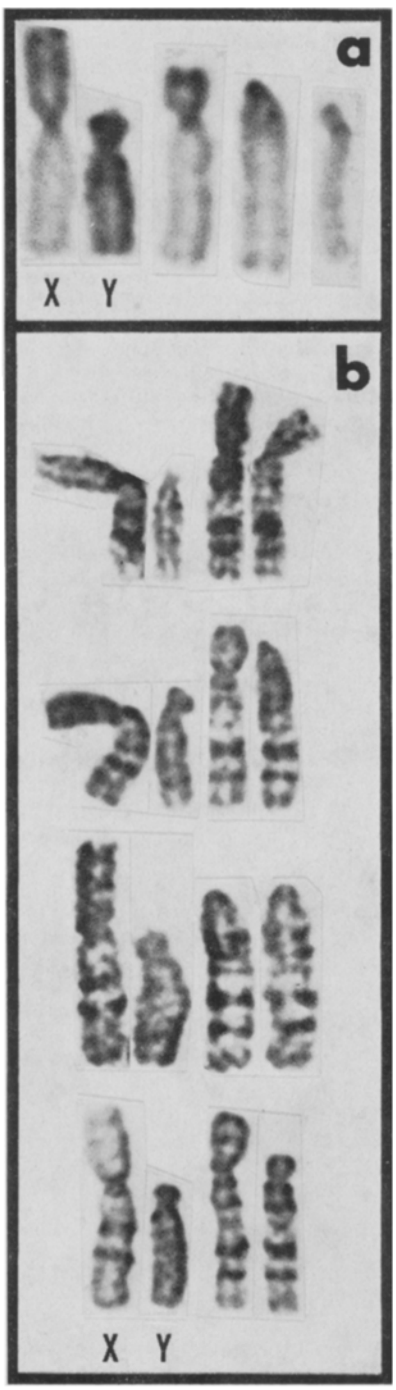
Chromosome Variations in the Plains Woodrat: A Pericentric Inversion Involving Constitutive Heterochromatin[1]

Many mammalian taxa, particularly the Cricetid rodents, have varying numbers of chromosome arms but constant diploid numbers. For example, all the species of the genus *Peromyscus* (deer mice) have a diploid number of 48 but their numbers of chromosome arms range from 56 (e.g., *P. boylei*) to 96 (e.g., *P. eremicus*)[2]. In woodrats, genus *Neotoma*, a similar but less extensive variability is found[3] but the striking fact is that the variability sometimes occurs among individuals belonging to the same species or even the same population. If every individuals in a taxon displaying this type of non-Robertsonian chromosome variation has the same amount of chromatin, then the changes in number of chromosome arms must have occured through pericentric inversions or through highly unequal reciprocal translocations. Such mechanisms should also give rise to distinctive meiotic anomalies but these have not been found[4].



a) G-band patterns of the sex chromosomes and of the 3 alternative morphologies of the autosome responsible for the observed karyotypic variations in specimens of *N. micropus*. b) G-band patterns of the sex chromosomes and the 4 observed configurations of the polymorphic pair. From top to bottom, the number of each combination examined was: 2,3,9,3.

Recently, several new karyological procedures, including heterochromatin staining (C-banding) and Giemsa banding (G-banding) have significantly furthered our understanding of these perplexing problems. Several investigators[5–7] have demonstrated that in *Peromyscus* every short arm is herochromatic and more recently PATHAK et al.[8] showed that the G-band patterns of the euchromatin in different species of *Peromyscus* were essentially the same even though the total arm numbers in these species differed drastically. These authors concluded that the karyotypic changes in *Peromyscus* were due mainly to the addition (or deletion) of heterochromatic chromosome arms and that pericentric inversions were less (if at all) involved.

BAKER et. al.[9] examined 100 individuals of the Plains Woodrat, *Neotoma micropus*, Baird, and learned that there existed a reciprocal relationship between the number of biarmed and acrocentric chromosomes, such that the diploid number was invariably 52. The number of large biarmed elements (including, for the sake of simplicity, the somewhat smaller Y in males) varied from 2 to 4 in 4 separate populations. One female had but a single large biarmed chromosome. The fact that acrocentrics large enough to be considered inversion products could not be found in individuals having less than 4 large biarmed chromosomes lead these authors to the consideration that mechanisms other than straightforward pericentric inversions were responsible for this variation.

We examined C-band and G-band preparations from 17 specimens of *N. micropus* and found that an inversion was almost certainly one of the events contributing to the karyotypic heterogeneity of this species and that this heterogeneity is somewhat more complex than previously described.

The X chromosome of this species is usually a large metacentric and the Y is a considerably smaller subtelocentric chromosome. In C-band preparations, 1 arm of the X and the entire length of the Y exhibited heterochromatic staining behavior (Figure a). The 2 largest autosomes are polymorphic. They may be biarmed with a long and a short arm or they may be acrocentric with a length equivalent to the total legth of the biarmed element. C-band patterns indicated that the short arm of the biarmed chromosome and the proximal $^1/_4$ of the acrocentric chromosome were heterochromatic (Figure a). Thus 3 types of individuals could be distinguished with respect to their two largest autosomes: those with 2

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biarmed chromosomes, those with 1 biarmed and 1 acrocentric chromosome, and those with 2 acrocentric chromosomes. In 3 cases, a fourth type was noted in which there were 2 biarmed chromosomes but one (a subtelocentric) had a distinctly shorter second arm than the other (a submetacentric). The chromosome with the shorter second arm apparently arose from a deletion, since the missing heterochromatin was not found translocated to any other chromosome of the complement.

G-bands revealed distinct homologies between the 2 largest autosomes in every case. The only regions not matching band-for-band were those regions that had stained positive for constitutive heterochromatin. Figure b illustrates the G-band patterns of the sex chromosomes and the 4 configurations of the polymorphic pair that were observed by us.

We interpret the C-band and G-band patterns of the heteromorphic autosomal pair as clear indications that the large submetacentric and the large acrocentric chromosomes are homologous except that one has sustained a pericentric inversion in the region containing constitutive heterochromatin. G-bands and C-bands themselves give no indication as to which form gave rise to the other but certain bits of circumstantial evidence indicate that the ancestral form is the submetacentric form. First, the fact that in *Peromyscus*, *Mesocricetus*, *Dipodomys*[5,10] totally heterochromatic short arms are abundant without any evidence for pericentric inversions suggests that the majority of events involving heterochromatin are additions/deletions or possibly translocations. Second, one of us (JWW) has examined over 400 *N. micropus* karyologically and found that the number of individuals homozygous for the submetacentric form of the polymorphic chromosome was greater (by over 20%) than the number of heterozygous individuals and more than 3 times as great as the number of individuals homozygous for the acrocentric form. This indicates that the acrocentric form may be less desirable and would be expected, if the placement of heterochromatin next to genes in the euchromatin has induced adverse position effects. Third, *N. micropus* and *N. floridana* appear to be very closely related (morphologically and karyologically) and probably have had a common ancestor[3,11,12]. Since the karyotypes of *N. floridana* and individuals of *N. micropus* with the two large submetacentric autosomes are identical, it seems logical to infer that the submetacentric is the ancestral form and that the subtelocentric and acrocentric forms are recent derivatives of it.

Résumé. Les bandes C et G des fivroblastes en culture de *Neotoma micropus*, Baird (le Plains Woodrat), indiquent que la base de la variation chromosomique de cette espèce est une inversion péricentrique comprenant un bloc d'hétérochromatine constitutive. On trouve aussi chez cette espèce une rature encore non-décrite affectant une partie de ce même bloc.

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A Sequential Caffeine-Cystein Treatment and Enhanced Radioprotection of *Vicia faba* Chromosomes

Recent studies reveal that, in addition to radical scavenging, cystein has a more important biochemical component to its pathway of radioprotection[1-3]. Caffeine (1, 3, 7 trimethylxanthine) is currently gaining importance as a radiosensitizer of γ- and x-ray-induced damage in a wide range of actively metabolizing test systems[4,5]; however, in dry barley seeds KESAVAN et al.[6] have recently found that it effectively decreases the magnitude of post-irradiation oxygen-dependent damage. There are also a few reports to the effect that caffeine has neither a sensitizing nor a protective action[7,8]. The radiosensitizing action of caffeine has been interpreted as due to its inhibition of either the repair replication[9] or a repair process confined to the replicative synthesis[10,11].

Since metabolic effects of a radioprotector may be expected to be antithetic to that of a radiosensitizer, we initiated a series of studies to investigate the mode and magnitude of modulation of gamma ray-induced chromosomal aberrations in *Vicia faba* pre- and post-treated with cystein and caffeine separately, sequentially or both simultaneously. The results presented here show that a treatment with caffeine applied before irradiation, and cystein immediately afterwards, gives greater protection than cystein alone to chromosomes irradiated at the G_1 stage of interphase.

Materials and methods. Solutions ($5 \times 10^{-3} M$) of caffeine and cystein separately, as well as a mixture of these in equimolar concentration, were used for treating the actively growing secondary roots of *Vicia faba* at 25 °C for 2 h. The roots were subjected to a total dose of 200 R γ-rays (3680 $Ci^{60}Co$) at a dose-rate of 38.5 R/sec. The pre-treatment solution were removed immediately after irradiation and the roots were washed in running water before placing them in the respective post-treatment solutions for 2 h. The time elapsed between irradiation and post-treatment was less than 10 min. Roots following all treat-

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