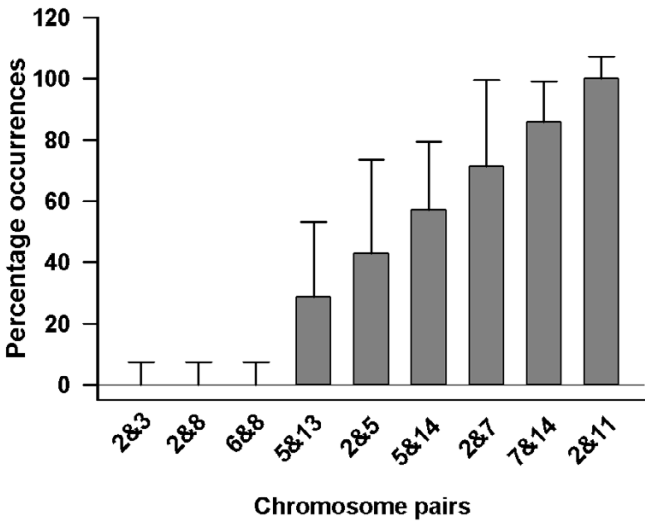

Figure 2
Frequency histogram showing centromere clustering of specific chromosome pairs in the Purkinje neurons (chromosome pairs 2&3, 2&8, 6&8 (n = 7 cells); 2&5, 2&7 (n = 10); 5&13 (n = 13); 5&14 (n = 19); 7&14 (n = 26); 2&11 (n = 29). Wide range of clustering is observed between one pair of homologues indicating trend towards varying combinatorial centromere association. Note that the second pair of homologues did not show clustering in any of the chromosome pairs studied. 95% confidence intervals are shown as error bars.

Previous work has shown that centromeres of chromosomes 2 and 11 were detected in close proximity in Purkinje neurons [11]. The results of the present work show that the additional chromosomes that contribute their centromeres to the same cluster that contains centromeres of chromosomes 2 and 11 vary. For example, the centromere of chromosome 2 has varying clustering with centromeres of chromosomes 3 and 8. It is still not known whether centromeres of some chromosomes are interchangeable with respect to the centromere cluster formation. Though the present work neither tested all the centromeric clusters nor analyzed one specific cluster to test the hypothesis that these are the same centromeres that contribute to a given cluster, our results using a pair of centromeric probes provides an indirect evidence that there is a varying contribution from different chromosomes towards a given centromeric cluster. Future experiments using primers to pericentromeric regions by new methods like capturing chromosome conformation (3C) [2,20] will help to explore the structural details.

Conclusion

The present study examined whether the centromere clusters in morphologically similar Purkinje neurons consist of same set of centromeres. Fluorescent *in situ* hybridization (FISH) with chromosome-specific para-centromeric probes provided an indirect evidence for a trend towards varying contributions from different chromosomes forming the centromeric clusters in Purkinje neurons. This structural organization among morphologically identical neurons may have physiologically important roles.

Methods

Experiments were performed under protocols approved by the University of Toronto animal care committee. Briefly, formaldehyde fixed (30 min) cerebellum of CD1 mice were Vibratome sectioned at 50 μm in a parasagital plane and used for both immunocytochemistry and FISH. For immunocytochemistry, all steps were carried out on floating sections. Sections were incubated in RNAase A (100 μg/mL PBS, 37°C, 2 hr), washed (PBS, 10 min) and blocked (4% BSA, PBS, 0.02% sodium azide, 2 hr, RT). The sections were then incubated in human CREST-type scleroderma anti-centromeric autoimmune serum (a gift from Dr. L. Rubin, 1:1000 in PBS, 2% Triton-X 100, 0.02% sodium azide, 3.2% BSA, 37°C, 24 hr, cross referenced with Centre for Disease Control Reference Serum, # 8). Following incubation, sections were washed (PBS, 3 × 20 min) and blocked again (4% BSA, 2 hr, RT) and stained by Alexa-conjugated goat anti-human IgG (Molecular Probes, 1:200, PBS, 0.02% sodium azide, 24 hr, 37°C). Sections were washed, (PBS, 3 × 10 min), counterstained (ethidium bromide 1 μg/mL, PBS, 10 min) and mounted.

For FISH experiments cerebellar vermis of adult CD1 mice was fixed (90 min) in 4% paraformaldehyde (PBS, pH 7.3). All the steps of the FISH experiments were carried out on sections that were adhered to aminopropyltriethoxysilane-coated glass cover slips. Sections were deproteinized (0.2 N HCl, 60 min, RT), washed (PBS, 3 × 5 min), permeabilized (1% Triton X-100, 1% Igepal CA-630, PBS, overnight, RT) and washed again (PBS, 3 × 5 min). Following incubation with RNAse-A (100 μg/mL, PBS, 37°C, 2 hrs), the sections were washed (PBS, 3 × 5 min), and equilibrated with Proteinase K buffer (1 mM Tris-HCl (pH 7.8), 0.5 mM EDTA, 0.05% SDS, 5 min, RT) followed by digestion with Proteinase K (Roche # 1964372), 40 μg/mL, Proteinase K buffer, 35 min, RT). Washed (PBS, 3 × 5 min) and digestion products were removed (1% Triton X-100, 1% Igepal CA-630, PBS, 20 min) and washed (PBS, 3 × 5 min). After equilibration (70% formamide, 2× SSC, overnight, RT), DNA in the cells was denatured (pre-heated 70% formamide, 2× SSC, 70°C, 12.5 min) and quickly chilled (ice-cold 50% formamide, 2× SSC, 10 min). The para-centromeric, chromosome-specific probes [21] were obtained as BAC clones (Research Genetics, MB 11300; now available from Open Biosystems, BMM 1036). The identities of the BAC clones were confirmed by *in situ* hybridization. BAC plasmids were isolated and haptens (biotinylated dATP (Invitrogen