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Constitutive heterochromatin (CH) is usually identified by classical C-banding. However, for a more detailed CH characterization, in terms of its location, detection of different subclasses and molecular composition, other methodologies are necessary. Among these, base-specific fluorochromes technique, which delimit chromosome regions rich in different types of DNA bases and *in situ* Restriction Endonuclease (REs) digestion, which cleave DNA at specific sequences, followed by C-banding, proved to be very useful.

In the present work, the CH of *Praomys tullbergi* (PTU) was described in detail, using specific fluorochromes for DNA regions rich in AT (DAPI) or GC (CMA3) and a panel of seven REs, followed by C-banding. These combined methodologies allowed the detection of 52 CH subclasses, distributed by three classes: centromeric, interstitial and telomeric.

It is now accepted that CH regions are hotspots for structural rearrangements being implicated in chromosome evolution. The CH map constructed can be used to predict the chromosomal rearrangements involved in the evolution of this genome. Constitutive Heterochromatin Maps can thus be used to analyse the chromosomal architecture of genomes.

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