strain the dose-frequency relation of these variants is reported in figure 1b-c, sulfur dioxide appears to be the mutagenic agent.

In grape must wild-type subcultures from 626 (sensitive) and 5215 (resistant) show the same sensitivity to sulfur dioxide as the original strains. In both strains the mutated subcultures have slower fermentation either in control or in treated samples.

Sulfur dioxide action on growing cells. During the fermentation of sulfited must (fig. 2a), cultures were plated every 24 h and the isolated colonies were examined.

The clones of the 5215 strain and its white mutants remained unchanged. On the other hand, the original populations of the 626 strain and its mutants were gradually replaced respectively: 1. by variants for colour (from black to brown, figure 2b), 2. by colonies, still brown but back-mutated for respiration and sporulation. Replacement took place with the same frequency in both.

All these subcultures, tested in sulfited grape must, showed the same resistance to sulfur dioxide as the 5215 strain.

Since SO_2-resistance is inherited by monosporial descendants of variants and back-mutants it may be suggested that this trait is due to genetic changes and not to the physiological effects of SO_2.

A preliminary investigation on the inheritance of 'colour in ABY' and of resistance to SO_2 was performed by crossing the resistant strain and the induced resistant mutant with 2 sensitive strains. The character 'colour in ABY' showed a 2:2 segregation ratio with complete dominance of black over brown in both crosses. As regards the character 'resistance', dominance was observed. The segregation of such a character, unlike colour, does not seem to fall under Mendelian inheritance; the tetrad analysis shows 2S:2R, 3S:1R, 4S:0R ratios.

Discussion. The results show that sulfur dioxide has a mutagenic action on resting cells of diploid *S. cerevisiae* strains. In buffer the compound always induces mutants for 'colour in ABY' and for respiratory deficiency either in a sensitive or a resistant strain. For the latter, higher doses or longer contact time are needed to get the same result as in the former. The alteration in methionine metabolism always appears as a colour change in ABY and always in the same direction; from black to brown in the sensitive strain and from brown to white in the resistant one.

On growing cells of the resistant strain the sulfur dioxide has no detectable effect, perhaps because of the low SO_2 dose used, but in the sensitive one, mutants for 'colour in ABY', resistance and revertants for respiration always appear. To sum up, 3 kinds of mutants have been obtained from the sensitive strain: 1. a respiratory deficient mutant in buffer only; 2. a mutant with generic alterations in the methionine metabolism in buffer and in grape must; 3. a mutant 'SO_2-resitant' in growing cells only.

The very high mutation frequency, determined on resting cells, might suggest that the respiratory deficient and colour mutants have cytoplasmic determination. The segregation ratios of mutants for 'colour in ABY', isolated from growing cells, suggest however a nuclear determination; SO_2-resistance shows various segregation ratios while brown coloured colonies, isolated from grape must, appear to be always SO_2-resistant.

The results still do not allow a clear explanation of the biochemical and genetic determination of 'resistance' and 'colour in ABY'; nevertheless, it seems probable that the 'resistance' is induced by SO_2 in the medium and that this character is heritable and constant in time. These results suggest the possibility of a selective action, consequent to the induction of SO_2-resistant mutants.

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XY_1Y_2, a new sex-chromosome system among caraboid beetles[1]

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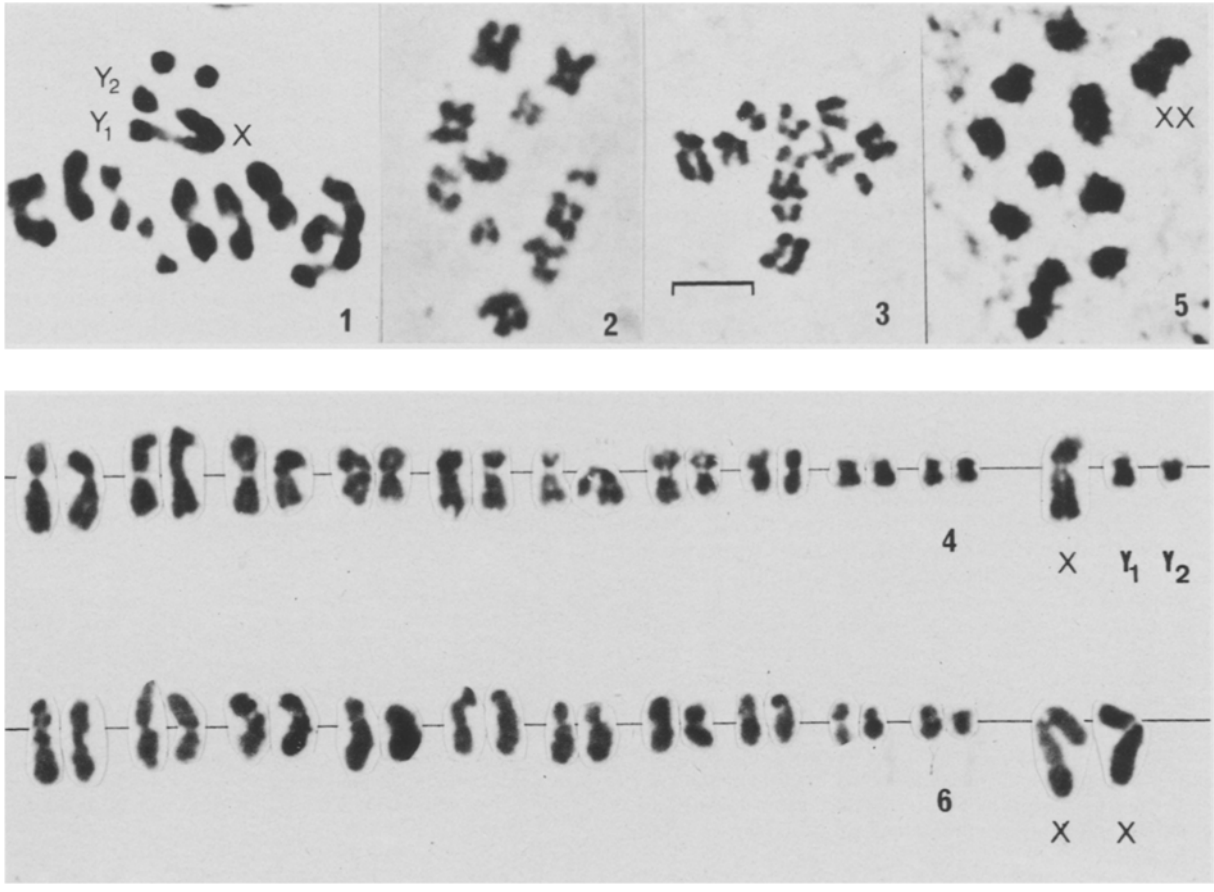
Summary. Calathus ascendens (Caraboidea, Pterostichidae, Sphodrini) has $2n=20$, XY_1Y_2:XX. This multiple sex-chromosome system seems to be derived from a neo-XY one through an X-autosome fusion.

The primitive male sex-chromosome system of Caraboidea, XO, has originated neo-XY and X_1X_2Y mechanisms after a fusion or a reciprocal translocation, respectively, between an autosome and the X-chromosome[2,3]. Neo-XY systems seem to be unstable within several groups of Caraboidea, giving raise to a new XO system after the loss of the Y-chromosome[2], but in the case of *Calathus ascendens* the evidence slightly points to an evolution towards an XY_1Y_2 mechanism through an X-autosome fusion.

Material and methods. 42 males and 11 females of *Calathus ascendens* Wollaston, 1862 have been analyzed. They were collected in 3 localities on the Island of Tenerife, Puerto de Erjós (UTM: 28R CS 2334), Fuente Joco (28R CS 5738) and Las Lagunetas (28R CS 6244). Gonads were dissected



Figures 1–6. Chromosomes of *Calathus ascendens*. Figure 1. Male 1st metaphase, $n = 10 + XY_1Y_2$. Figure 2. 2nd metaphase, $n = 10 + X$. Figure 3. 2nd metaphase, $n = 10 + Y_1Y_2$. Figure 4. Male karyotype, $2n = 20 + XY_1Y_2$. Figure 5. Female diakinesis, $n = 10 + XX$. Figure 6. Female karyotype, $2n = 20 + XX$. The bar represents 5 μm.

out in the laboratory and hypotonized with deionized H_2O, fixed with ethanol-acetic acid (3:1), stained with lacto-propionic orcein and squashed.

Results. Spermatogonial cells of *Calathus ascendens* have $2n = 23$, whereas 1st meiocytes have $n = 10 + III$ (figure 1) and 2nd meiocytes have $n = 11$ (figure 2) or $n = 12$ (figure 3). The male karyotype is made up of 10 pairs of autosomes and 3 odd heterosomes (figure 4). The chromosomes are metacentric and submetacentric except for the smallest subtelocentric heterosome. Pairs 2, 4 and 5 frequently show secondary constrictions. The largest heterosome is submetacentric and somewhat larger in size than the largest pair of autosomes; the other 2 heterosomes are about the size of pairs 9 and 10 respectively. 1st metaphase cells show 10 pairs of rods and rings and 1 trivalent (figure 1). This is made up of a large element in the center which we have called the X-chromosome, a metacentric Y_1-chromosome, whose short arm pairs firmly with the short arm of the X-chromosome by means of a distal chiasma, and a Y_2-chromosome, whose very short arm is paired with the long arm of the X-chromosome by a terminal chiasma loosed early during metaphase (figure 1).

Female ovogonia have $2n = 22$ and 1st meiocytes have $n = 11$ (figure 5). The female karyotype is made up of the expected 10 pairs of autosomes and 1 pair of large X-chromosomes (figure 6).

Discussion. The study of the karyotype and the meiotic stages of *Calathus ascendens* has led us to conclude that this species has a sex-chromosome system unusual among caraboid beetles, XY_1Y_2:XX. Its origin is uncertain and necessarily obscure because the karyotype of the species must have undergone a major revolution from the 2n ($\delta\delta$) = 36 + X karyotype which is actually found in 4 *Calathus* spp. from Tenerife (Serrano, unpublished results). We think that a pausible explanation would be that in which the long arm of the X-chromosome and the whole Y_2-chromosome represent the neo-XY chromosomes existing before an X-autosome fusion, as they are very different in size and have very small pairing segments (we have found such neo-XY chromosomes in 3 *Calathus* spp. from Tenerife), whereas the short arm of the X-chromosome and the Y_1-chromosome are of autosomic origin, as they are of the same size and show a more well-developed pairing.

In any case, the finding of this new mechanism is another remarkable indication of the primary role that sex-chromosomes play in the karyotypic evolution of caraboid beetles.

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