The standardization of the laboratory environment, their relatively long time in captivity, and the relatively similar body weights suggest that the difference in s.c. gas tension between the 4 chromosomal species is intrinsic and has a genetic basis in accord with the climatic selection of the species ranges. It is remarkable that significant differences were found between the 4 chromosomal species in their swimming ability in the following decreasing order: $2n=52$, $54>58>60$[12]. This result seems to support the hypothesis that differential swimming ability of individuals among the chromosomal species may be associated with the extent and level of flooding and free-standing water, since, as indicated earlier, $2n=52$ and 54 range in high-precipitation regimes, whereas $2n=58$ and 60 are from areas characterized by lower precipitation. The geographic variation in the swimming ability of the chromosomal species displays a pattern parallel to that in the differential respiratory adaptations described here. Furthermore, both phenomena may be explained by the very same climatic-edaphic model described earlier. Both the selection for better swimming ability and the ability to withstand extreme hypoxic-hypercapnic conditions characterizing the $2n=52$ and 54 chromosomal species may be due to climatic selection.

The chromosomal speciation of *S. ehrenbergi* in Israel has several adaptive physiological correlates, including metabolism[13], thermoregulation[14], and nonshivering thermogenesis NST[15]. The progressive change of s.c. gas tension discovered in this study represents another important parameter in the aforementioned adaptive physiologic syndrome, thereby reinforcing the physiological correlates of ecological speciation through chromosomal rearrangements in the *S. ehrenbergi* complex.

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Biological effects of singlet delta oxygen on respiratory tract epithelium[1]

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Summary. Exposure of hamster tracheal organ cultures to gas phase singlet delta oxygen, $^1O_2$, at atmospheric pressure produced significant alterations in the mucociliary epithelium resulting in changes in ciliary activity and cellular morphology.

Singlet delta oxygen is recognized as a potential oxidant in polluted atmospheres[2]. The health related effects of $^1O_2$ have not been reported due to the lack of adequate atmospheric generation methods. A laboratory apparatus for the gas phase generation of $^1O_2$ at 1 atmosphere has recently been described[3]. Chemical studies indicate that $^1O_2$ is sufficiently long lived to be a predisposing factor in respiratory tract disease. Since tracheal epithelium is a target tissue for the induction of acute toxicity by oxidant gases, experiments were conducted to evaluate the short term effects of $^1O_2$ on hamster tracheal organ culture.

Materials and methods. The $^1O_2$ generator consisted of a 13 mm outer diameter × 20 cm water-jacketed, Pyrex flow tube lined with a thin film of rose bengal which was prepared by evaporation from a methanol solution of the dye. A mixture of 95% N_2:5% O_2 was passed through the flow tube at 8 l/min while the dye film was exposed to strong visible radiation from four 1000 W projection lamps (G.E. Model DPT) enclosed in an air-cooled reflector. The exit gas was passed directly into a cube shaped exposure chamber (1′ × 1′ × 1′) from the top and down onto culture dishes supported on a rack in the middle of the chamber.

The concentration of the gas phase $^1O_2$ entering the chamber was monitored for each run by the intensity of the 1.27 micron emission[4], using a 'chopped' germanium diode detector system with sensitivity of 0.0025 ppm. The mean gas phase $^1O_2$ concentration for all experiments at the normal operating conditions was 0.121 ± 0.005 ppm.

Organ cultures were prepared from tracheas of 4–6-week-old male Syrian golden hamsters by previously described methods[5]. Tracheas were initially cultured for 24 h, allowing explants time to adjust to external environment before treatment. Culture dishes containing tracheal ring explants in L-15 medium were exposed to $^1O_2$ in a controlled atmosphere chamber (Bellco Glass Inc., Vineland, NJ). The chamber was placed on a rocker platform that rocked 10 cycles per min, allowing the tracheal rings contact with both $^1O_2$ and culture medium. Ring explants were exposed for 2 h to $^1O_2$ from the top of the controlled atmosphere chamber. The evaporation loss was compensated for by the periodic (once an hour) addition of medium to the dishes. The effects of $^1O_2$ on cilia beating frequency and cytology[5] were assessed immediately after the 2-h exposure, and rings processed for histology.

Results and discussion. The data presented in the table show that a concentration of 0.121 ppm singlet oxygen causes a significant decrease in cilia beating frequency and significantly greater cytological alterations than in control exposures. The gas entering the exposure chamber in the control exposures was identical to that used in the $^1O_2$ exposures except no $^1O_2$ was present in the gas (N_2/O_2, 95%/5%). In the singlet oxygen

Cilia beating frequency and percentage of normal epithelium in tracheal ring cultures after termination of a 2-h in vitro exposure to singlet oxygen

Treatment	Cilia beating frequency[a]				Mean percentage of normal epithelium[b]	
	0 time		Post-exposure		0 time	Post-exposure
	Mean	SE	Mean	SE		
Control	1238	4	1206	6	94	87
Singlet oxygen (0.121 ppm)	1244	4	847*	38	97	43**

[a] Values represent mean ± SE from 16 ring cultures (64 determinations). Cilia beating frequency measured as beats per min; [b] Mean data from 16 separate ring cultures; * Significantly different from both 0 time baseline values and the blank 2-h post-exposure ($p < 0.05$; Dunnett's test[7]); ** Significantly different from 0 time baseline values and the blank 2-h post-exposure ($p < 0.05$; χ^2 distribution test).

treated cultures, the general appearance of the epithelium no longer had its sharp outline; it had a swollen appearance with some sloughing of epithelial cells. Ciliary arrest was observed in approximately 25% of the epithelium. In this area, normal cellular morphology was observed.

The singlet oxygen induced alterations in the ciliated respiratory epithelium are different from other environmental toxicants previously studied, since both cytopathology and ciliostasis were observed. Exposure of tracheal cultures to 2 ppm NO_2 for 1.5 h/day for 5 consecutive days caused reduced ciliary activity without significant changes in morphology[6]. Since the mucociliary escalator depends on the functioning of the ciliated epithelium and rate of mucus transport, exposure to singlet oxygen could cause ciliary dysfunction and epithelial damage resulting in an altered host defense system. Although the mechanism of $^1O_2$-induced damage remains to be elucidated, these data reflect an important physiological response that warrants further investigation. To the best of our knowledge, this is the first report on $^1O_2$-induced damage to respiratory tract epithelium.

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Inhibition of platelet ADP and serotonin release by carbon monoxide and in cigarette smokers[1]

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Summary. The release of ^{14}C-serotonin by ADP, epinephrine and arachidonic acid and the release of ADP by kaolin were measured in normal platelets in the presence and absence of carbon monoxide and in smokers' platelets. It is shown that carbon monoxide inhibits significantly the platelet release reaction. This function is also decreased in platelets obtained from heavy cigarette smokers.

The incidence of atherosclerosis is significantly enhanced in heavy cigarette smokers[2-4]. Platelet aggregation is also enhanced in this population[5-7]. This enhancement of platelet aggregation has been suggested as a possible cause for vessel wall endothelial cell damage[8-10].

Contrary to the previous findings by others, our recent work has shown that platelet aggregation is decreased in the presence of cigarette smoke and carbon monoxide (CO)[11]. Because aggregation and release reaction are not necessarily linked[12,13], we have investigated the platelet release reaction in platelets obtained from smokers compared to non-smokers and in normal platelets (obtained from non-smokers) in the presence and absence of CO.

Methods. Blood was obtained from healthy cigarette smokers and non-smokers who were not on any drugs known to affect platelet function. Platelet rich plasma (PRP) and platelet poor plasma (PPP) were prepared as reported previously[11]. Carboxyhemoglobin (CO-Hb) levels were determined on a Co-oximeter 282, Instrumentation Laboratory. Platelet counts were obtained on an Ortho ELT-8 cell counter.

Serotonin release was measured using a modified technique of Valdorf-Hansen and Zucker[14]. The PRP was incubated for 20 min at 37 °C with 5.2×10^{-4} μCi/ml ^{14}C-serotonin with a specific activity of 58.5 μCi/mmol. Volumes of 0.5 ml PRP were placed in glass tubes (according to the original method[14]) and incubated for 30 min at room temperature in the presence of CO or air which was bubbled through the sample. A releasing agent (1.2 μmol of ADP, 1.3 μmol of arachidonic acid (AA) or 0.022 μmol of epinephrine (EP) in 0.05 ml) or 0.05 ml of non radioactive PPP for the control sample, was then added to every tube. The remainder of the procedure was the same as described[14]. The percent of ^{14}C-serotonin release was calculated as follows: (sample (cpm) − control (cpm))/sample (cpm) × 100. The control sample was taken as zero percent released. Samples were prepared in duplicate or triplicate and the results averaged.

Serotonin release in smokers and non-smokers was determined as above except that samples were not preincubated with CO or air.

ADP release, (by exposure to kaolin), was measured in the