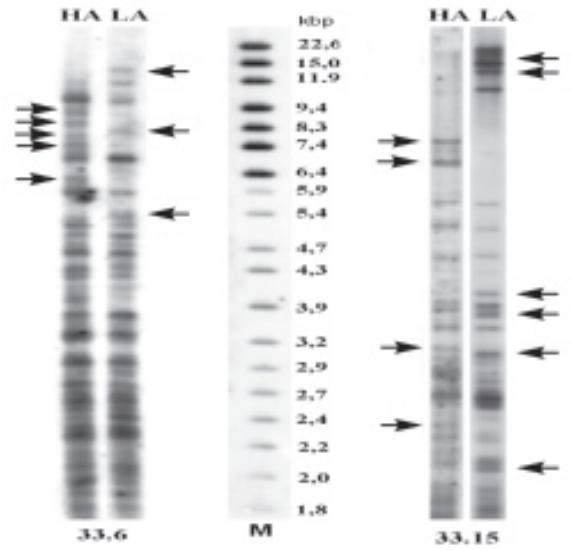

Photo 2. DNA fingerprints of sixteen individuals from the HA and LA lines as revealed by 33.6 probe.

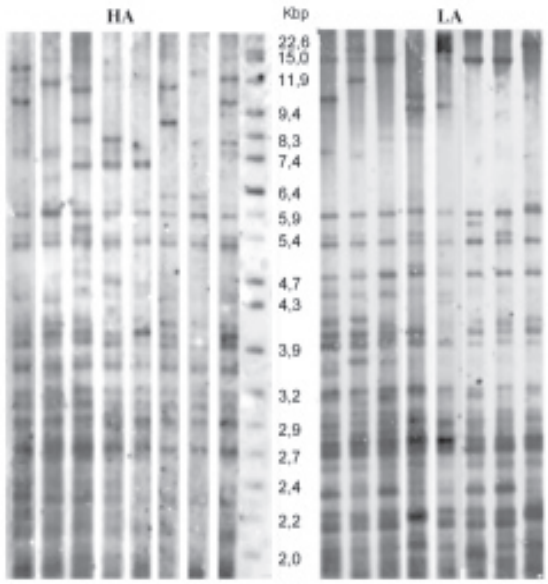

Photo 3. DNA fingerprints of sixteen individuals from the HA and LA lines as revealed by 33.15 probe.

Table 1. Means and their standard deviations (SD) for genetic parameters of diversity in mice lines selected for high (HA) or low (LA) stress-induced analgesia

Line	Probe		BS	N	L	P	AVB	H
HA	33.6	mean	0.692	15.125	15.886	4.930×10^{-4}	0.527	0.349
		SD	0.088	3.044				
	33.15	mean	0.722	20.000	24.032	2.211×10^{-5}	0.560	0.249
		SD	0.069	3.460				
	33.6+33.15	mean	0.707	17.263	19.959	2.270×10^{-4}	0.544	0.295
		SD	0.046	3.252				
LA	33.6	mean	0.751	17.556	13.756	1.766×10^{-3}	0.451	0.311
		SD	0.102	2.920				
	33.15	mean	0.781	26.444	22.938	6.765×10^{-7}	0.534	0.222
		SD	0.053	2.186				
	33.6+33.15	mean	0.765	22.000	18.347	8.875×10^{-5}	0.493	0.267
		SD	0.056	2.553				

BS – band sharing; N – average number of bands; L – number of independent loci; P – probability of identity patterns; AVB – average variation of bands; H – heterozygosity.

Table 2. DNA fingerprinting comparison of lines HA and LA

Parameter	Line HA		Line LA
BS	0.917[a]	0.909[b]	0.912[c]
DL	0.083[a]	0.091[b]	0.088[c]
Number of specific bands			
probe 33.6	5		3
probe 33.15	4		6

[a] Probe 33.6.
[b] Probe 33.15.
[c] Probe 33.6+33.15.
BS – band sharing; DL – genetic distance.

HA from LA mouse lines (Photo 1 and Tab. 2).

The frequency of specific DNA bands in selected lines is limited by the distance of mini-satellites to the critical genes and depends on the frequency of crossing-over. For this reason, some of the characteristic bands for the selected lines were observed in all examined individuals. These bands may represent minisatellites located near the genes or located within them as introns.

The principal question concerns the mechanism of line-specific minisatellites cosegregation during selection. Are they merely linked to genes determining the selected trait or do they participate in their expression? Although minisatellites are usually noncoding, some of them may play role in gene expression. Because of the fact

that minisatellites take part in many important cellular processes, such as regulation of transcription and translation as well as maintaining the stability of mRNA or modification of protein activity, they may be engaged in various physiological dysfunctions or in predisposition to diseases. Forms of lengths causing malfunction are created as a result of mutations of the original, progenitor forms [Benjamin *et al*. 1996, Lesh *et al*. 1996, Langdon and Armour 2003].

It seems that individual and cellular sensitivity to stress are not independent forms of stress. If this is true, any situation that is stressful to the animal (or human) causes changes at the cellular level. Similarly, the genetic composition of cells is closely connected with the status of stress perception [Shavit *et al*. 1985]. This may suggest that HA mice are more sensitive to stress, due to the lower adaptation buffer of cells [Sacharczuk *et al*. 2003a,b]. The selected lines can be used for the studies of genetic basis of variation in several behavioural traits.

The studies presented here are the first to provide evidence of the genetic background of stress and of the linkage between stress susceptibility and specific minisatellites. These results demonstrate that studies on the HA and LA lines may give rise to new strategies of attenuating potentially harmful effects of stress.

REFERENCES

1. ABRAMOVICI A., WOLMAN M., 1995 – Inbred strains of laboratory animals: superior to outbred mice? ***Journal of the National Cancer Institute*** 87, 933.
2. BENAVIDES F., CAZALLA D., PEREIRA C., FONTANALS A., SALAVERRI M., GOLDMAN A., BUGGIANO V., DRAN G., CORLEY E., 1998 – Evidence of genetic heterogeneity in a BALB/c mouse colony as determined by DNA fingerprinting. ***Laboratory Animals*** 32, 80-85.
3. BENJAMIN J., LI L., PATTERSON C., GREENBERG B.D., MURPHY D.L., HAMER D.H., 1996 – Population and familial associations between D4 dopamine receptor gene and measures of novel seeking. ***Nature Genetics*** 12, 81-84.
4. BLASZCZYK J.W., TAJCHERT K., LAPO I., SADOWSKI B., 2000 – Acoustic startle and open-field behavior in mice bred for magnitude of swim analgesia. ***Physiology & Behavior*** 70, 471-476.
5. BRUFORD M.W., HANOTTE O., BROOKFIELD J.F.Y., BURKE T., 1992 – Single locus and multilocus DNA fingerprinting. In: Molecular genetic analysis of populations: a practical approach (Hoelzel AR, ed). Oxford: Oxford University Press, pp 225-69.
6. CRABBE J.C., WAHLSTEN D., DUDEK B.C., 1999 – Genetics of mouse behavior: interactions with laboratory environment. ***Science*** 284, 1670-1672.
7. HAWKINS P., 2002 – Recognizing and assessing pain, suffering and distress in laboratory animals: a survey of current practice in the UK with recommendations. ***Laboratory Animals*** 36, 378-95.
8. JEFFREYS A.J., MACLEOD A., TAMAKI K., NEIL D.L., MONCKTON D.G., 1991 – Minisatellite repeat coding as a digital approach to DNA typing. ***Nature*** 354, 204-209.
9. JEFFREYS A.J., MORTON D.B., 1987 – DNA fingerprints of dogs and cats. ***Animal Genetics*** 18, 1-15.
10. JEFFREYS A.J., WILSON V., THEIN S.L., 1985 – Hypervariable „minisatellite" regions in human DNA. ***Nature*** 314, 67-73.

11. KUHNLEIN U., DAW Y., ZADWORNY D., GAVORA J.S., 1989 – DNA fingerprinting: a tool for determining genetic distances between strains of poultry. ***Theoretical and Applied Genetics*** 77, 669-672.
12. LANGDON J.A., ARMOUR J.A., 2003 – Evolution and population genetics of the H-ras minisatellite and cancer predisposition. ***Human Molecular Genetics*** 12, 891-900.
13. LESCH K.P., BENGEL D., HEILS A., SABOL S.Z., GREENBERG B.D., PETRI S., BENJAMIN J., MULLER C.R., HAMER D.H., MURPHY D.L., 1996 – Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. ***Science*** 274, 1527-1531.
14. LYNCH M., 1990 – The similarity index and DNA fingerprinting. ***Molecular Biology and Evolution*** 7, 478-484.
15. MOGIL J.S., 1999 – The genetic mediation of individual differences in sensitivity to pain and its inhibition. ***Proceeding of the National Academy of Sciences*** USA 96, 7744-7751.
16. MOGIL J.S., FLODMAN P., SPENCE M.A., STERNBERG W.F., KEST B., SADOWSKI B., LIEBESKIND J.C., BELKNAP J.K., 1995a – Oligogenic determination of morphine analgesic magnitude: a genetic analysis of selectively bred mouse lines. ***Behavior Genetics*** 25, 397-406.
17. MOGIL J.S., MAREK P., FLODMAN P., SPENCE M.A., STERNBERG W.F., KEST B., SADOWSKI B., LIEBESKIND J.C., 1995b – One or two genetic loci mediate high opiate analgesia in selectively bred mice. ***Pain*** 60, 125-135.
18. MOSKOWITZ A.S., TERMAN G.W., LIEBESKIND J.C., 1985 – Stress-induced analgesia in the mouse: strain comparisons. ***Pain*** 23, 67-72.
19. PANOCKA I., MAREK P., SADOWSKI B., 1986a – Differentiation of neurochemical basis of stress-induced analgesia in mice by selective breeding. ***Brain Research*** 397, 156-160.
20. PANOCKA I., MAREK P., SADOWSKI B., 1986b – Inheritance of stress-induced analgesia in mice. Selective breeding study. ***Brain Research*** 397, 152-155.
21. PANOCKA I., MASSI M., LAPO I., SWIDERSKI T., KOWALCZYK M., SADOWSKI B., 2001 – Antidepressant-type effect of the NK3 tachykinin receptor agonist aminosenktide in mouse lines differing in endogenous opioid system activity. ***Peptides*** 22, 1037-1042.
22. RUSSELL R.J., FESTING M.F., DEENY A.A., PETERS A.G., 1993 – DNA fingerprinting for genetic monitoring of inbred laboratory rats and mice. ***Laboratory Animal Science*** 43, 460-465.
23. SACHARCZUK M., JASZCZAK K., SADOWSKI B., 2003a – Chromosomal NOR activity in mice selected for high and low swim stress-induced analgesia. ***Behavior Genetics*** 33, 435-441.
24. SACHARCZUK M., JASZCZAK K., SADOWSKI B., 2003b – Cytogenetic comparison of the sensitivity to mutagens in mice selected for high (HA) and low (LA) swim stress-induced analgesia. ***Mutation Research*** 535, 95-102.
25. SACHARCZUK M., JEZIERSKI T., DANIEWSKI W., GÓRECKA A., PARADA R., ŚWIERGIEL A.H., JASZCZAK K., 2005 – DNA fingerprinting analysis of rabbits from lines divergently selected for high and low open-field activity. ***Animal Science Papers and Reports*** 22 (2), 107-117.
26. SAMBROOK J., RUSSELL D.W., 2001 – Molecular Cloning: A Laboratory Manual, 3rd edn. New York: Cold Spring Harbor Laboratory Press.
27. SAS Institute 1990 – SAS/STAT Users Guide, 4th edn. Cary N.C., SAS Institute.
28. SELYE H., 1946 – The general adaptation syndrome and the disease of adaptation. *The* ***Journal of Clinical Endocrinology*** 6, 117-230.
29. SHAVIT Y., TERMAN G.W., MARTIN F.C., LEWIS J.W., LIEBESKIND J.C., GALE R.P., 1985 – Stress, opioid peptides, the immune system, and cancer. ***Journal of Immunology*** 135, 834-837.
30. SMITH J.A., JENNINGS M., 1998 – Ethics training for laboratory animal users. ***Laboratory Animals*** 32, 128-136.

31. STAFLEU F.R., TRAMPER R., VORSTENBOSCH J., JOLES J.A., 1999 – The ethical acceptability of animal experiments: a proposal for a system to support decision-making. ***Laboratory Animals*** 33, 295-303.
32. STEPHENS J.C., GILBERT D.A., YUHKI N., O'BRIEN S.J., 1992 – Estimation of heterozygosity for single probe multilocus DNA fingerprints. ***Molecular Biology and Evolution*** 9, 729-743.
33. WETTON J.H., CARTER R.E., PARKIN D.T., WALTERS D., 1987 – Demographic study of a wild sparrow population by DNA fingerprinting. ***Nature*** 327, 147-152.

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Porównanie techniką DNA-fingerprinting linii myszy selekcjonowanych rozbieżnie na wysoką i niską analgezję wywoływaną stresem pływania

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

Analiza DNA fingerprinting z wykorzystaniem sond wielopozycyjnych jest użyteczną techniką w szacowaniu parametrów zmienności genetycznej zwierząt laboratoryjnych. Wykorzystując tę technikę, sporządzono profile genetyczne myszy należących do dwóch linii - jednej selekcjonowanej na wysoką (HA), a drugiej na niską (LA) analgezję wywoływaną stresem pływania. Myszy tych linii odbiegają od siebie także wynikami szeregu innych testów behawioralnych oraz poziomem hipotermii po pływaniu w zimnej wodzie, który jest wyższy w linii HA. W poprzednich badaniach wykazano dodatnią korelację między genetycznie zdeterminowaną wrażliwością na stres myszy HA a ich podatnością na mutageny (określaną częstością występowania mikrojąder i aberracji chromosomowych po napromienieniu promieniami γ lub pod działaniem mitomycyny C, a także aktywnością ich obszarów jąderkotwórczych). Obecne badania dowodzą, że selekcja na wysoką i niską wrażliwość na stres mierzona poziomem uzyskiwanej analgezji postresowej doprowadziła do powstania odrębnych genotypów, które cechuje występowanie specyficznych sekwencji minisatelitarnych dla każdej z dwóch linii. Utrwalenie się lub eliminacja alleli minisatelitarnych, specyficznych dla linii, może być wynikiem ich sprzężenia z *loci* genów determinujących selekcjonowaną cechę.