tion. Recent reports also suggest that Ca^{++} is a prerequisite for glucose-induced accumulation of cAMP in rat islets[5, 24].

Calcium ions may stimulate the accumulation of cAMP in pancreatic islets exposed to high concentrations of glucose in several ways. One possibility is that Ca^{++} inhibits the degradation of cAMP. This seems unlikely in view of the observation that glucose-induced accumulation of cAMP requires that the phosphodiesterase activity be inhibited in the present islet preparation[11]. Furthermore, Ca^{++} has not been found to affect the phosphodiesterase activity in mouse islets[25, 26]. Omission of extracellular Ca^{++} does not significantly reduce the islet oxidation of glucose at glucose concentrations below 5 mM[27], i.e. the concentration range in which ATP is critically dependent on glucose[28]. Therefore the promoting effect of Ca^{++} on cAMP accumulation might reflect changes in the adenylate cyclase activity rather than being the result of increased β-cell levels of ATP.

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Phylogenetic Position of the American *Timarcha* Latr. (*Coleoptera*, Chrysomelidae) Based on Chromosomal Data

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Summary. The chromosomal analysis of *T. intricata* Hald. has shown a diploid complement of 44 chromosomes, the highest number found in *Timarcha* and clearly different from those of the taxa previously studied. This complement favours a derivative origin of the *Americanotimarcha* subgenus from a hypothetical ancestral species of 20 chromosomes. The implications of this karyotype are also discussed with some morphological, biogeographical and ecological points of view and observations.

Since 1968, the karyological analyses carried out on 31 taxa of the genus *Timarcha* Latreille have revealed a rather wide range of variation in chromosome numbers from $2n = 20$ to $2n = 30$[2–5]. By using morphological criteria, the genus appears as sharply homogeneous; thus these unsuspected karyological differences have provided valuable tools to establish the primitiveness of the genus and have thrown light on evolutionary lineages. The karyological results were in a good agreement with some others obtained in various aspects of taxonomic interest[6].

Most *Timarcha* have a diploid complement of 20 chromosomes, a number which has been considered as the primitive among the *Coleoptera polyphaga*[7]. All taxa cytologically examined belong to the subgenus *Timarcha s. str.* (including in this sense the subgenus *Timarchostoma* Mots.), but representatives of the two other subgenera, *Metallotimarcha* Mots. and *Americanotimarcha* Jolivet, were not chromosomically analyzed.

According to several characters of external morphology and male genitalia structure, the *Metallotimarcha* and the *Americanotimarcha* are generally considered as the most ancient *Timarcha*. This aspect is presumably related with the relict geographical distribution of both subgenera, the *Metallotimarcha* in hercynian mountains of Central Europe and in the Caucasus, and the *Americanotimarcha* in western coast of North America from Vancouver (Canada) to northern California[8, 9]. However, ecological observations on the *Americanotimarcha* suggest a derivative origin of this subgenus from the ancestral generic source, since the two species of *Americanotimarcha* feed on Roseaceae while most of the other *Timarcha* feed, actual or potentially, on Rubiaceae though they may be secondarily adapted to plants of other families[8, 9]. From this point it seemed particularly interesting to know some cytological data on the *Americanotimarcha* to determine its phylogenetic position within the genus.

Material and method. A small sample of male living individuals of *T.* (*Americanotimarcha*) *intricata* Hald. were air-mailed in June 1974 from Oregon to Barcelona and immediately studied on arrival. The chromosome analysis was performed on meiotic and mitotic metaphases of spermatogonial cells by aceto-orcein squash preparations. Some microphotographs were also taken of the best metaphase spreads which complemented the microscopic observations.

Results. Two individuals were cytologically examined and both showed a diploid complement of 44 chromosomes. The karyotype of this species is constituted by chromosomes of small size, mostly acrocentrics (Figure 1). 22 rod/round shaped bivalents were easily recorded in

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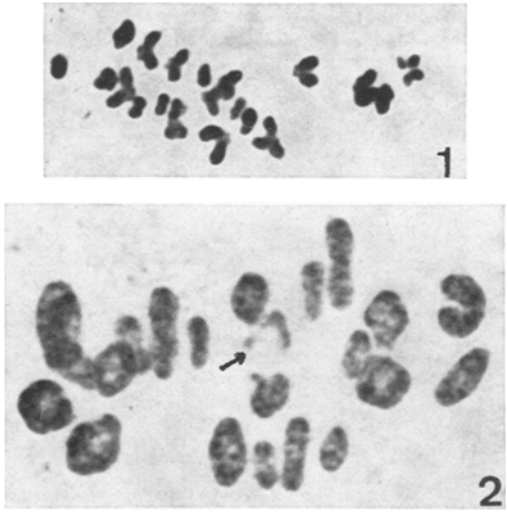
metaphases I. Among these bivalents a sex-determining system of 'parachute-like' type, Xy_p, was clearly apparent as in all other taxa of *Timarcha* (Figure 2).

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[3]. 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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