# Presence of intrachromatidic bands in mitotic chromosomes of *Triturus marmoratus* (Amphibia: Caudata)[1]

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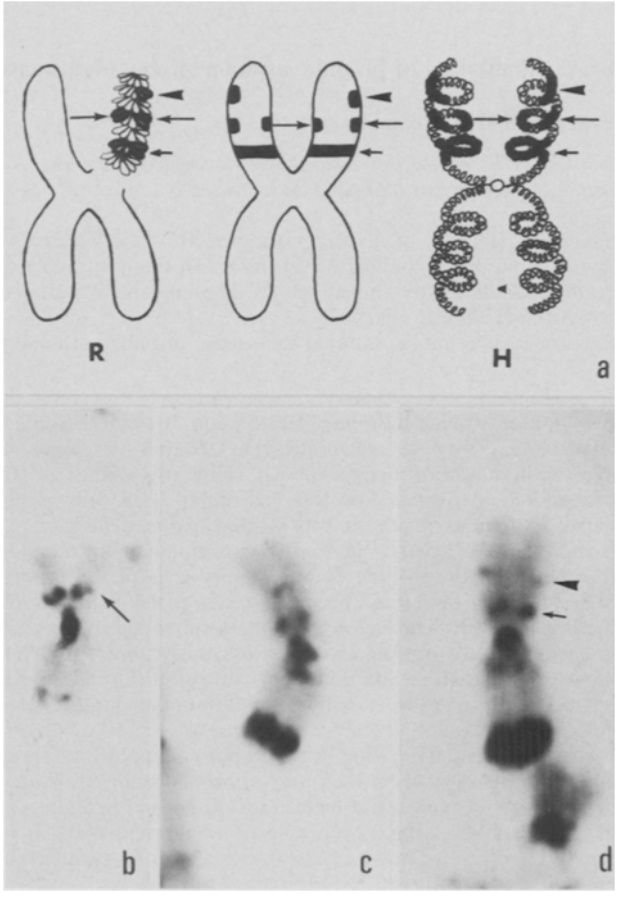
*Summary.* The C-banding technique has revealed the existence of single and double intrachromatidic bands in mitotic chromosomes of *Triturus marmoratus*. The presence of the bands is discussed on the basis of the helical coil model versus the radial loop model, which assumes a different packing of the DNA fiber.
*Key words.* Chromosome structure; vertebrate cytogenetics.

Cytological procedures do not yield a fine characterization of heterochromatic regions since the techniques applied, even those of banding, offer only a gross approach to this topic. Nevertheless, the banding techniques are useful for analyzing the patterns of karyotypic divergence existing not only between different species or populations, but also between individuals within a population[2].

Particularly in amphibians, with which the present note deals, closely related species within the same genus may differ largely in their patterns of C-banding[3–5], whereas intraspecific polymorphisms are not so commonly found[6]. The detection of populational variations will be achieved more accurately if the resolution of these bands can be improved. In this note a particular type of C-banding obtained in chromosomes of *Triturus marmoratus* by using a procedure reported elsewhere[7] is described.

*Triturus marmoratus* presents a biarmed chromosome complement ($2n = 24$). The pattern of C-banding, as described by Mancino[8], shows centromeric regions intensely stained together with pericentric, subterminal and interstitial C-positive regions, most of them weakly stained. In our case, these latter bands may occupy a total region crossing the whole chromatid region when they are large enough, and so coincide with those normally found by other authors. On the other hand, some particularly fine C-bands are not transversely extended in the whole chromatid region and appear either as single dots on one side of each sister chromatid (fig., c, d) or as, double dots, side by side, in the same sister chromatid (fig., b). Both kinds will be referred as to intrachromatidic bands. When a single intrachromatidic band is observed it can be located either in an external or in an internal position of the chromatid, such as is depicted in the figure (c and d); both representing the same region of the chromatin fiber. It is necessary to emphasize the regular symmetrical disposition of such bands in the sister chromatids. That is, in no case was an external-internal disposition of these bands in a single chromosome observed. When two heterochromatic dots per chromatid are observed, they would represent two different and individualized heterochromatic regions located very close together, but not continuous with each other, along the chromatin fiber (fig., a). In any individual these intrachromatidic bands appear in all mitotic metaphases of the tissues studied from gonads and intestines, and their visualization seems to depend, at least in these cell types, on the level of condensation reached by the chromosomes.

Obviously, it is difficult to give a plausible explanation to this particular visualization of some heterochromatic regions. Nevertheless, and on the basis of a supersolenoid structure of the chromatin fiber[9–11] with a differential packing in the regions enriched with repetitive DNA, and possibly with a particular system of distribution of clusters of repeated sequences along the chromosome, the different position of intrachromatidic bands along the chromatids as well as the appearance of double intrachromatidic bands may be supported (fig., a). The topological interpretation of intrachromatidic bands according to the radial loop model as proposed by Marsden and Laemmli[12] could be valid assuming a differential but sequential condensation of the chromatin fiber loops with different levels of compaction of the fiber (fig., a) On the other hand, the symmetry observed between the single intrachromatidic bands of sister chromatids leads us to suggest the existence of an interdependence in the structuring of the chromatin between these chromatids.




C-banded chromosomes of *Triturus marmoratus*. *a* Diagrammatic representation of a chromosome according to helical coil (H) and radial loop (R) model with a single intrachromatidic band (arrow head), a double intrachromatidic band (long arrow) and a standard band (small arrow). *b* Selected chromosome of the pair 8 showing a double intrachromatidic band. *c* and *d* Two selected chromosomes of the pair 2 showing a single intrachromatidic band: internal (c) and external (d). Small arrow shows a standard C-banded region.

Anyway, the fact that up to the present these intrachromatidic bands have been shown only in this group and not in others is perhaps related to the special chromosome organization (high spiralization) in the amphibians[5]. In this sense, the high spirilization proposed for the DNA in these species couples with the banding ability of certain chromosome regions, and so the pattern of C-banding is easily obtained while G or Q bands are difficult to show in these vertebrates.

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## Brain stimulation of juvenile hormone production in insect larvae[1]

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*Summary.* The titer of juvenile hormone (JH II) in *Galleria mellonella* decreases from 3 pmol/g b.wt in the penultimate to less than 0.1 pmol/g in the last larval instar. Hormone production is resumed when newly ecdysed last instar larvae either receive brain implants or are chilled on ice. The implanted brains as well as the chilled in situ brains stimulate JH production by a blood-borne allatotropin.
*Key words.* Wax moth; *Galleria mellonella;* juvenile hormone production; corpora allata; allatotropin, cerebral.

It has been known for fifty years that in insects a decrease or cessation of juvenile hormone (JH) secretion from the corpora allata is necessary for the termination of larval development and the initiation of metamorphosis[2]. How this change in JH production is effected, however, has never been fully elucidated. Experiments with the wax moth, *Galleria mellonella,* indicated that the larval corpora allata are stimulated by an allatotropin from the median neurosecretory cells of the brain[3]. Stimulation by the brain appears to cease at the beginning of the last larval instar. The corpora allata subsequently become insensitive to allatotropin as well as nervously inhibited[4]. This model of stimulatory and inhibitory control of corpora allata has been confirmed in experiments with another moth, *Manduca sexta*[5].

Evidence for the stimulation of the corpora allata by a cerebral allatotropin derives from the observation that brain implantations induce an extra larval molt in intact, but not in allatectomized, last instar larvae. According to an alternative explanation of these results, however, the implanted brains stimulate a precocious secretion of ecdysteroids which in turn elicit a prompt molt[6]. The nature of the molt is determined by the presence or absence of corpora allata in the larval host, because in intact larvae these glands are producing enough JH at the beginning of the last instar to support a larval molt. By contrast, allatectomized larvae lack JH and therefore undergo a pupal molt. The present study provides proof that the original interpretation of the results of the implantation studies was correct: implanted brains stimulate an increase in the body concentration of JH. It is also shown that chilling of freshly ecdysed last instar *Galleria* larvae, a treatment which causes an extra larval molt[6,7], elicits this effect by increasing the JH titer.

*Materials and methods.* This study utilized penultimate and last instar larvae of *Galleria mellonella* (Lepidoptera, Pyralidae) reared under standard conditions[8] and staged within ± 6 h. Last (VIIth) instar larvae 12 h after ecdysis were taken for brain implantations and chilling. Those selected for the implantation each received three brains dissected from 1-day-old last instar larvae[3,4]. Chilling involved placing larvae on melting ice for 30 min.

Duplicate or triplicate samples for JH determination each weighed about 2 g and contained 15–100 larvae, depending on their developmental stage. The larvae were frozen on dry ice, lyophilized, and homogenized in 3 ml methanol (p.a. grade, Lachema). The homogenate was left overnight at 0 °C, then centrifuged and the pellet washed three times with methanol. The pooled supernatants (30 ml) were reduced to 3 ml under vacuum at 50 °C, sealed in glass ampoules and stored for 1–6 months at −20 °C before further processing.

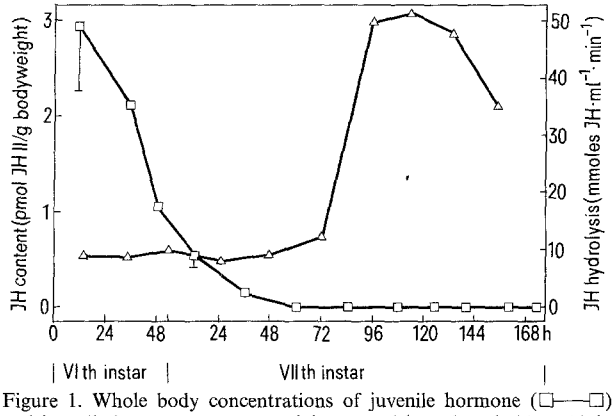


Figure 1. Whole body concentrations of juvenile hormone (□—□) and juvenile hormone esterase activity per ml hemolymph (△—△) in penultimate and last instar larvae of *G. mellonella.* Data on JH-esterase activity is reproduced from Hwang-Hsu et al.[15].

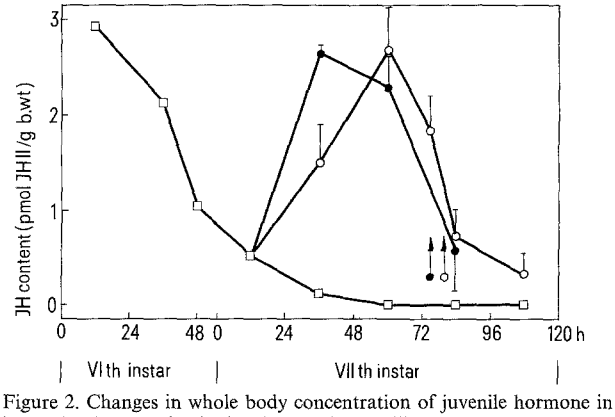


Figure 2. Changes in whole body concentration of juvenile hormone in larvae implanted with brains (●—●) or chilled (○—○) 12 h after the seventh (normally last) larval ecdysis. Hormone content of control larvae is also shown (□—□). Vertical bars indicate standard deviations and the arrows (e) the times of the supernumerary larval ecdysis.