the same methods described previously[6]. M-8 and 002 strains were cultured in shaking flasks containing Allen's medium[2] with added glucose (0.5% final) under 2,000 lux fluorescent light at 38 °C. These materials were collected by centrifugation, washed with distilled water, then fixed in 0.6% glutaraldehyde dissolved in buffer-S[5] and stored at 4 °C. They were mixed in equal quantities with DAPI dissolved in buffer-S on slide glass and squashed gently against the samples. The stained samples were observed with an Olympus BHS-RFK epifluorescence microscope equipped with phase contrast objectives. They were excited with 200 W Hg lamp through a green filter (560 nm) or a UV filter (350 nm) in combination with a 420 nm suppression filter. Phase-contrast, green-excited and UV-excited fluorescent micrographs were taken in the same field in each strain with Fuji Neopan 400 black and white films.

Results and discussion. Figure 1a and 1d show phase contrast micrographs of *Cyanidium* RK-1 and 001 strains, respectively. They are 2–4 μm in diameter and grow by formation of 4 endospores[3]. Under green light excitation, only 1 ovule chloroplast emitting red fluorescence, which occupied the greater part of the cell, was seen per cell in each strain (fig. 1b and 1e). When the cells were excited with UV instead of green light, one bluish white rod-shaped chloroplast nucleoid appeared in the center of a pink chloroplast adjacent to a bluish white nucleus (fig. 1c and 1f).

The rod-shaped chloroplast nucleoids were observed at in every stage of the cell cycles of 2 strains. The central part of the *Cyanidium* chloroplast where a chloroplast nucleoid occurs, appears to correspond to the electron opaque region of electron micrographs of its thin sections[3]. Figure 2a and 2d show phase contrast micrographs of *Chroococcidiopsis* M-8 and 002 strains which are 9–11 μm in diameter and multiply by formation of 4, 8, 16 and 32 endospores[3]. In electron micrographs of thin sections of strain M-8[3] it sometimes seemed that there were several chloroplasts per cell. However, fluorescent microscopic observation with green light excitation (fig. 2b and 2e) indicated that only a single multilobed chloroplast was present per cell and surrounded a vacuole. When glucose was eliminated from the culture medium, the chloroplast occupied a greater part of the cytoplasm than when cells were grown in complete culture medium, in each strain. When the cells were excited with UV light, a circular white nucleoid of the chloroplast could be observed along the periphery of the pink chloroplast, in addition to a bluish white nucleus (ca. 1.7 μm in diameter) (fig. 2c and 2f). The chloroplast nucleoid looked like a chain of small spherical particles as in the case of a brown alga *Ectocarpus indicus*[7]. This observation is consistent with the previous one[8] as to *'Cyanidium caldarium'* strain M-8, and with that by electron microscopy, where an electron-opaque region is recognized inside the peripheral girdle lamella of the chloroplast of strain M-8[3] or strain 002 (= forma B)[9]. These results confirm the previous conclusion that M-8 and 002 strains are very different from *Cyanidium* RK-1 and 001, and must belong to a different genus *(Chroococcidiopsis)*[3]. Kuroiwa et al[8] have proposed that chloroplast nucleoid structure may be classified into at least 5 types as a result of observation on various algal and higher plants by fluorescent microscopy. According to their view, *Cyanidium* RK-1 and 001 may belong to the CN-type in which *Glaucocystis nostochinerum* (Glaucophyta) and *Acetabularia calyculus* (Chlorophyta) are included. On the other hand, *Chroococcidiopsis* M-8 and 002 may belong to CL-type in which some brown algae such as *Sphacelaria* and some diatoms such as *Melosira* are included. The chromophyta *Olisthodiscus luteus* also possesses a ring-shaped chloroplast nucleoid[11]. Therefore, these 2 hot spring algae may be considerably distant from each other phylogenetically, and *Cyanidium* may be more primitive than *Chroococcidiopsis* in its chloroplast nucleoid structure.

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Karyotypes of three species of Caviinae (Rodentia, Caviidae)[1]

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Summary. Chromosomes of *Cavia aperea aperea* (2n = 64; FN = 116 *Galea spixii* (2n = 64; FN = 118) and *Kerodon rupestris* (2n = 52; FN = 92) are described with data on banding patterns. Comparisons with karyotypes of others species of Caviinae are taken into consideration.

The subfamily Caviinae is composed of 4 essentially South American genera; *Cavia, Galea, Microcavia* and *Kerodon*. Chromosomal data available for this subfamily consist of conventional stained karyotypes of 7 species and 2 subspecies[2–12] plus banding patterns of some of these taxa[8–12]. From these data characteristic features shared by these species can be observed; for example a large number of biarmed autosomes (more than 80% of the complement); biarmed Xs in all species; a considerable karyotypical symmetry; a high frequency of species with an identical diploid number of 64 (only 2 species behave differently: *Galea musteloides,* 2n = 68 and *Kerodon rupestris,* 2n = 52). Our investigation provides a description of the karyotype of subspecies *Cavia aperea aperea,* with G and C banding patterns, not published so far, and additional data about karyotypes of *Galea spixii* and *Kerodon rupestris*. The taxon *Cavia aperea* has a wide range of geographical

distribution in Brazil, spanning about 3500 km, while *Galea spixii* and *Kerodon rupestris* are confined to some localities of the Northeastern region.

4 specimens of *C.a. aperea,* 6 of *G.spixii* and 6 of *K.rupestris* were collected in the State of Pernambuco (8° 44′ S, 35° 11′ W; 8° 44′ S, 35° 11′ W and 7° 31′ S, 39° 43′ W; 7° 52′ S, 37° 58′ W) and the skins and skulls are kept in the collection of Departamento Biologia Geral, UFPe, Brazil. Mitotic plates were obtained from bone marrow cells following a modification of the technique outlined by Ford and Hamerton[13]. G and C bands were obtained by means of trypsin Giemsa[14] and $Ba(OH)_2$ treatment[15].

Chromosome analysis of 20 metaphases per specimen showed a diploid number of 64,FNa = 116 in *C.a.aperea,* with an autosomal complement composed of 27 pairs of biarmed chromosomes, graduated in size, and 4 pairs of small acrocentrics. The X is submetacentric. Our data are not in agreement with those reported for *C.aperea*[7] and *C.a.pamparum*[10] whose karyotypes included only biarmed autosomes. Nevertheless, by the use of the trypsin Giemsa technique, which allowed us to identify all the chromosome pairs in our material (fig. 1) a great similarity of G banding patterns between the largest chromosomes of *C.a.aperea* and *C.a.pamparum* can be observed. Comparisons between the smallest chromosomes were not possible. An interesting fact to be pointed out is that as in *C.a.pamparum, C.fulgida* and *C.magna*[10], G banded chromosomes of *C.a.aperea* showed large segments which were not stained with Giemsa. C banding showed a large amount of constitutive heterochromatin in the genome, mainly forming the short arm of several chromosomes, besides being present in all centromeric regions (fig. 2). This pattern of C-bands seems

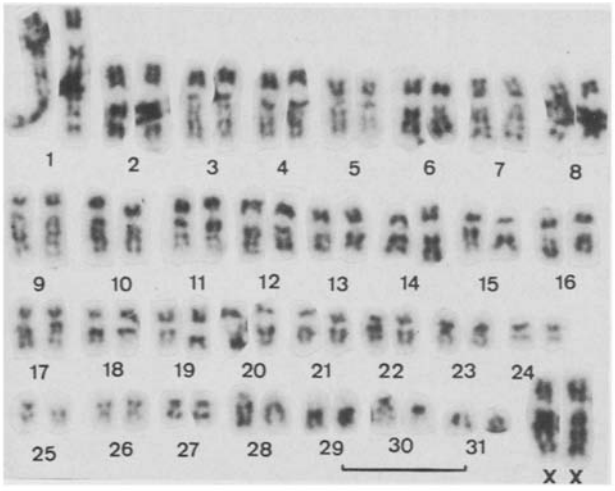

Figure 1. G-banded karyotype of *Cavia aperea aperea* (♀); Bar 5 µm.

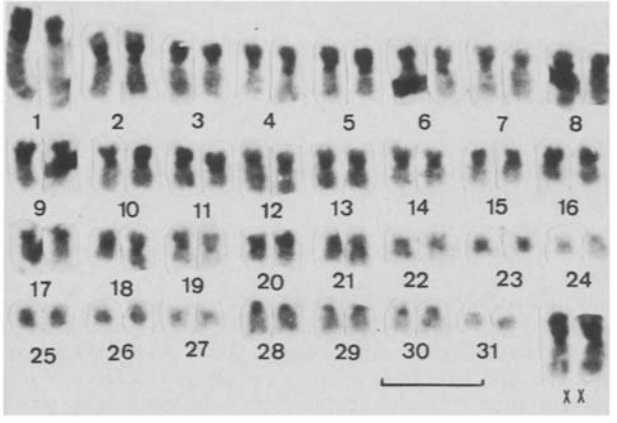

Figure 2. C-banded karyotype of *Cavia aperea aperea* (♀); Bar 5 µm.

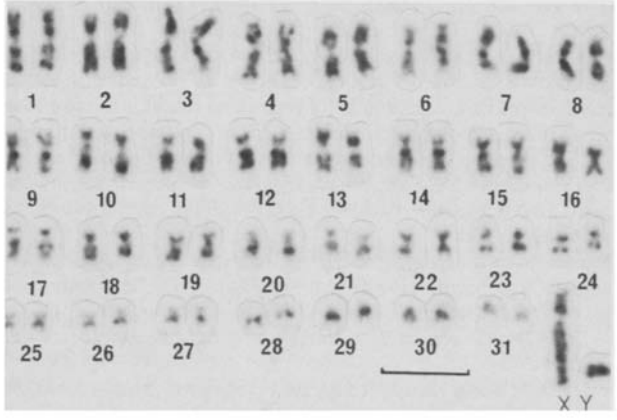

Figure 3. G-banded karyotype of *Galea spixii* (♂); Bar 5 µm.

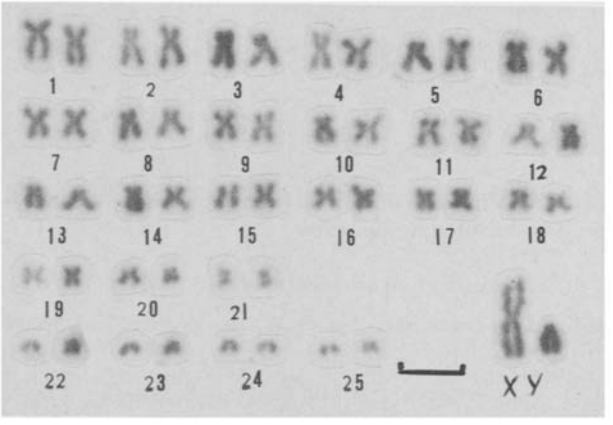

Figure 4. Conventionally-stained chromosomes of *Kerodon rupestris* (♂); Bar 5 µm.

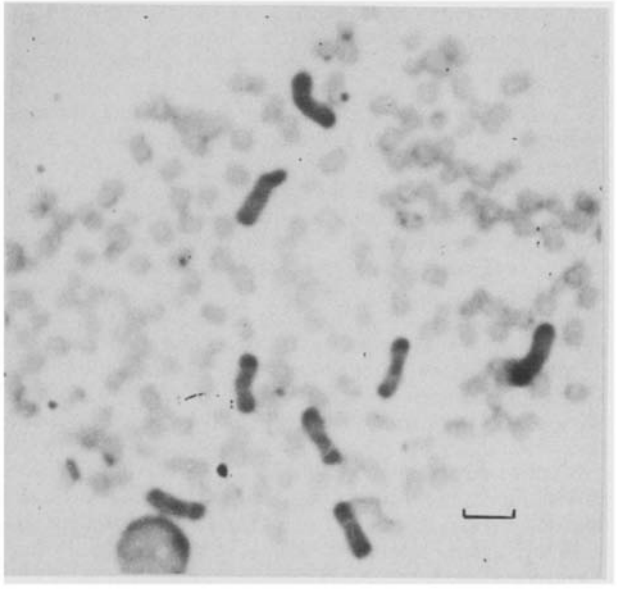

Figure 5. C-banded octoploid metaphasic chromosomes of *Kerodon rupestris* (♀), showing extensive heterochromatics regions of X chromosomes; Bar 5 µm.

to be characteristic of the genus *Cavia*, since a somewhat similar one has also been reported in *C. porcellus*, *C. a. pamparum* and *C. fulgida*.

In *Galea spixii*, whose conventionally stained karyotype has already been described[11], we intend to report further observations on other specimens collected in distinct localities and on G-banded karyotype. 70 metaphases showed 2n = 64, FNa = 118, with an autosomal complement composed of 28 pairs of biarmed autosomes and 3 pairs of small acrocentrics. Large unstained segments in G-banded karyotypes, as found in *C. a. aperea*, were not observed in this species. Chromosome X is submetacentric – the biggest one in the complement – and Y is a small acrocentric (fig. 3). A submetacentric X is also the biggest element in *G. musteloides*[7]. In *Kerodon rupestris*, the analysis of 87 metaphases showed 2n = 52, FNa = 92 with 21 pairs of biarmed chromosomes and 4 pairs of small acrocentrics in the autosomal complement. X is metacentric and also the biggest one in the complement, and Y is a medium-sized acrocentric chromosome (fig. 4). C banding reveals that practically all constitutive heterochromatin in this genome is confined to the X chromosome, shaped as symmetrically-positioned large pericentromeric and telomeric blocks (fig. 5), a very distinctive pattern in comparison to those observed in species of genera *Cavia* and *Galea*.

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Differential sensitivity of *Drosophila melanogaster* and *Drosophila simulans* to chronic exposure to carbon dioxide during development[1]

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Summary. Drosophila melanogaster is less sensitive to low concentrations of CO_2 (2–20%) than *Drosophila simulans* with regard to egg-to-adult mortality, duration of development and adult size measured by wing length, thorax length and dry weight. This difference may be related to the better adaptation of *D. melanogaster* to alcoholic fermentation.

Effects of carbon dioxide upon *Drosophila* have been extensively studied. Besides narcosis, the most classical effects concern the sensitivity of adults which is either due to viral infection[2], or to genetic factors[3]. Other consequences in adults have been studied; reduction of longevity[4], and decrease of egg deposition for a few h after anesthesia[5]. Such exposure to pure CO_2 does not occur in the field, and we decided to examine the developmental effects of lower doses (2–20%) in *D. melanogaster* and *D. simulans*.

Material and methods. Stocks of *D. melanogaster* and *D. simulans* were founded from 2 sympatric Tunisian populations (Nasrallah) and kept in the laboratory by mass-breeding. Before the experiments, they were tested for their lack of sensitivity to short exposure to pure CO_2.

For the experiments, lots of 50 eggs were deposited each in 100 × 2.5 mm vials containing 20 g of corn-yeast medium[6] and closed with a wire netting allowing easy gas circulation.

Treatment chambers were tightly closed clear plastic boxes (35 × 25 × 13 cm), each of which could receive 14 breeding vials (7 for *D. m.*, 7 for *D. s.*). The gaseous mixtures were delivered by gas-mixing pumps (Wösthoff). Pure CO_2 was mixed in the required ratio with atmospheric air previously filtered, dried over silica gel, cleared of CO_2 on KOH columns, then moistened again up to 60 ± 5% relative humidity by bubbling through a saline solution. Gaseous mixtures were conveyed to the treatment chambers; the gas flow rate was regulated up to 300 ml/min using flowmeters and needle valves. The insects were reared at 25 °C with a photophase (LD 12:12). The actual relative humidity inside the treatment chambers averaged 65%.

Emerging adults were counted and sexed twice a day. Egg-to-adult viability was calculated in each vial as the ratio of emerging flies to the initial number of eggs. Duration of development was measured in each vial separately for the 2 sexes. At each concentration, 25 males were randomly chosen in each species for individual measurement of their wing length, thorax length and dry weight. CO_2 tests were run simultaneously with 0% (control), 2%, 5%, 10% and 20% concentrations. Control tests were run on *D. melanogaster* in air enriched with 10% and 20% nitrogen.

Results. As shown in figure A, increasing CO_2 concentration reduced the overall viability. Analysis of variance shows a highly significant effect in both species ($p < 0.01$), the decrease being more striking in *D. simulans*. In this species, reduction of viability occurs for concentrations above 5% and reaches 43.4% at 20% CO_2. In *D. melanogaster* viability decreases linearly and reduction reaches only 11%. This mortality affects both sexes equally.

Mean developmental time for the sexes increases significantly with CO_2 concentration ($p < 0.01$) (fig. B). The lengthening is gradual and reaches 63.6 h (+28%) in *D. melanogaster*, 60.9 h (+27.8%) in *D. simulans*. Differences between sexes are not significant.

Wing length and thorax length decrease significantly ($p < 0.01$) and linearly (figs C and D). Curiously, the thorax length of controls (0%) is higher in *D. simulans*, which is rather un-