# Structure Analysis of Two Toxoplasma gondii and Neospora caninum Satellite DNA Families and Evolution of Their Common Monomeric Sequence 

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RE: J Mol Evol (2004) 58:557-567. Figures 2, 3, and 5 of this article were presented incorrectly in the print issue, in which the photographic segment of each figure was inadvertently omitted. The figures appear here correctly along with their captions.


Fig. 2. Cloning of Sat680 element. A Genomic DNA from T. gondii RH (virulent, type I), Me49 (avirulent, type II) strains, and $N$. caninum ( Nc ) was amplified by PCR with 350 F 1 and 350 R 2 primers. The figure shows the PCR products electrophoresed in an agarose gel containing ethidium bromide. C-control without DNA. Arrowheads on the left indicate bands observed in RH and Me49 (Me) lanes that are approximately 350 bp , or a multiple of 350 bp ,
long. Bands of $350-$ and $680-\mathrm{bp}$ regions from RH, Me, and Nc were recovered, cloned in pGEM T easy (Promega) vector, sequenced, and deposited in the GenBank database. B Sequence analysis of 680-bp repetitive elements. Numbers indicate nucleotide positions. The 680-bp elements were split around position 334 , giving the halves . 1 and .2. Identity percentages were obtained by Blast2 analysis.


## T. gondii N. caninum

Fig. 3. Tandem organization of Sat 350 and Sat 680 elements. DNA from $T$. gondii and $N$. caninum were digested with SalI, blotted on a nylon membrane, and hybridized with ${ }^{32} \mathrm{P}-\mathrm{TgSat} 350-3$ and ${ }^{32} \mathrm{P}-\mathrm{TgSat} 680-2$ probes. T. gondii bands of $\sim 350 \mathrm{bp}$ or multiples, are clearly observed with Sat 350 probe. Arrows on the right of $N$. caninum Southern blot indicate detected bands. In the case of $T$. gondii DNA hybridization, membranes were exposed for 3 h in a phosphoimager, whereas in the case of $N$. caninum DNA hybridization membranes were exposed for 48 h to autoradiographies. 1 Kpb-plus and $\lambda$-HindIII were used as DNA standard (Invitrogen).


Fig. 5. PCR amplification and cloning of new $350-\mathrm{bp}$ related structures. Genomic DNA from T. gondii (A) and N. caninum (B) was amplified by PCR with different combinations of AF, AR, CF, and CR primers. The figure shows the PCR products electro-
phoresed in an agarose gel containing ethidium bromide. C-control without DNA. Arrows on the right indicate bands recovered from the gel, which were cloned in pGEM T easy (Promega) vector, sequenced, and deposited in the GenBank database.

