A chromosome study of *Scarites occidentalis* (Coleoptera, Caraboidea)[1]

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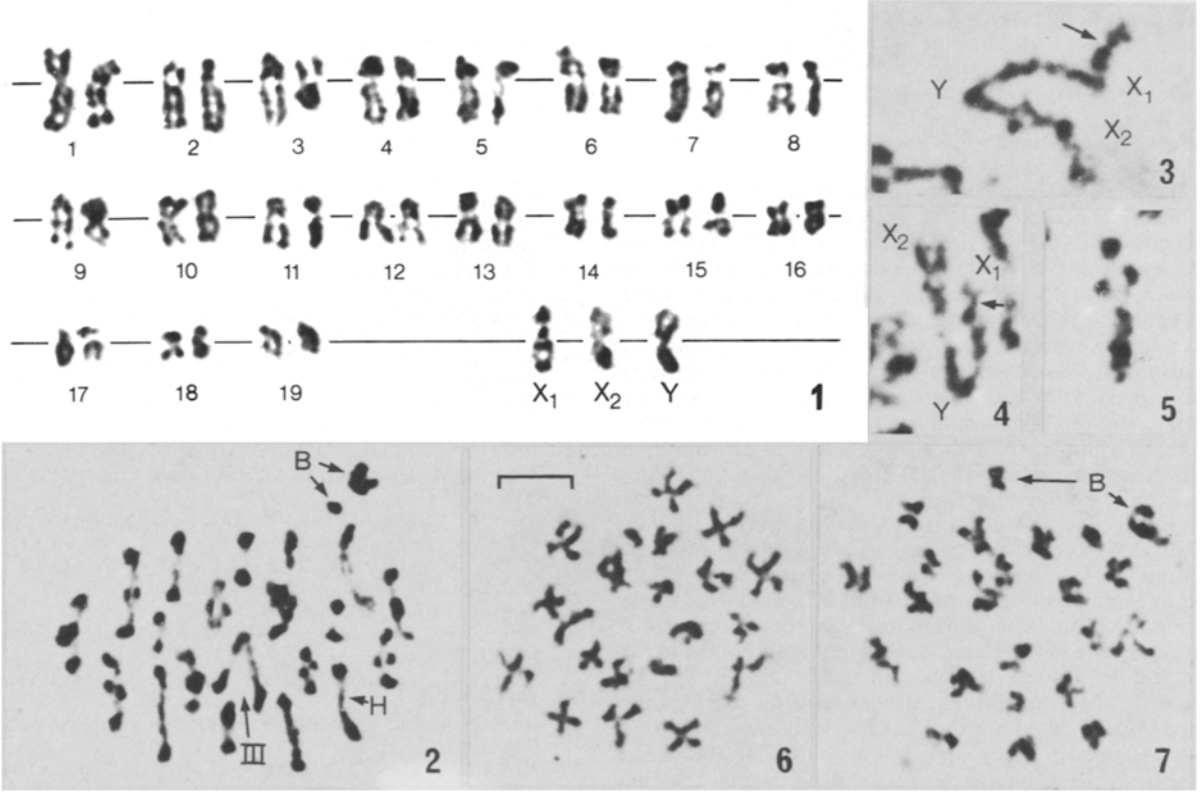
Summary. Scarites occidentalis has 2n = 41 (females 2n = 42), n = 19 + X_1X_2Y. This multiple sex-chromosome system and other karyotypic characteristics suggest that this species and *S. buparius* share a recent common ancestor in which the sex trivalent was probably originated.

Morphology and geographic distribution of *Scarites occidentalis* and *S. buparius* indicate that they are closely related species sharing a recent common ancestor. The finding of an X_1X_2Y system in *S. buparius*[2] provides a test for this hypothesis, that is, looking for this system in *S. occidentalis*, as these multiple heterosomes are unknown among other Scaritini[3-6] and rare among the Caraboidea[7].

Material and methods. 17 males and 2 females of *Scarites occidentalis* Bedel, 1895 have been analyzed. They were collected in 2 localities of Huelva (South Spain), La Palma del Condado (UTM: 29SQB1740) and Doñana (29SQA2897). Routine squashing methods have been described earlier[2]; in addition, Giemsa staining of dry cell preparations was performed.

Results. Spermatogonial cells of *S. occidentalis* have 2n = 41 (fig. 1) whereas 1st meiocytes have n = 19 + III (fig. 2) and 2n meiocytes have n = 20 or n = 21 (fig. 6). A few ovogonial cells showed 2n = 42. Chromosomal polymorphism is very common, 2-3 heteromorphic bivalents being found in most specimens (figs. 2 and 5), although it is not known whether they are always the same ones. Tentative karyograms (fig. 1) have not helped in identifying them; they show, however, that size ranges between 1.2 µm and 4.7 µm, and that the species has more asymmetrical pairs (about 7 submetacentric and 3 subtelocentric) than are usually found among caraboid beetles. One specimen from Doñana showed 2 B-chromosomes, 1 of large size (5 µm) and 1 very small (fig. 2). They form univalents at the 1st meiotic division and orientate and move polewards independently, so that a quarter of the 2nd metaphasic cells have 23 chromosomes (fig. 7). The 3 heterosomes appear to be metacentric (figs 1, 3, 4). The X_1-chromosome shows a small constriction in the pairing arm and a marked secondary constriction in the free arm, which is probably fully heterochromatic (fig. 3). The Y- and X_2-chromosomes are frequently paired by means of a subterminal chiasma not observed with certainty between the Y- and the X_1-chromosomes. Orientation of the trivalent is very regular, without abnormal segregations.

Discussion. Scarites occidentales shows a close likeness with *S. buparius* (*2n* = 37♂) in relation to their karyotypes, the former species being somewhat more advanced in the trend towards numerical increases observed within the Scaritini (2n = 37-52[2-6]). They also differ in the heterochromatic



Chromosomes of *Scarites occidentalis*. Figure 1. Tentative male karyogram, 2n = 41. Figure 2. Metaphase I, n = 19 + III. Figures 3 and 4. Details of the trivalent; arrow shows secondary constriction in the free arm of the X_1-chromosome. Figure 5. Detail of a heteromorphic bivalent. Figure 6. Metaphase II, n = 21. Figure 7. Metaphase II, n = 21 + 2 Bs. H, heteromorphs; B, B-chromosomes. The bar equals 5 µm in figures 2, 6, 7; 4.2 µm in fig. 1; 3.6 µm in fig. 5 and 3.3 µm in figs 3 and 4.

blocks, more developed in *S. buparius*. On the other hand, they share moderately asymmetrical karyotypes, high numbers of bivalents with interstitial chiasmata (8–10) and male X_1X_2Y sex-chromosomes. These show a morphology and a pattern of pairing which suggest that they are an homologous character for the 2 species and therefore must have arisen in a common recent ancestor. This point will be clarified by future studies of related species of this genus.

The fact that the X_1-chromosome shows a peculiar non-pairing arm without homologue in the Y-chromosome, and that this one and the X_2-chromosome show a higher degree of affinity (as judged by the mode of pairing), indicate that the X_1-chromosome was possibly the primitive X, the Y- and the X_2-chromosomes being of autosomal origin. This interpretation is thought to be more consistent than the one we proposed earlier about *S. buparius*[2].

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Tumorigenic potential of endoperoxide analogs

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Summary. The endoperoxide analogs known as U-46619 and U-44069 significantly enhanced the carcinoma formation and cellular atypicality initiated by a chemical carcinogen in mice. Studies of DNA radioactivity demonstrated that endoperoxides exerted their cocarcinogenic action by stimulating DNA synthesis. Thus, they play an important role in tumor cell proliferation.

Recent investigations suggest that prostaglandins play an important role in cancer development and the modulation of skin tumor promotion[2,3]. Some prostaglandins, such as PGD_2 and prostacyclin (PGI_2) also exert a cytoprotective activity and are powerful antimetastatic agents against B_{16} amelanotic melanoma colony formation in mouse lung[4,5].

The endoperoxide analogs known as U-46619 (15(s)-hydroxy-11α,9α-(epoxymethano) prosta-5Z, 13E-dienoic acid) and U-44069 (15(s)-hydroxy-9α, 11α-epoxymehtano) prosta-5Z, 13E-dienoic acid) are intermediary products in the synthesis of prostaglandins. They exert strong pharmacologic and physiologic effects on vascular and respiratory smooth muscle[6]. Thus, they are powerful vasoconstrictors, bronchoconstrictors and platelet aggregating agents[7].

As their role in carcinogenesis is unknown, this study was designed to investigate the effects of the stable endoperoxide analogs (U-46619 and U-44069) on tumor formation, DNA synthesis and cellular evolution of squamous cell carcinomas induced by 3-methylcholanthrene (MCA) in mice.

Materials and methods. The experiments were carried out on male Swiss mice weighing 25–30 g. They were divided into 6 groups of 20 mice as follows: 1. mice which received only the solvent and served as controls; 2. mice treated topically with 0.2 ml of a 0.3% acetone solution of MCA on a marked region of the shaved dorsal skin twice a week for 5 months; 3. mice treated topically with MCA as above and injected i.m. concomitantly with 5 µg of endoperoxide U-46619, twice a week; 4. mice treated with MCA as above and injected i.m. concomitantly with 5 µg of endoperoxide U-44069 twice a week. Groups 5 and 6 were treated only with U-46619 and U-44069 respectively, as above. The doses of U-46619 and U-44069 were similar to the dosage regimen used by other investigators in studying the bronchoconstrictor effect in dogs[8].

At the end of 5 months and 2 h before sacrifice under anesthesia with ether and nembutal, 6 mice from each experimental group received an i.m. injection of 7 µCi per g b.wt [^{3}H]-thymidine for the study of DNA synthesis. The period of 2 h prior to sacrifice was selected for the isotope studies because it was found in previous experiments that prostaglandins exert their maximum effect on cell structure and metabolism in that time[9].

DNA synthesis was comparatively studied in control epidermal and neoplastic cells from groups 2, 3 and 4, on at least 5–6 specimens, which were removed from experimental group, dissected, homogenized, using a Potter-Elvehjem homogenizer, and washed several times with 0.4 M per-

The incidence of carcinomas in mice following MCA and endoperoxide analog (U-46619 and U-44069) administration

Group	Treatment	Time (months)	No. of mice	Epithelial hyperplasia	Carcinomas	Percent of tumors
1	Controls + solvent	5	20	0	0	–
2	MCA + solvent	5	20	12	8	40
3	MCA + U-46619	5	20	0	20	100
4	MCA + U-44069	5	20	0	20	100
5	U-46619	5	20	10*	0	–
6	U-44069	5	20	9*	0	–

The data presented are based on counts of tumors visible to the naked eye, as well as on diagnosis made by light microscopy.
* Mild epidermal hyperplasia.