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GUM DAMAR AS A SUBSTITUTE FOR CANADA BALSAM IN MOUNTING MEDIA FOR MICROSCOPICAL SPECIMENS

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ABSTRACT

A number of substitutes for Canada balsam have been suggested in the literature with varying degrees of success for the entomologist. This paper re-evaluates classic Gum Damar media as a substitute for Canada balsam

INTRODUCTION

Canada balsam is the natural exudate of *Abies balasmea* (Gray, 1973) and it is considered a permanent light microscopy mountant for whole mounts of insects as well as genitalia and dissected insect parts; its archival properties are widely known. Brown (1997) states Canada balsam slides can survive over 150 years. Brown (1997) also notes that Canada balsam has a refractive index (1.52–1.54) close to that of glass (1.53). Canada balsam has a few disadvantages; firstly it is dissolved in xylene and Gray (1973) notes that it is often a 40% solution of Canada balsam in xylene. It is a difficult solvent to obtain for the amateur entomologist due to the possible health effects; indeed, Brown (1997) raised questions as to whether the use of xylene within museum environments might be restricted in the future as a result. Secondly, it is known to become acidic and yellow with age (Gray, 1973); the acid nature has been linked to degradation of tinctorial stains. Finally, Canada balsam is becoming more difficult to obtain and costly *in lieu* of cheaper synthetic resins.

Two such resins in common usage are Euparal and D.P.X.

Gray (1973) states that Euparal was first disclosed by Gilson in 1906 and is a proprietary formula the exact composition of which is still a secret. Gray (1954) notes the problems of proprietary secrets, and discourages the use of such formulae. Brown (1997) notes that, dependent upon place of origin, Euparal can vary in quality; it is also noted that the refractive index is lower than that of Canada balsam at 1.48.

D.P.X. (sometimes referred to as DePeX) is made of the Distrene-80 (a polystyrene), tricresyl phosphate and xylene, and was first described by Kirkpatrick and Lendrum in 1939. It is often marketed as 'Canada balsam substitute'. Brown (1997) observes the refractive index of D.P.X as 1.53, and notes that due to excessive shrinkage, changes in refractive index and optical distortion of specimens, D.P.X is not considered a permanent mountant for natural history specimens. From the authors personal experience D.P.X appears fine with sectioned histological material due to the reduced thickness of the mounted material, but for natural history specimens the material used is often too thick for D.P.X to be considered as a suitable mountant. Indeed Kirkpatrick and Lendrum (1939) recommended D.P.X for use in bacteriology and 'probably' histology as

D.P.X preserves staining, due to its more neutral nature. Also, given that D.P.X uses xylene as a solvent its use may be further restricted in the future. Brown (1997) also notes that other synthetic resins are not used in the Natural History Museum, London as they present similar problems to D.P.X.

Gum Damar (sometimes referred to as Gum Dammar) it is the natural exudate of *Shorea wiesneri* (Gray, 1973) with a refractive index of approximately 1.52–1.55 (Brown, 1997). Fowell (1946) considers Gum Damar as a substitute for Canada balsam in permanent zoological and botanical preparations. Gray (1973) notes that it contains solid impurities and the user 'is to be particular over supply'. It is also noted that, with a good supply, Damar is less likely to become yellow or acid than Canada balsam and thus preserve staining better. Gray (1973) notes two formulae for Gum Damar medium, one being a mix of Damar gum and Canada balsam, and one using benzene and turpentine as solvents. Mayer (1981) notes that Damar dissolved in turpentine is used as a picture varnish due to its archival properties.

MATERIALS AND METHODOLOGY

For a stock solution

Gum Damar (2 parts) Turpentine (3 parts) Acetone (small amount)

As previously stated Gum Damar is soluble in turpentine. As a matter of clarification between turpentine and turpentine substitute, turpentine is the distilled resinous sap of pine trees, and is still used as a solvent by artists. Whereas turpentine substitute (sometimes called mineral spirits) is a solvent composed of petroleum distillates, Damar is noted as being insoluble in turpentine substitute (Mayer, 1982) and as such only artists grade distilled turpentine should be used to dissolve Damar Gum.

The authors personal preference is to dissolve two part picked lumps of Gum Damar in three parts turpentine; the Damar is suspended in the turpentine in a cloth bag to filter out impurities for a number of days at room temperature. After the gum has dissolved, the solution is inspected for clarity; if it appears cloudy Mayer (1982) recommends adding a small amount of acetone and stirring vigorously; upon addition a white wax precipitate forms which re-dissolves upon stirring. The acetone should be added in very small amounts because if too much is added the waxes can be permanently removed from the solution. This can make the mountant brittle in the long term. If the acetone leaves the mountant too fluid for use, evaporate the excess solvent for a few days, protecting the mountant from dust. The final mountant should be clear to straw coloured.

In use, Gum Damar is similar to Canada balsam, being a similar compound, with a long drying and curing time. The author's personal method for slide mounting Calliphoridae larvae using Gum Damar is as follows.

- 1. After making a small incision along the ventral surface of the larva, submerge in 10% sodium hydroxide (or potassium hydroxide) on a warm heat mat overnight to macerate the specimen.
- 2. Remove to a small tile and gently extract the remains of the body contents. Place the specimen in glacial acetic acid to which a few drops of stain have been added (either acid fushin, or methylene blue) and leave for at least 10 minutes.
- 3. Transfer to 70% alcohol for 10 minutes gently to dehydrate the specimen.
- 4. Place specimen in 100% alcohol for 10 minutes to remove final traces of water.
- 5. Transfer to either turpentine or clove oil* for 10 minutes to clear and remove alcohol present in the specimen.
- 6. Place a suitable sized drop of Gum Damar on a microscope slide, carefully position the specimen in the mountant and apply a coverslip and leave to dry (may take several weeks).

*On the choice of clearing agent, personal experience has shown that clove oil appears to over-clear specimens when used with Gum Damar. Also, turpentine appears to preserve stains better than clove oil; indeed, Brown (1997) suggests that the fading of stains in Canada balsam may in part be due to the remnants of clove oil in the mount. However, Gray (1954) notes that clove oil is more tolerant of incomplete dehydration than turpentine (clove oil will clear from 75% alcohol to turpentine's 95% alcohol); for this reason Fowell (1946) states clove oil is the clearing agent of choice for elementary work.

COMPARISON TO OTHER RESINOUS MEDIA

Gum Damar is similar in composition to both Canada balsam and indeed D.P.X, in that it is a mixture of resin, plasticizer and solvent. It behaves in similar ways to the aforementioned mountant media in use, being a viscous fluid, with a fairly slow drying time. It is less prone to shrinkage under drying than D.P.X and other synthetic media used with entomological specimens. Its natural clarity and lower cost make it a more economical medium than Canada balsam and it has the favourable traits of being less likely to yellow or become acid with age, possibly preserving staining of specimens better than clove oil cleared balsam mounts. While the recommendation of Gum Damar as a substitute for Canada balsam in permanent preparations of zoological materials and its archival qualities are recorded in the literature, it would be wise to maintain a watching brief as advocated by Brown (1997).

REFERENCES

Brown, P.A., 1997, A review of techniques used in the preparation, curation and conservation of microscope slides at the Natural History Museum, London, *The Biology Curator*, **10**: Special Supplement, 33pp.

Fowell, R.R., 1946, *Biology staining schedules for first year students*, H.K. Lewis and Co. Ltd, London.

Gray, P., 1954, The Microtomist's Formulary and Guide, Constable and Company Ltd, London.

- **Kirkpatrick, J. & Lendrum, A.C.**, 1939, A mounting medium for microscopical preparations giving good preservation of colour, *Journal of Pathological Bacteriology*, **49**: 592–594.
- Mayer, R., 1982, *The artist's handbook of materials and techniques* (4th edition), Faber and Faber, Norfolk.
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