LASCAUX INTERNATIONAL SCIENTIFIC THINK-TANK
(LIST)

POST-SYMPOSIUM REPORT ON THE LASCAUX CRISIS

July 15, 2009

Preliminary note

This report is based on informations gathered at the Lascaux Symposium organized in February 2009 by the Ministère de la Culture as well as informations obtained from professionals present on the site at various periods between 1960 and 2009. It has been prepared and was approved by all members of the **LIST** at the date of its finalization,

Several independent scientists and personalities concerned by the proliferation in the cave of Lascaux, since the beginning of the previous decade, of various microorganisms liable to damage its paintings became involved since the onset of the crisis in informing the public and the administration from a critical, scientific and independent point of view. They spontaneously formed a group aimed at leading a reflection on curative and preventive measures likely to stop the microbial proliferation. Convinced that only a multidisciplinary approach, involving "hard sciences" like microbiology (bacteriology, mycology, biofilms), physics, chemistry, hydrogeology, climatology and physical-chemistry, led independently from the administration was likely to identify the real causes of the crisis and to propose the most appropriate measures to treat it, they created the **Lascaux International Scientific Thinktank (LIST)** in January of 2009.

At the time of the preparation of this report, the LIST consisted in the following personalities:

François Bourges (Geologist - expert of decorated caves
CEO of « Géologie-Environnement-Conseil»)

Pascale Cossart (Prof. Institut Pasteur – Member of the French Academy of Sciences – Member of the National Academy of Science of the United States - expert molecular microbiologist)

Henri Dabernat (Prof. Toulouse University – expert in Hygiene Bacteriology)

Antoine Danchin (Prof. Institut Pasteur – expert in Bacterial Genomics)

Dominique Genty (Research Director at Commissariat à l'Energie Atomique *(CEA)* - Paleoclimatologist)

Michel Goldberg (Honorary Prof. at Institut Pasteur and at the Paris VII University – Chairman of the Pasteur-Weizmann Council – Biochemist, expert in enzymology and biotechnological applications)

Paul-Marie Guyon (Research Director Emeritus at the Centre National de la Recherche Scientifique *(CNRS)* – expert of the climatology of Lascaux – created the climate control system that rescued Lascaux during the 1960 crisis)

Alain Mangin (Emeritus Research Director at the Centre National de la Recherche Scientifique *(CNRS)* – expert in hydrogeology)

Ralph Mitchell (Prof. at Harvard University - expert in biofilms and biotcchnological applications)

Pierre Vidal (engineer - expert of decorated caves – worked for years at Lascaux)

Jean-Philippe Rigaud (former curator of Lascaux)

For additional information about the LIST and/or this report, please contact the LIST spokesman :

Prof. Michel Goldberg
Institut Pasteur – 28 rue du Dr Roux – 75724 Paris Cedex 15 - France
Tel: + 33 1 45 68 83 86
Fax: + 33 1 40 61 30 43
e-mail: goldberg@pasteur.fr

POST-SYMPOSIUM REPORT ON THE LASCAUX CRISIS

July 15, 2009

From information collected by the LIST members as well as from what we heard during the symposium, we understood the following:

- The paintings in the cave could be preserved for such a long time because of the existence in the cave of *1*- a "climatic equilibrium" and *2*- a "microbial equilibrium" between a large number of bacteria and fungi present in the cave. By equilibrium, we mean a stable or slowly evolving state that prevents visual degradation of the paintings (no calcite deposition, no visually observable microbial proliferation, no pigment deposition).
- These two equilibriums have been perturbed on various occasions by important morphological and climatic changes resulting from human interventions. A state of equilibrium could be reestablished after the crisis of the 60's and lasted until the year 2000-2001.
- For reasons that have not been specified in a clear way (contradictory information have been provided relative to its condition) the equipment that helped maintaining a climatic equilibrium from the 60's until the end of the 90's was replaced by a machine that was implicitly criticized during the symposium as being misconceived, inadequate and not in conformity with the initial specifications. Though claimed to be "based on the same principles" as the previous machine, it included heat exchangers and fans that were requested for "proper efficiency". Contradictory information was provided concerning the fans (the use of which might have introduced air currents disrupting the existing climatic equilibrium and might have contributed to the spreading of spores). During the symposium, M. Geneste stated very unambiguously that these fans were never activated. Shortly later, M. Malaurent stated that the fans were activated for about two weeks, but at very low speed, "making sure that no air flow could be detected by use of a heated electric wire". Later, in a private discussion, he stated that the fans were activated during a year. ***These inconsistencies should be clarified if one has to find the real causes of the crisis rather than start on a wrong basis***. Moreover, whereas the plans of the machine did not include it, some parts of the machine were covered with polystyrene foam. It was indicated that the polystyrene contained spores of white fungi, but the time at which this observation was made and the polystyrene coating removed was not specified during the symposium. At one point during the symposium, it was suggested that these spores were introduced in the cave by the polystyrene, but this seems to contradict the conclusions from the microbiologist of the LRMH, Genevieve Orial, that the contaminating microorganisms observed in the cave were "preexisting", rather than introduced at the time of the crisis. This conclusion is based on the differences in the subtypes of *Fusarium* found in various locations of the cave. ***Thus, there still seems to be a debate on the origin of the "white disease":*** *introduction of Fusarium spores during the installation of the machine, or "spontaneous" development of pre-existing spores due to an increased humidity in the cave generated by a flood in the water lock preceding the cave (sas 2)?*
- Whatever the exact cause, there is no doubt that the implementation of this new machine and the important works associated with its installation coincided in time with the disruption of the climatic and microbial equilibriums.

- **Concerning the disruption of the climatic equilibrium**, it has been suggested by M. Malaurent that a climatic change outside the cave (progressive increase in outside temperature reported by the nearby meteorology station, not necessarily associated with the "global warming") was the main cause of the disruption of the climatic equilibrium inside the cave. The "dramatic" climatic disruption referred to by M. Malaurent consists in the following facts: before the crisis, the lowest point in the cave (the end of the "diverticule axial") was at a temperature higher by a fraction of a degree Celsius as compared to that of the highest point in the cave (the entrance to the "salle des Taureaux"). According to M. Malaurent, this was enough to produce convection currents resulting in a permanent, slow natural movement of the air inside the cave that participated in creating local climatic conditions presumably favorable to the local microbial equilibrium in any part of the cave. Nowadays, the low point is colder than the high point, leading to a stagnation of the air inside the cave. That changes in the outside temperature would be the cause of the change in the climatic regime inside the cave is based mainly on computer simulations of the air flow inside the cave at two outside temperatures, those prevailing at two dates, one well before and an other after the crisis. Not enough information has been provided during the symposium to assess the validity of these simulations which, if we understand it correctly, must model not only the air conditions in the cave but also the temperature transmission (heat transmission temperature gradient) through the rock above and around the cave. **The predictive value of the simulation must in our opinion be validated before a serious conclusion can be made**. One of the predictions that might be checked is the temperature gradient between the ground and the vault at various points of the cave and the local convection currents thus created, that had been previously measured and observed. *An intriguing question is that, according to oral reports on measurements performed in the 1960's, there was no difference to within +/- 0.1 °C in the average temperatures measured at the high and low points of the cave, while no deleterious microbial proliferation was observed until 2000. It is therefore questionable whether the climatic regime supposedly created (according to the simulation) by the temperature difference between the high and low points is critical or not*. **An additional, very important (in the eyes of all climate experts in the LIST) problem related to the simulation is that it does not take into account the natural air exchange between the inside and outside of the cave.** This air input was said to exist to an unknown extent, and might well perturb the very slow convection movements predicted by the simulation. Moreover, according to a report dated April 2001 by Pierre Vidal, it seems that the "temperature inversion" inside the cave occurred within a very short time interval, between December and February 2001. In this period, the outside temperature was unusually low during about a month and went down to -11 °C during two freezing nights. The sliding doors supposed to isolate the cave from the airlock (sas 2) were out of order and could not be closed. As a consequence, masses of very cold air penetrated inside the cave and moved to its lower parts, resulting on the vault in very heavy water condensation originating from the warm air chased out from the lower parts of the cave by the denser cold air. This warm air may have been particularly loaded with water because of the temperature increase (the temperature at 2/3 of the height at the end of the "diverticule axial" was as high as 13.8 °C) created by the quicklime treatment that had been applied shortly before (exact dates need to be checked). It is very likely, in our opinion, that this influx of very cold air was responsible for the observed temperature inversion and the putatively resulting disruption of the climatic equilibrium. Even if one assumes that

the simulations mentioned above are precise enough and right in predicting the effects of changes in outside temperature on the internal climatic equilibrium, the fact that the temperature inversion was very rapid and coincided in timing with the brutal introduction of cold air strongly suggest that the absence of the sliding door and of a proper air assistance machinery are the triggering event of the disruption of the climatic equilibrium. It seems that the previous climatic assistance equipment was able to maintain the proper climatic environment in spite of the "unfavorable" (according to the simulation) outside conditions.

- In order to reach a better understanding of the potential effectiveness of the simulation, we talked to Mrs. Lacanette, whose lecture at the symposium dealt with this approach. She promised to send all relevant papers on this work. We still look forward getting them.
- A final remark concerning the simulation deals with its specific objectives, which did not appear obvious to us. In particular, is the aim to reestablish "proper" convection movements of the air in the cavity, or is it to help preventing a mold outbreak? Is it to help regaining a climatic equilibrium in the absence of human interventions, or is it to predict and help controlling the effects of human presence in the cave?

- **Concerning the microbial equilibrium**, the information we obtained clearly showed the presence of a large variety of microorganisms in the cave, both in the "sick" as well as in the "healthy" parts of the walls. It seems impossible to reconstruct the history of the importation of each germ. Claude Alabouvette indicated in his talk that today the "predominant" fungus in the cave is *Scolicobasidium sp*. By "predominant", he means "the species that gives rise to the largest number of clones". The relation between number of clones obtained and number of microbes present (dead or alive) for a given species is affected by the efficiency of the PCR (polymerase chain reaction) used to amplify the DNA in the samples, and by the cloning efficiency. These efficiencies may vary significantly from species to species. It therefore seems that the quantification should be taken with a grain of salt. In our opinion, at least quantitative PCR should be used to gain a more reliable estimate of the amounts of each DNA type present in the samples.
- The melanin deposits were previously attributed by the LRMH to the presence of *Ulocladium* and most of the communication related to the "black fungi" refers to that organism as being the cause of the melanin black spots. How come that this fungus was not mentioned by Claude Alabouvette as a component of the population? *In an e-mail exchange with Claude Alabouvette, his answer to this question is that the initial identification of the black fungus as Ulocladium was probably a mistake since:* i- *the initial sample he saw did not exhibit all the morphological features of Ulocladium;* ii- *his DNA-based analysis produced only one clone of Ulocladium;* iii- *his cultures contained no Ulocladium; rather, only Scolecobasidium were observed by an expert mycologist in his cultures of black fungi. However, Genevieve Orial as well as other members of the present Lascaux scientific commission stick to the conclusion that Ulocladium is involved rather than Scolecobasidium, since they failed to isolate the latter.* ***This discrepancy between the conclusions of expert microbiologists within the scientific commission sounds surprising and I am amazed that such an important question could not be unambiguously answered. Expertise from outside the scientific commission should be sought to settle that question.***

- It seems hopeless to identify in a reasonable time range all the species present. Nor does it seem feasible to kill all the germs present. And even if all living organisms

could be totally destroyed, the cave would be rapidly contaminated again by new germs brought either by visitors or by infiltrations, and could not be kept sterile.

- The goal one should therefore aim at is to reach a new microbial equilibrium (stable dynamic state) where visually polluting fungi or bacteria would not be able to take over.
- Letting the present microbial population evolve by itself under the present climatic conditions would probably have disastrous consequences, as the fungi that have been taking over recently (and are probably still present after the treatments) are likely to be favored in the present environment.
- It has been mentioned during the Symposium, and it is written in one of C. Alabouvette's papers, that the use of the biocide benzalkonium chloride may have contributed to the spreading of the fungi since this molecule can serve as nutrient for the fungi. This assumption is based on two papers cited by Alabouvette (Nagai et al. 1996, Biol. Pharm. Bull. 19, 873-875; Hamada 2005, Seikatsu Eisei, 49, 161-167). The first paper reports the characterization of a *Pseudomonas* strain isolated from a benzalkonium chloride solution. Though shown to indeed be resistant to the biocide, this strain is also reported in this paper NOT to be able to grow on benzalkonium chloride as a source of carbon, nitrogen, or both. In the second paper, the author reports the growth of various fungi, including *Scolecobasidium*, on various media using different surfactants (all structurally quite different from benzalkonium chloride) as a nutrient. This paper concludes that the microbial flora found in washing machines using detergents is determined by non-ionic surfactants, while benzalkonium chloride is clearly an ionic molecule. Thus, to our knowledge, no evidence contradicts the observations reported by Genevieve Orial and Thomas Warscheid that benzalkonium chloride is not degraded, and cannot be used as nutrient, by the fungi they studied.

***Suggested general strategy**:*

The members of the LIST consider that three parallel, yet distinct steps must be made to deal with the Lascaux crisis:

- define as rapidly as possible an appropriate working method, that should include the "ethics" of the preservation and the precise sharing of responsibilities between the various parties involved in the preservation of the cave. In particular, the scientific and administrative aspects of the management of the crisis should be separated and the scientific committee in charge should include a solid majority of independent (from the administration) experts in "hard sciences" under the leadership of an external expert of international standing.
- stop as rapidly as possible the progress of the present invasion of the cave by black fungi. **This should be based on a solid, concerted multidisciplinary scientific analysis of all information available about the cave since the 1960s**.
- undertake long-term research projects (on the climate, microbial composition, interactions within microbial communities, nutrient inputs via infiltrations, …) and a long-term surveillance of the cave to help in the prevention, early detection and treatment of future microbial outbursts. Note that the conditions under which experiments should be conducted for such long term research have to be defined: is a non-decorated cave somehow resembling Lascaux an appropriate approach in view of

the very narrow specificity of each cave, or should some experiments be performed within the Lascaux cave? This question should be the object of future discussions.

With the aim of helping the cave to reach as rapidly as possible a new microbial equilibrium based on climate conditions which will help to prevent and control the proliferation of biocorrosive and biofouling microorganisms as much as possible, we have defined a possible general strategy that can serve as a starting basis for its treatment.

1- We have not heard during the symposium any convincing evidence that the "temperature inversion" that occurred in the cave is the real **cause** of the crisis. Contradictory information was provided to us concerning the status of the temperatures inside the cave along the years. According to some statements, it seems that in the 60's, at a time when the cave was "sound", there was no significant temperature difference between the low and high points. The latter observation would cast serious doubts on the hypothesis that the global convection movements due to temperature differences at two ends of the cave are a major player in the climatic equilibrium. The planning of a new machine aiming at creating a "cold spot" in the cave to reestablish the convection movements that existed (according to the simulation) before the crisis therefore seems to rely on a shaky basis. As a consequence, we would suggest:
    - *to examine in details the evolution of the temperature difference between the entrance of the Salle des Taureaux and the end of the Diverticule Axial, based on the temperature recordings since 1965 until 2000.*
    - *to disregard the convection regime as an important factor, unless the evolution of the temperature difference (see preceding paragraph) confirms the existence of a "favorable" gradient throughout the period from 1965 till the onset of the white crisis and an "unfavorable" gradient thereafter.*
    - *Thus, the aim of the "new" machine should be redefined as that which turned out to be effective during 35 years: maintain the humidity of the air at approximately 1% below the condensation point. As an additional aim of the machine, and in view of the slow drift of the temperature toward higher values during the last decades which might continue during the next decades, one might perhaps consider regulating the temperature.*
    - *to reconstruct the walls and doors of the "Sas Bauer" that may have played a role in the humidity control. Though no precise data exist on the way in which these large plastic surfaces may have served as a condenser for water vapors, restoring the configuration of all elements present at the time when the cave was "healthy" seems a pragmatic approach.*
    - *to rebuild the metal grids (walkway) that used to protect the ground of the cave, so as to avoid possible spreading of microbes by people working in the cave or by visitors.* In this respect, one should remember the assumption put forward by Mrs. Orial (LRMH) that spores from fungi are present everywhere in the ground of the cave, and could develop lavishly at the onset of the "white disease" because of an important humidity increase that resulted from a flood in the water lock before the "Salle des Taureaux" (sas 2). Thus, extreme care should be taken in order not to spread spores present in the ground.

2- Taking the prevention of additional visual pollution of the walls (and primarily of the paintings) as the most important and urgent goal, one should as soon as possible

undertake the destruction of the presently polluting microbes. We understood that they are essentially one of the melanized fungus *Scolecobasidium* or *Ulocladium*. Rather than using a drastic treatment (physical, or chemical) likely to kill all microbes, one should focus, if possible, on biocides specific for fungi. This is still a matter of debate (see the "Conclusions" section), but for immediate application this seems the best option. And rather than spreading such a biocide in the whole cave, one should apply it only locally, where pollution is visible. This implies that a very strict **permanent** surveying of the cave should be implemented to detect as soon as possible any resurgence of visual pollution for immediate identification and treatment. Thus we suggest:

- *to apply, on and around all visible pollution spots, a biocide treatment effective on fungi.*
- *to use a biocide as specific as possible for Scolecobasidium/Ulocladium. The staff of the LRMH, together with Thomas Warscheid, seems to master the use of biocides in conservation well enough to give the conservators the time to develop a long-term strategy.*
- *it should be noted that one expert microbiologist from the LIST has the opinion that "no treatment is better than a blind treatment" as long as one does not know enough on the flora of the cave and on what controls the microbial equilibrium. This opinion is not shared by most LIST members who fear a spreading of the black spots, and think that a knowledge-based, empirical approach likely to block the spreading should be used as soon as a provisional study of its impact has been conducted. It should be emphasized that, in the present state of our knowledge there is no evidence in the literature that the detailed knowledge of a microbial community in a biofilm can provide predictive information about subsequent mold infestation.*
- *to implement in the cave a* ***scientific observation method of the painted walls*** *that should supplement the visual observation and allow for a rigorous, objective follow up of their evolution.*
- *to pursue long term systematic follow up of the composition of the microbial flora of the cave with the aim of getting a good understanding of the interactions responsible for the microbial equilibrium. This might enable us to more effectively handle future crisis likely to occur in the long term.*
- *to develop, in the long term, new fungicides with very narrow specificities to fight future ruptures of the microbial equilibrium that could not be treated with the present fungicides.* One track to consider may be the use of a novel class of biocides developed by Prof. Yechiel Shai, from the Weizmann Institute in Israël. Y. Shai has been studying and developing powerful antimicrobial lipopeptides (Makovitzki et al. – 2008 – Biochemistry, vol 47, pp 10630-10636). Some are active on fungi while poorly active on bacteria; one example is palmitoyl-lys-leu-leu-lys which has been tested on *Aspergilus fumigatus, Aspergilus Niger, Candida,* and some agricultural fungi. It also destroys spores and hypae of *A. alternata, B. cinerea, C. heterostrophus* (Makovitzki et al. 2007 – Appl. Environ. Microbiol. Vol 73, pp 6629-6636). An other approach might be to discuss with David Martin (David Martin, Director of AvidBiotics Inc) the possibility of developing analogs of pyocins for fungi and rendering them specific for *Scolecobasidium* or *Ulocladium* ? It is clear that such "specific" fungicides might become available, and their effects studied in depth, only in the long term. In the meantime, more classical fungicides with properties well known from conservators should be applied.

Conclusions

It is important to realize that this strategy, based on the information presently available to the LIST members, may need some modifications that will depend on **additional information that MUST be obtained**. Indeed, it is clear to all of us that a precise diagnostic of the causes of the crisis must be reached for finding the best, long lasting, possible cure. In particular, the following elements are badly needed:

- a precise calendar of all actions conducted in the cave before and during the crisis (from 1999 until now)
- the complete data recordings of the temperatures, relative humidity, pressure, CO_2 content, that have been measured in the cave over the years.

Two important points need be emphasized.

1- related to the climate simulation and its applications:

It is widely accepted by the LIST members that **any decision based only on the climate simulations (as they stand now) would be premature and even dangerous**. Indeed, in the absence of detailed information on the simulation algorithm, the parameters used and the tests performed to ascertain the validity of the simulations, we must question ~~its~~ their exactness and precision. A major reason why the validity of the simulations must be tested by real measurements *in situ* is that the exchanges of gases between the cave and its surroundings are not taken into account in the simulations. Yet, in most caves (and Lascaux is likely not an exception), such exchanges are known to play an important role. Exchanges of air between the cave and the outside (via the access to the cave and the large clefts in the rock) due to atmospheric pressure variations and to air density differences (related to temperature differences between inside and outside), as well as exchanges between the cave and the karst (which are known to control the air composition in caves) can drastically alter the air movements in the cavity. Omitting these contributions in the model thus may lead to considerable deviations as compared to reality.

An additional reason for not putting too much emphasis on the need for a higher temperature in the bottom of the cave as compared to the entrance is the situation in the Chauvet cave. There, the bottom is colder by about 1° Celsius than the cave entrance, and in the galleries the ground is colder than the vault, thus precluding the type of convection regime predicted by the present simulation model during the "pre-1980" period. Yet the conservation is fine. A global convection regime therefore does not appear as an important, general requisite for the conservation.

These are the reasons why the LIST members recommend not to experiment any simulation-based, important modification of the climate regulation paradigm (like introducing artificial cold spots in the cave) that might have unpredictable effects on a cave which "breathes" and has important and complex exchanges with its surroundings. Rather, they recommend the implementation of a minimalist climate regulation based on the same requirements as those that proved efficient in the past.

2- related to the need to rapidly control fungal growth:

The opinions on this subject are not as unanimous as on the climate problem.

One LIST member emphasizes that attempts to "blindly" treat the disease without having first a good understanding of the microbial community and of the interactions between its components may be dangerous and may lead to advert effects. He therefore recommends not applying any treatment for the time being. However, several LIST members consider

that, in the present state of advancement of science, a knowledge-based preventive or curative treatment of complex biofilms is still out of reach. Therefore, all but one of us think that a treatment is urgently needed to stop the progression of melanized fungi that, if left alone, are likely to cause irreversible visual damage to the paintings. A real, objective follow up of the recent progress of the "black disease" should be available. If it confirms the (likely) mold proliferation, a fungicide treatment should be applied urgently.

The way to eventually stop the fungal growth is no more consensual. One original approach that has been suggested is to gently blow locally, on infected areas, a flow of humidified anoxic argon. An other approach that was suggested during the symposium as well as by some LIST members, and was briefly discussed is the use of radiations. A very serious drawback of these two approaches is that they are likely to kill all microbes present in this area, thus disrupting to an extreme the initial microbial equilibrium and opening the door to the development of a new unknown microflora. Most LIST members therefore reject such "non-specific" physical treatments as long as the invasion by black fungi can be controlled by more specific means. An alternative approach, widely preferred by most microbiologists in the LIST, aims at destroying only (if possible) the contaminating fungi by using a biocide with the highest possible specificity for fungi of the species identified as visually polluting. These options should be discussed further and experimentation outside the cave might perhaps be envisaged to determine the pros and cons of each approach as well as the properties of the various biocides considered.

Finally, we want to emphasize that it seems unlikely to us that outbreaks of molds will be controlled in the long term without extensive, detailed and continuous surveillance:

- of the environment at different locations in the cave. There is clear evidence of plural microclimates in the cave. They need to be monitored and controlled.
- of the microbial flora, as discussed above, to detect as soon as it appears and fight immediately any resurgence of polluting microbial growth.

This will require the implementation of modern, adapted monitoring equipment for temperature, relative humidity and CO_2, and of a systematic microbiological sampling and analysis procedure.

One last observation: the LIST members have noted numerous discrepancies in the information provided, and felt very serious "tensions" among persons in charge of the cave, should they be members of the present Lascaux Scientific Commission or administrators at different levels. This appeared to us as extremely harmful to the serene and productive search for a solution to the Lascaux crisis.