INFLUENCE OF SELECTION FOR REPRODUCTIVE LONGEVITY ON DNA FINGERPRINTING PATTERNS IN MICE

M.P. Sabour, C.Y. Lin and J. Nagai

Centre for Food and Animal Research
Agriculture and Agri-Food Canada,
Ottawa, Ontario, Canada K1A 0C6

SUMMARY

The frequencies of hypervariable DNA markers in three mouse lines, two selected for reproductive longevity and one unselected control, were examined using multilocus DNA fingerprinting (DNFP). Frequency of nine of 31 bands analyzed differed significantly ($P < .05$) between the selected and control lines. The changes in the frequency of DNFP bands for the two selected lines were consistent. These results indicate that the differences in DNFP patterns were due to the selection practised, suggesting a putative association of the DNFP patterns with reproductive longevity in mice.

INTRODUCTION

Reproductive inefficiency is the second most frequent cause of culling in livestock following low yield. Improvement of reproductive longevity offers one of the greatest opportunities for increasing the productive efficiency of livestock and poultry.

The two major components of reproductive longevity, aging (lifespan-limiting processes) and reproduction (adult fertility) are closely related and are both under multigenic control (Rose *et al.*, 1990). One strategy for the identification of such quantitative trait loci relies on the use of DNA sequence polymorphisms as genetic markers in linkage studies. This approach, which is referred to as reverse genetics, has been used to identify genes or DNA markers that are closely linked to quantitative traits of interest (Hilbert *et al.*, 1991).

Selection of animals for a trait is expected to increase the frequency of the favourable alleles at the segregating quantitative trait loci. By measuring the allelic frequency differences of any locus between the selected and unselected control populations, marker loci associated with the trait can be identified.

In farm animals selected for traits such as reproductive performance, significant changes in frequencies of specific DNA fingerprint bands (loci) have been observed when compared with unselected control strains (Kuhnlein *et al.*, 1989; Sabour *et al.*, 1992). In some cases, these DNA fragment polymorphisms show significant associations with reproductive traits under selection (Plotsky *et al.*, 1990). A comparison between reproductive performance and the DNA fingerprint (DNFP) patterns of the selected and unselected controls should reflect their genetic differences as a response to selection. The objective of the present study was to compare the DNFP patterns of lines of mice selected for length of reproductive life with those of an unselected control strain and seek evidence for an association of specific DNA markers with length of reproductive life.

Materials and Methods

Mice: The two lines of mice selected for increased reproductive longevity (days from first mating to the last parturition) and an unselected control line were developed from a common base population (Nagai *et al.*, 1990; 1994). In one selected line (designated 121) and the unselected control (221), litter size was adjusted to eight at birth, but not adjusted in the second selected line (141) during the generations of selection. Each line consisted of 30 pairs of breeders and were mated at 7-8 weeks of age, avoiding full-

sib mating. Pairs co-habitated continuously throughout their reproductive life. The progeny in the first parity of the control line and of the 5-9th parity in the two selected lines were used for line maintenance. Thus, the number of generations in the selected and control lines differed at a particular time. In the present study the selected lines at generation 12 and the control line at generation 34 were compared with respect to their DNFP patterns.

DNA Fingerprinting: Males and females were sampled at about 500 days of age from the three lines (11 mice per line) for DNA extraction. Either pooled or individual DNA was digested with *Hin*fI restriction endonuclease and subjected to fingerprinting according to a previously reported procedure (Sabour *et al.*, 1992) using Jeffreys minisatillite probe 33.15.

Analysis of Data: Frequencies of DNFP bands of pooled DNA were analyzed by computer assisted densitometric measurement (Sabour *et al.*, 1992) and by analysis of Southern blots of DNA from individual animals. Chi-square test of significance was used to detect differences in the frequencies of the DNA markers among lines.

RESULTS

Lifetime reproductive performance of the three lines at generations 12 and 16 is presented in an accompanying paper (Nagai *et al.*, 1994). Briefly, the selected line 121 at generation 12 had significantly longer (48%) reproductive days; a larger (62%) number of parturitions, and a concomitant increase (41%) in total number of young born alive during lifetime of parents. There was a further increase in

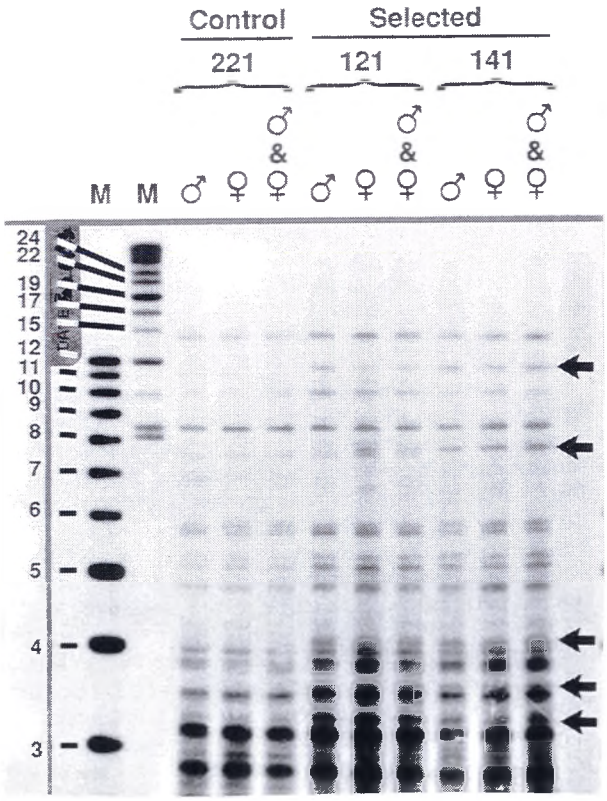

Figure 1. Autoradiogram of DNA fingerprinting patterns of mice selected for length of reproductive life (line 121 and 141) and unselected control (line 221). For each line, pooled genomic DNA from 11 individuals from each sex and 20 individual DNAs of each of two sexes were digested with *Hin*fI restriction endonuclease and hybridized with Jeffreys probe 33.15. Only 5 of the nine DNA fragments that significantly differed in their frequencies are marked (←), M: DNA size markers in kb.

reproductive life at generations 16. The two selected lines showed a significantly ($P < .01$) longer (ca. 80%) reproductive life than the contemporaneous control line at generation 16.

Fig. 1 shows the DNFP patterns of pooled DNA from the three lines. Thirty one bands ranging from 3.5 to 25 kb were analyzed. Both selected and control lines showed a similar number of bands (25-29). Densitometric evaluation of DNFP bands of the pooled DNA showed major intensity differences in nine bands which were further analyzed by individual DNFP to determine their frequencies. The frequency of these nine DNFP bands differed significantly ($P < .05$) between the selected and control lines (Table 1). There were no significant differences between the DNFP patterns of males and females and therefore only male data were presented in Table 1.

Table 1. **Frequencies of DNA bands (loci) of male mice from lines selected for reproductive longevity (line 121 and 141), or from an unselected control (line 221)**

	Control line 221[1]		Selected line[2] 121		141		
Locus (kb)	Number[3]	Frequency[3]	Number	Frequency	Number	Frequency	
25.0	1	.09	10	.91	5	.45	**[4]
14.5	4	.36	9	.82	11	1.00	**
11.5	1	.09	3	.27	10	.91	*
11.0	5	.45	0	.00	0	.00	**
7.9	1	.09	8	.73	8	.73	**
6.8	0	.00	9	.82	9	.82	**
6.4	0	.00	7	.64	8	.73	**
4.8	3	.27	11	1.00	8	.73	**
3.5	1	.09	7	.64	10	.91	**

[1] At generation 34, with litter size adjusted to 8.

[2] At generation 12 of selection with litter size adjusted to 8 (121), and not adjusted (141).

[3] Number of individuals that had the specific band (out of 11 males) and its frequency.

[4] Indicates pooled band frequencies of the selected strains that were significantly different from the unselected control (Chi-square test) * $P < .05$, ** $P < .01$.

DISCUSSION

In this study the two selected lines had significantly longer reproductive life than the control and also differed significantly from the control in the frequencies of DNA markers in 9 out of 31 bands studied. This suggests that divergent difference in reproductive life between the selected and the control lines may be due to the 9 DNA markers. A well designed study of a larger scale is needed to confirm the association and detect separate and combined effects of these markers.

Genetic analysis of identified DNFP bands and their linkage to reproductive longevity is

underway. Isolation and characterization of these bands and their use as locus specific probes in F2 and backcrosses would confirm the validity of linkage of these DNFP bands and reproductive longevity. Our preliminary survey of several other multilocus probes also reveals significant changes in the frequency of certain DNFP bands in the selected lines when compared with the control.

CONCLUSIONS

Selection for increased length of reproduction seems to affect the frequencies of certain DNA markers. These differences suggest a possible association between these DNA markers and the length of reproductive life in mice.

ACKNOWLEDGMENTS

The authors thank A.J. Jeffreys for his generous gift of the probe 33.15.

REFERENCES

Hilbert, P., Lindpaintner, K., Beckman, J.S., Serikawa, T., Soubrier, F., Dubay, C., Cartwright, P., De Gouyon, B., Julier, C., Takahasi, S., Vincent, M., Canten, D., George, M. and Lathrop, G.M. 1991. Nature 353:521-529.

Kuhnlein, U., Sabour, M.P., Gavora, J.S., Fairfull, R.W. and Bernon, D.E. 1989. Poultry Sci. 68:1161-1167.

Nagai, J., Lin, C.Y. and Sasaka, H. 1990. Theor. Appl. Genet. 79:268-272.

Nagai, J., Lin, C.Y. and Sabour, M.P. 1994. Proc. 5th World Cong. on Genetics Appl. to Livestock Prod. Submitted.

Plotsky, Y., Cahaner, A., Haberfeld, A., Lavi, U. and Hillel, J. 1990. Proc. 4th World Congr. on Genetics Appl. to Livestock Prod. XIII :133-136.

Rose, M.R. 1990. Molecular Biology of aging. C.E. Finch and T.E. Johnson (eds.) Wiley-Liss, New York. pp. 19-30.

Sabour, M.P., Chambers, J.R., Grunder, A.A., Kuhnlein, U. and Gavora, J.S. 1992. Poultry Sci. 71:1259-1270.