Searching for Blood in Chinese Lacquerware

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"Before applying such varnish over wood, the Chinese use – but not always – to give it a bed or primer, as painters usually do before painting, in the following way. Take the blood of a pig [...] and mix it with powdered quicklime, and coat the wood with this mixture [...] once it is dry, smooth it down with pumice stone or something similar."


It is significant for the conservation of lacquer objects to know the type of binding medium used for the ground layers as it influences the choice of consolidation medium. For more than 2000 years pig's blood as well as blood from cattle was used as binding medium, not only for lacquerware and not only in China. When mixed with fitting materials such as brick powder it made an appropriate binder to prepare the ground for lacquer, due to its adhesive property and good water-resistance. Also, it was most likely cheaper than using lacquer itself.

Different methods of the blood identification:

**Benzidine and Luminol**

A sample from the foundation layer of the Chinese lacquer tea box was analysed by both microchemical test and gas chromatography/mass spectrometry (GC/MS). The sample was firstly treated with hydrogen peroxide and benzidine; the positive presence of blood was indicated by a blue coloration appearance.

Further investigations on the selectivity of the benzidine test, showed a possible bleeding of pig skin, as it is not 100% distinctive. Therefore, the GC-MS analysis was necessary to confirm the obtained results. The comparison of chromatographic profiles of amino acids gained by hydrolysis (6M HCl, at 105°C for 24 hour) of a blood reference standard and the sample proved that the ground contains blood.

GC-MS analysis were performed using a 6890N gas chromatograph connected to a quadruple MS (model 5973N, Agilent Technologies, USA).

**DNA Analysis**

A set of samples from foundation layers of different lacquer panels from the Chinese Cabinets were DNA extracted using the E.Z.N.A. Advanced (Omega, Hilden, Germany) following the manufactures' protocols.

The cytochrome b gene was sequenced to identify the animal species used as binder as well as the quality of the selected primer. The 105 base pair products were sequenced on an ABI 3730xL automated sequencer (Applied Biosystems, USA) using the Big Dye v3.1 reagent and the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit. The fluorescently labeled sequencing reactions were separated on an ABI 3730xl automated sequencer and the sequence data was analyzed with Geneious software (Biomatters Ltd., New Zealand). The presence of a blast homology match with an identified specie was considered positive evidence of blood.

**THM-Pyrolysis-GC/MS**

Ten samples of ground and lacquer layers were examined at the Serological Department of the Institute for Forensic Medicine in Vienna (Prof. Dr. Georg Bauer / Dr. Elisabeth Friedrich) with regard to traces of blood components. The samples were tested with a solution of benzidine in glacial acetic acid with addition of some drops of hydrogen peroxide.

The blood test, a luminol test was executed in a dark room; the samples were treated with a reagent according to Paler: a slightly alkaline solution of 3-amino-phenylhydrazide with the addition of hydrogen peroxide (= Luminol). Three samples, which underwent the benzidine test, showed a slightly blue reaction, what indicated the presence of blood, while the other seven samples gave negative results with both the benzidine and the Luminol test as well.

**Nano LC-MS-MS**

A set of samples from foundation layers of different lacquer panels from the Chinese Cabinets were DNA extracted using the E.Z.N.A. Advanced (Omega, Hilden, Germany) following the manufactures' protocols.

The cytochrome b gene was sequenced to identify the animal species used as binder as well as the quality of the selected primer. The 105 base pair products were sequenced on an ABI 3730xL automated sequencer (Applied Biosystems, USA) using the Big Dye v3.1 reagent and the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit. The fluorescently labeled sequencing reactions were separated on an ABI 3730xl automated sequencer and the sequence data was analyzed with Geneious software (Biomatters Ltd., New Zealand). The presence of a blast homology match with an identified specie was considered positive evidence of blood.

**Conclusion:**

The benzidine reaction is not distinctive, but informative for the first screening. GC/MS analysis confirms the presence of blood according to the profile of amino acids of the reference.

The presence of blood can be confirmed in lacquer samples by DNA extraction and sequencing and the presence of blood in lacquer objects can be confirmed by THM-Pyrolysis-GC/MS. Good water-resistance. Also, it was most likely cheaper than using lacquer itself.

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