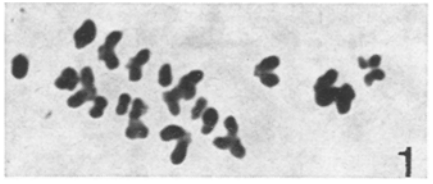
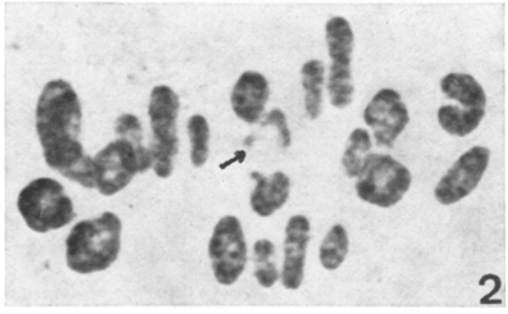
metaphases I. Among these bivalents a sex-determining system of 'parachute-like' type, Xy_p, was clearly apparent as in all other taxa of *Timarcha* (Figure 2).

Discussion and conclusions. The chromosome number of *T. intricata* allowed us to separate the *Americanotimarcha* from the other *Timarcha* previously studied, since this species shows the highest number of the genus ($2n = 44$) and its chromosomal features are not similar to those of any other species of the genus. This high chromosome number and the acrocentric shape of most elements in the karyotype of *T. intricata* suggest a derivative origin of *Americanotimarcha*, probably through an ancestral species having a primitive karyotype of 20 chromosomes by 12 chromosomal dissociations plus other possible chromosome rearrangements. The best candidate as an ancestral species of *Timarcha* would be one of the *Metallotimarcha*, because this subgenus includes the least evolved species from morphological and ecological points of view. Nevertheless, it would be necessary to verify this assumption on cytological grounds by examining one or more species of this group.





Figs. 1 and 2. Spermatogonial metaphases of *T. intricata*. *1*. Metaphase II showing 22 chromosomes mostly acrocentrics. *2* Metaphase I with 22 bivalents and among them the sex-determining mechanism, Xy_p, is indicated (×2.300).

From the chromosomal results obtained in *Americanotimarcha*, it could be concluded that this subgenus can no longer be phylogenetically considered at the *Timarcha* basis, in spite of its external morphology and male genitalia primitive characteristics. These aspects probably point towards a direct relationship between *Americanotimarcha* and *Metallotimarcha*, but since food-plant preferences and especially the karyotype of *T. intricata* is perfectly distinguishable from the others, it is necessary to assume a great genetic divergence from the hypothetically most primitive *Timarcha*.

According to some authors[10,11], the geographical origin of the genus was in Central Asia and from this area it spreaded to Europe and Northern Africa in several evolutionary lineages, and reached North America by an independent lineage, although after the Ice Age the *Timarcha* became extincted in almost the whole of Asia. The chromosomal results are not against this interpretation, which with our present knowledge, therefore, is in agreement with all data of evolutionary interest.

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Differential Giemsa Staining of the Holokinetic Chromosomes of the Two-Spotted Spider Mite, *Tetranychus urticae* Koch (Acari, Tetranychidae)

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Summary. The chromosomes of the spider mite *Tetranychus urticae* can be stained differentially with Giemsa-staining methods for G-bands. C-band patterns representing constitutive heterochromatin could not be detected. Their absence may be related to the holokinetic condition of the chromosomes.

The two-spotted spider mite *Tetranychus urticae* Koch (Acari, Tetranychidae) reproduces by arrhenotokous parthenogenesis. 3 chromosomes are found in the haploid male eggs and 6 chromosomes in the diploid female eggs[1-3]. The metaphase chromosomes differ in length and measure 1.3, 1.5 and 1.7 μm[2]. Primary constrictions are not present because of the holokinetic nature of the chromosomes[3]. Secundary constrictions have not been observed. Consequently the length is the only criterion to distinguish the individual chromosomes within the complement. In order to facilitate the identification of the spider mite chromosomes, it was decided to investigate whether the chromosomes could be stained by Giemsa techniques that have been developed to produce characteristic banding patterns on monocentric mitotic chromosomes[4,5]. In the present paper results will be reported which were obtained with procedures for C-banding to stain constitutive heterochromatin and for G-banding to produce differential staining along the length of the chromosomes.

The staining techniques were carried out on air-dried chromosome slides which were prepared from eggs in 2nd or 3rd cleavage division[3,6]. Samples of eggs were collected from detached leaf cultures of wild type *T. urticae* (strain Sambucus) between 3–4 h after oviposition. The eggs were placed, 5 in a row, on a slide, pricked, and smeared with a needle. They were then air-dried at room temperature, fixed in Carnoy 3:1 (alcohol-acetic acid),

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and air-dried again. The slides were treated with one or more of the various chemical solutions[7–16] that have been used to induce G-bands[7–12] or C-bands[12–16] in monocentric mammalian and plant chromosomes. The chemical treatments, followed by Giemsa staining, were carried out according to the given methods, or with slight modifications. Reproducible and satisfactory results were obtained only with the ASG technique for G-bands[12] in which the incubation in 2 × SSC and the Giemsa staining each lasted for 30 min. However, we found that identical results were obtained when the chromosomes were incubated in 5 *N* HCl at room temperature. Since this HCl procedure could be carried out more quickly than the ASG technique, the former was used ultimately. The schedule was as follows: 1. Treat slides with 5 *N* HCl for 10 min at room temperature; 2. Wash with tapwater, then with distilled water, and air-dry; 3. Stain with 2% Giemsa (Gurr) in Sörensen buffer adjusted to pH 6.9 at room temperature for 15 min.; 4. Same as 2; 5. Place in xylol, and mount in DPX under a coverglass.

It appeared that, in the chosen strain, the most prominent banding patterns were found in chromosomes which were clearly split into the 2 chromatids (Figure 1a). Since the chromosomes are very small and the chromatids do not appear until metaphase, the yield of usable plates was very low (about 1% of the eggs), making this research rather time-consuming. The results have been summarized in a diagram (Figure 1b). It was also found that the last connections between the parallel disjoining chromatids always were located at the same places. These connections caused characteristic curves in the chromatids just before complete separation.

In addition to the Sambucus strain, the strain Baardse J12 which is considered to carry chromosome mutations[17], was investigated. It appeared (Figures 2a, b, c) that the terminal bands of the chromosomes of the Sambucus strain could easily be found back in the Baardse J12 strain, but that the intercalary bands could not be located with certainty. However, it is very clear that reciprocal translocations have taken place in all three chromosomes.

The results with the 2 Giemsa staining techniques reported here show that banding patterns can be produced along the chromosomes of *T. urticae*. They can be used very well for the identification of the 3 individual

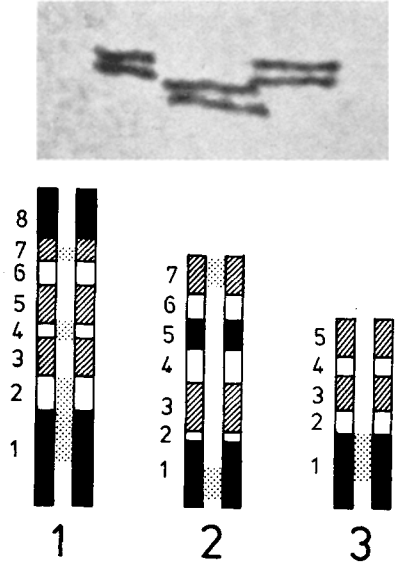

Fig. 1. Giemsa banded meta-anaphase chromosomes of *Tetranychus urticae* from cleavage divisions of haploid male eggs. ×2500. Complement of the Sambucus strain (a) and idiogram (b).

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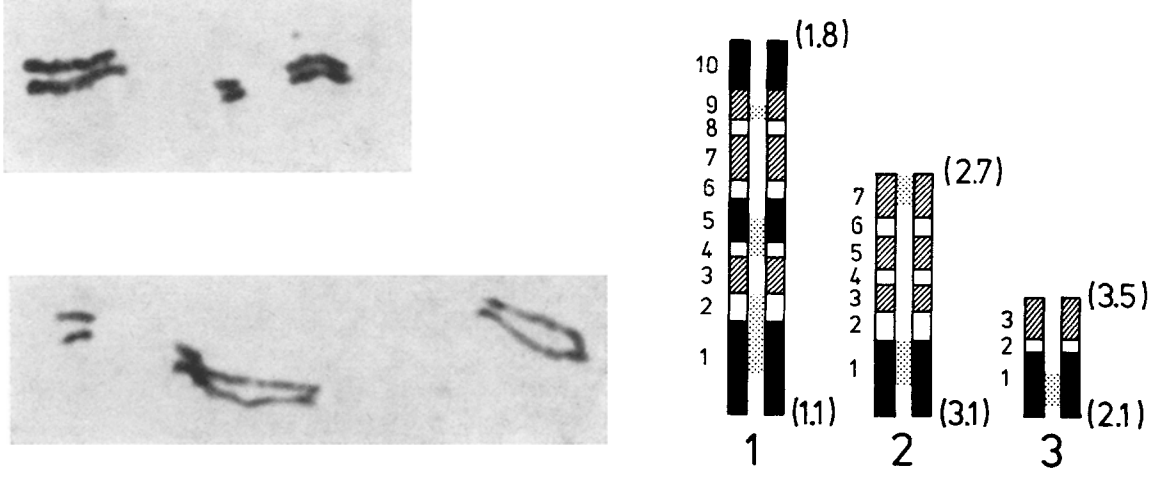

Fig. 2. Complement of the Baardse J12 strain (a), with prominent connections between the chromatids (b), and idiogram (c). The relative band sizes and distributions in the idiograms can be taken to be approximately correct. Positions of prominent connections between the chromatids are marked by dots. Solid area: very heavily stained band; crosshatched area: heavily stained band; clear area: unstained band. Figures in parentheses in Figure 2c refer to the bands in Figure 1b.

chromosomes as well as for the detection of translocations, and thus can form a helpful tool for the studies on genetic control methods of spider mites[18].

The results further demonstrate for the first time that bands can be induced in holokinetic chromosomes. According to the procedures employed the bands are G-bands. C-band patterns, however, could not be seen. In monocentric mammalian chromosomes C-bands are typically found adjacent to the centromeres and may represent constitutive heterochromatin with highly repetitive DNA[4,5]. In holokinetic chromosomes centromeres do not occur and the spindle attachment sites (kinetochores) extend the entire length or almost the entire length of the chromosomes[19–21]. Repeated DNA sequences, as found in centromeric constitutive heterochromatin, were found to be scattered throughout the holokinetic chromosomes of the milkweed bug *Oncopeltus fasciatus*[22]. The absence of C-bands in the chromosomes of *T. urticae* may thus be related to the holokinetic condition of the chromosomes. The centromeric type of constitutive heterochromatin may then be either not present at all or scattered throughout the genome in units too small to form visible bands.

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Contraction and Volume Reduction of the Glycerolated *Carchesium* Spasmoneme: Effects of Alkali Earth Cations

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Summary. The series Ca>Sr>Ba>Mg represents the relative activities of the alkali earth cations causing contraction of glycerolated *Carchesium* stalks. During contraction, spasmonemal volume is reduced by 37%.

Mature colonies of *Carchesium*, a sessile colonial peritrich ciliate, may consist of more than 100 zooids each with an individual spasmoneme. There is no structural continuity between spasmonemes of different zooids and individual stalks may retract independently. Stalk coiling in the peritrich ciliates results from spasmonemal shortening. Studies of the *Zoothamnium* spasmoneme have revealed mechanical, stress-optical, chemical and ultrastructural characteristics incompatible with either actomyosin or microtubule-based cell motile systems[2]. Specifically, contraction of the glycerolated spasmonemes in *Vorticella*[3–5], *Carchesium*[6] and *Zoothamnium*[2] can be induced simply by increasing the ambient free calcium ion concentration from 10^{-8} to 10^{-6} g ion/l. Neither ATP[7] nor magnesium ion is required. Spasmoneme re-extension follows the removal of free calcium ion. This is distinct from primitive actomyosin systems which require ATP and both calcium and magnesium ions, and from interacting microtubule mechanisms in which ATP and magnesium are essential and calcium inhibitory[8,9].

Materials and methods. Carchesium sp. were cultured at 20 °C in C solution (10^{-3} *M* $CaCl_2$, 10^{-4} *M* $MgCl_2$, 10^{-4} *M* KCl, 10^{-3} *M* $NaHCO_3$, in distilled water) to which was added 15% v/v 0.03% lettuce infusion. Glass microscope slides provided a convenient substrate. Cultures of young carchesia were obtained by treating mature colonies with 0.6 *M* urea in C solution. This treatment induced shedding from the colony of most zooids within 30 min, and shed zooids, when returned to C solution, developed new contractile stalks within 24 h[10]. In this report, young colonies are those with 1–2 zooids, mature colonies those with more than 20 zooids. The youngest stalks in mature colonies originate farthest from the pedal end.

The same glycerolation technique was used for both young and mature colonies. Slides bearing suitable colonies were washed and left overnight in C solution prior to extraction. The slides with attached carchesia were incubated for 24 h at 0 °C in relaxing solution (10^{-1} *M* KCl, 2×10^{-2} *M* *tris*-maleate buffer pH 6.8, 4×10^{-3} *M* EGTA[7], in distilled water) to which was added 50% v/v glycerol, then passed through 2×30 min washes in relaxing solution at 0 °C, and finally allowed to warm to room temperature. Cells remained anchored to the culture slide throughout extraction.

The threshold divalent cation concentration for spasmonemal contraction was determined by titration, using the spasmoneme itself as the indicator of end point. Individual glycerolated colonies or single cells were dislodged from the substrate and transferred to a glass, flat-bottomed, 50 mm petri dish containing 10.0 ml of relaxing solution. A coverslip shard resting on the main stalk pinioned the colony within the dish. Stalk coiling was prevented as required by similarly restraining both ends of the colony. Contraction was observed with a Zeiss Universal microscope equipped with phase contrast and Nomarski differential interference contrast optics with the aid of a water-immersion cap[11]. Threshold was measured by adding from a burette drop by drop a solution of divalent cation (4×10^{-3} *M* $MeCl_2$[7], 10^{-1} *M* KCl, 2×10^{-2} *M* *tris*-maleate buffer pH 6.8, in distilled water)

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