Number of foetuses in the rabbit uterus (mean ± SEM)

	Total foetuses		Percent males		Combined	
	Left uterine horn	Right uterine horn	Left uterine horn	Right uterine horn	Males	Females
Number	4.11 ± 0.26	3.87 ± 0.28	56.5 ± 4.6	42.4 ± 4.5	157	154
Student's 't' statistical value	0.61		2.22			
Degrees of freedom	37		37			
Level of significance	N.S.		< 0.05		N.S.	

determine whether a similar asymmetry occurred during normal pregnancy.

Materials and methods. New Zealand white rabbits were housed individually with food and water available ad libitum. 38 does were mated between November and July using 2–4 fertile bucks each time. Pregnant does were sacrificed at days 22–31 of pregnancy and the foetuses removed from each uterine horn. The sex of each foetus was determined by microscopic examination of haematoxylin-eosin stained sections of one gonad, and the testosterone content of the other. Data were analyzed by the Student's paired t-test.

Results and discussion. The number of males was expressed as a percentage of the total number of foetuses in each uterine horn. A larger percentage of males was present in the left horn ($p < 0.05$, table). Similarly more females were present in the right horn ($p < 0.05$). There was no significant difference in the number of foetuses present in each horn or in the total number of male and female foetuses. These data confirm that there is no sex difference in the number of offspring produced by the domestic rabbit (157 males:154 females) and the normal 1:1 sex ratio is present. Sex selection or controlling the sex ratio could have a number of benefits especially in animal husbandry where one sex may be more desirable.

As far as we are aware there has not been a study of the sex distribution of foetuses in the uterus of animals with large litter size. Buchanan[8] has observed that there is an asymmetrical distribution of foetuses in the rat uterus but the sex of the foetuses was not recorded. Slob et al.[9] have tried to increase the probability of litters of one sex by hemihysterectomy of rats prior to mating. They found 11 out of 30 pregnancies with males greater than females and 14 out of 30 with females greater than males. Overall, the ratio was not different from 1:1.

The possibility of unequal loss of embryos from one sex can be ruled out since the overall sex ratio was 1:1, the average litter size (7.98) was similar to that (7.39) reported by Hagen[10], and the average number of foetuses per horn was approximately the same.

The significant difference in the sex ratio between the uterine horns of the rabbit suggests that hemihysterectomy may be a method for regulating the sex of rabbits. However, the number of rabbits required to achieve this difference may preclude this procedure being a feasible one.

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Mutagenic effects of sulfur dioxide on *Saccharomyces cerevisiae* diploid strains

Daniela Guerra[1], Patrizia Romano and C. Zambonelli

Institute of Genetics, University of Bologna, Via F. Selmi 1, I-40126 Bologna (Italy), and Institute of Microbiology and Industries, University of Bologna, Coviolo, I-42100 Reggio Emilia (Italy), 24 November 1980

Summary. In resting cells of diploid *Saccharomyces cerevisiae* strains sulfur dioxide induces at very high frequency: a) respiratory deficient mutants; b) mutants with altered methionine metabolism. In growing cells the following kinds of mutants appear: a) revertants for respiration; b) mutants altered in the methionine metabolism; c) SO_2-resistants. It is suggested that sulfur dioxide acts as a selective agent through the induction of SO_2-resistant mutants.

Sulfur dioxide is commonly used in wine-making; this compound selectively inhibits the growth of acetic and lactic bacteria, allowing desirable yeast strains (*S. cerevisiae* and related species) to dominate the fermentation[2]. These strains are generally different from the wild-type for methionine biosynthesis and particularly in sulfate reduction. Studies on the mutagenic effects of sulfur-dioxide have been reported for phage[3,4] bacteria[5] and yeast[6,7].

The primary biochemical lesion of sulfur-dioxide is reported to involve deamination of cytosine to uracil[8,9], transamination[10], free radical damage to DNA[11] or an indirect effect on RNA[12] and on protein metabolism. (For an extensive review see Shapiro[13]). Recently, the effects of sulfite on resting cells of *S. cerevisiae* were investigated by Schimz[7]. The author has studied the influence of various factors, including the extracellular pH, and has determined that

prior to cell inactivation sulfite induces the formation of respiratory deficient cells both in aploid and diploid strains. The work reported in the present paper was performed in order to investigate the effects of sulfur dioxide on *S. cerevisiae* diploid strains and to find out the relations between mutagenic action, methionine biosynthesis and SO_2-resistance.

Material and methods. Organisms. The following strains, belonging to the Microbiology Institute's collection, were examined: *S. cerevisiae* strain 626 = isolated from wine, SO_2-sensitive, diploid, wild-type: in synthetic medium it releases traces of sulfite and produces detectable amounts of H_2S, exhibits a black streak in ABY. *S. cerevisiae* strain 901 = isolated from wine, SO_2-sensitive, diploid, wild type. *S. cerevisiae* strain 5215 = isolated from sulfited must, SO_2-resistant, diploid, in synthetic medium it releases small but detectable amounts of sulfite (12–15 mg/l) and traces of H_2S; it exhibits a brown streak in ABY.

For each strain we used single spore, self-diploidizing descendants.

Nutritive medium. Agar YEPD was used for plate count.

Test media. H_2S production was tested on ABY medium (DIFCO; see Nickerson[14]) where the streaks remain white or turn brown or black (wild-type) after 24 h at 25 °C; brown or white streaks are related to generic alterations in methionine biosynthesis. The determination of nutritional requirements and SO_2 and H_2S extracellular production were tested in minimum medium Williams, supplemented with the following growth factors: biotin (25 µg/l), thiamine-HCl (300 µg/l), nicotinic acid (300 µg/l), mesoinositol (25 mg/l), Ca-pantothenate (300 µg/l), pyridoxine-HCl (300 µg/l). The sulfite production (as SO_2 mg/l) was determined with the iodine test; the H_2S released from growing cultures was estimated from the blackening of lead acetate papers. The deficient respiratory mutants (petite) were assayed on a lactate medium, according to Ogur and St. John[15].

Test in must. Grape must clarified, diluted (12% total reducing sugars), pH = 3, autoclaved at 100 °C for 30 min in 125 ml flasks (100 ml of must), was inoculated with 3 ml of 48 h preculture in the same medium. Sulfur dioxide (as $K_2S_2O_3$) was added to grape must up to a final concentration 150 ppm. The fermentation was measured by determining the rate of CO_2 evolution, according to Castelli[16].

Test in buffer. 48 h cells, taken from solid YEPD medium, were suspended in 100 ml citrate buffer (pH = 3) and serially diluted in the same buffer to a final concentration of 200 cells/ml. The sulfur dioxide was added in different concentrations. Cell suspensions were incubated at 25 °C in reciprocal shakers. The colony-forming cell count was carried out in YEPD plates. The same low pH as grape must, giving a strong antimicrobial action to sulfur dioxide, was used.

Genetic analysis of the strains. Asci dissection, spore isolation, crosses (spore conjugation) and tretrad analysis, were performed with a de Fonbrune pneumatic micromanipulator.

Results. Sulfur dioxide action on resting cells. Cell suspensions in citrate buffer pH = 3 of 626 and 5215 strains were incubated with different doses of SO_2 (60, 90, 120, 150, 180, 210 mg/l); contact time 90 min.

The regression lines of survival vs log. dose are shown in figure 1a. The cell mortality seems to be related to a different sensitivity of the 2 strains towards sulfur dioxide; a similar trend is observed with the same dose of SO_2 (100 mg/l) at different contact times (60, 120, 180, 240, 300 min).

Colonies of surviving cells, tested in ABY, have shown a colour variation from black to brown in the 626 strain, from brown to white in the 5215 strain with a dose-dependent increase.

All subcultures, brown and white respectively, are respiratory deficient and also unable to sporulate. These results are in agreement with observations of Ephrussi[17]. For each

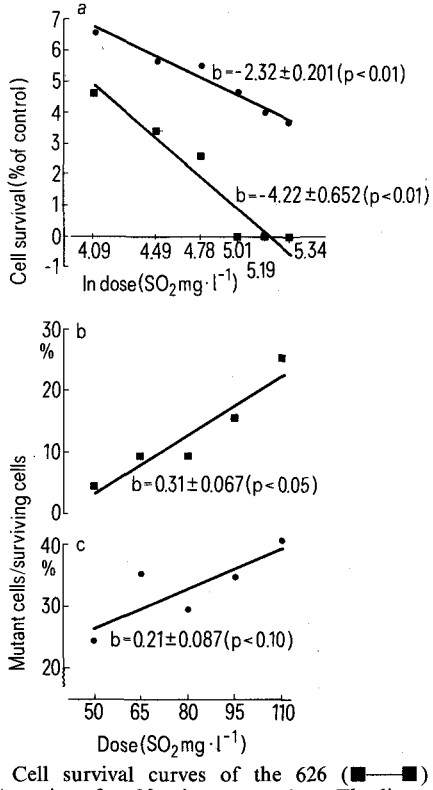

Fig. 1. *a* Cell survival curves of the 626 (■——■) and 5215 (●——●) strains after 90 min contact time. The linear regression was estimated on probits; *b* and *c* percentage of mutation frequency of the 626 (contact time 90 min) and 5215 (contact time 180 min) strains. The coefficients of linear regression (b) are reported.

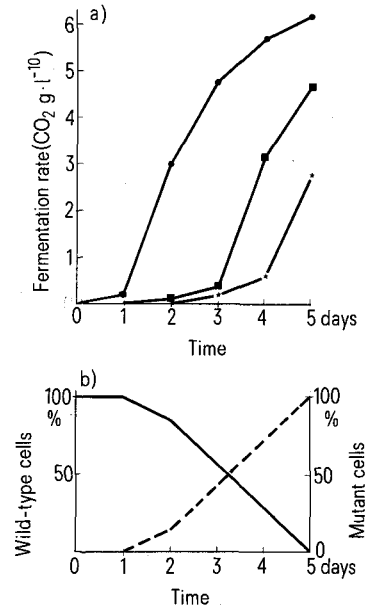

Fig. 2. *a* Fermentation rate of 5215 strain (●——●), wild type (■——■) and mutant (*——*) 626 strain in sulfited grape must (SO_2 150 mg·l^{-1}); *b* frequency of wild type (———) and mutant (- - - -) cells of the 626 strain during the fermentation.

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]

J. Serrano

Museo Nacional de Ciencias Naturales, Castellana 80, Madrid 6 (Spain) 1 December 1980

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