Diversity in the length of macronuclear chromosomes in the phylum Ciliophora : rumen ciliates and Nyctotherus – a case study

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the duodenum. Ruminal and duodenal samples were pooled from two Holstein cows fed either low forage or typical forage diets. Genomic DNA was extracted and purified, and two ciliate-specific primer sets were used to amplify hyper-variable regions within the 18S rRNA gene producing 223- and 297-bp amplicons with a G:C clamp. Denaturing gradients and running conditions were optimized for each type of amplicon. The DGGE banding profiles were markedly similar within rumen and duodenal samples from the same animal within diet but were less similar for the respective sites among the animal. After electrophoresis, the bands were excised, re-amplified, and sequenced. Sequence similarity searches were performed using BLASTn of GenBank. The presumptive identification of the sequences from excised bands from both amplicon types corresponded with predominant generic distributions observed microscopically for each animal. Based on these data, the use of protozoal standards collected from the rumen seems appropriate for a quantitative PCR assay measuring duodenal protozoal N.

Diversity in the length of macronuclear chromosomes in the phylum Ciliophora; rumen ciliates and *Nyctotherus* –a case study. N.A. Thomas[a], M. Regensbogenova[b,c], R.M. de Graaf[d], E. Devillard[a], P. Pristas[b], G.W.M. van der Staay[d], P. Javorsky[b], J.H.P. Hackstein[d], C.J. Newbold[e], N.R. McEwan[a] ([a] Rowett Research Institute, Aberdeen, Scotland, UK; [b] Institute of Animal Physiology, Košice, Slovakia; [c] University U.P.J.S. Department of Molecular and Cell Biology, Košice, Slovakia; [d] Department of Evolutionary Microbiology, University of Nijmegen, Nijmegen, The Netherlands; [e] The Institute of Rural Studies, University of Wales, Aberystwyth, Wales, UK).

Ciliates possess two types of nuclei: the micronucleus and the macronucleus. Micronuclei contain the cell's complete genetic complement and macronuclei contain a sub-population of the DNA present in the micronuclei. Despite comprising a sub-population of the micronuclear DNA complement, the macronucleus is larger, and contains more DNA. This apparent anomaly is because the macronucleus contains hundreds to thousands of copies of each macronuclear, highly processed chromosome. Ciliate cells were harvested, embedded in agarose plugs, and subjected to proteinase K treatment. Plugs were added directly to wells in agarose gels. After electrophoresis, a smear of DNA was observed, which for the rumen ciliates indicated the presence of DNA substantially larger than 10 kb. In the case of *Nyctotherus ovalis*, as with certain hypotrichous/stichotrichous ciliates, the size of the DNA observed was typically 0.5–10 kilobases (kb), suggesting that its macronuclear chromosomes are gene-sized molecules. A more accurate size of the chromosomes from rumen ciliates was determined by pulse field gel electrophoresis (PFGE). Typically the DNA seen following PFGE was around 40–50 kb, demonstrating these cells do not have gene-sized chromosomes. PFGE gels were blotted onto membranes and probed with ciliate genes. These blots produced discrete bands, rather than smears, implying the size of the chromosomes on PFGE was genuine, and not a minimum size resulting from degradation of larger chromosomes. We conclude that both these taxa possess highly processed chromosomes in their macronucleus, but that only *Nyctotherus ovalis* possesses chromosomes which are gene-sized in length. This work was supported by the EU infrastructure grant QLK3-2002-02151: CIMES.

Gene-sized macronuclear chromosomes in the anaerobic ciliate *Nyctotherus ovalis*. A.H.A.M. van Hoek, T.A. van Alen, G.W.M. van der Staay, S.Y. Moon-van der Staay, B. Boxma, J.H.P. Hackstein (Dept. Evolutionary Microbiology, Fac. Sci., University of Nijmegen, Toernooiveld 1, NL6525ED Nijmegen, The Netherlands).

Ciliates, unicellular protists with a world-wide distribution, are characterised by a nuclear dimorphism. They possess two types of nuclei, i.e. a "germ-line" micronucleus and a "somatic" macronucleus. The macronucleus of all ciliates studied so far contains highly amplified, rearranged chromosomes. In certain ciliates, micronuclear chromosomes are processed in a way that gene-sized DNA molecules are generated in the course of macronuclear development, which contain only a single open reading frame (ORF). This ORF is flanked by short runs of non-coding leader and trailer sequences; of course, these