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Basic Technique for Preparation of Down for Examination with the Scanning Electron Microscope

ROXIE C. LAYBOURNE, 1 BETH ANN SABO, 2 AND ANN MORNINGSTAR 2

1Smithsonian Institution, Division of Birds, Washington, D.C. 20560, USA; and
2U.S. Fish and Wildlife Service, National Fish and Wildlife Forensics Laboratory, Ashland, Oregon 97520, USA

The scanning electron microscope (SEM) has aided researchers studying feathers in describing unique structural features (Dyck 1973, Schmalz 1982), making identifications from microscopic characters (Davies 1970, Robertson et al. 1984), and pursuing taxonomic studies (Brom 1990, Reaney et al. 1978). While a number of authors (see above citations) have described preparation of material for SEM examination, their focus was not on material-preparation technique. We have developed a preparation method for plumulaceous feather structures (i.e. down) to be examined with the SEM. Our method is simple, safe, and re-
requires no special equipment or material not normally available in an SEM laboratory.

The shape and arrangement of plumulaceous structures (basal cells or flanges, internodes and nodal structures) vary among species. Structure also varies with location of the feather on the bird (tract), location of the barb on the feather (vane), orientation of the barbules (vanule), and position of the barbules along the rachilla or ramus. Feathers recently removed from the bird often bear foreign particles, such as dust, oil, dirt, and powderdown fragments. Plumulaceous morphology is obscured by these particles, and cleaning often reveals significant structural features of the down (Fig. 1).

We clean feathers prior to SEM examination by blowing the entire feather with compressed air to remove the bulk of foreign material. Next, the feather is washed at least twice in a warm, mild soap solution. Slight manual agitation of the feather will remove obstinate particles. After washing, the feather is rinsed in several changes of warm water until the rinse water is completely clear. The structure is then dried with compressed air. Feathers are washed twice in ethanol and again blown dry. We have found no differences in results using ethanol solutions of 70%, 80%, 90%, 95% and 100%. Foreign material that is difficult to remove may require additional washings, a higher alcohol concentration, or both.

Ultrasonic cleaning damages microstructure in the plumulaceous barbules of some species (Barton and Weik 1986). Thus, it is not recommended.

Because the morphology of vanules on a single barb may vary, standardization in the removal and mounting of barbs is critical to the study of plumulaceous structures. We use microforces to remove barbs from the left and right vanes of the feather. Each barb is mounted separately, dorsal side up, on a Cambridge-style stub coated with an adhesive tab. The barbules are spread to facilitate visibility. If there is sufficient space, we place more than one barb on each stub.

Samples to be preserved for further study are best mounted on 12-mm round coverslips designed specifically for use with the SEM (Carolina Biological Supply). Coverslips are cleaned with ethanol before applying double-sided tape or adhesive tabs and labeled with a species code (e.g. Edwards 1982, 1986) in permanent, carbon-based ink. Coverslips are mounted on stubs with carbon paint. After the barbs have been coated and examined, the coverslips are removed from stubs and stored. We use paleontological microslides and slide holders (Curtin Matheson).

Feather material sputter-coated with approximately 15 nanometers of gold-palladium shows minimal charging, little specimen damage, and features of taxonomic significance. We do not recommend carbon coating for feather material, because the heat of carbon evaporation can damage feather structures. Specimens should be studied as soon after coating as possible.

In our experience, plumulaceous material is best studied with accelerating voltages between 10 and 15 KV, depending on the microscope. Higher accelerating voltages increase chances for specimen damage, while lower accelerating voltages yield poor resolution. Standard working distance is 15 mm, but larger samples may require increased working distance.

Micromorphology of plumulaceous structures typically varies with the position on the barb and barbule. Therefore, we confirm the orientation of the barb at magnifications low enough to include both vanules. Details of nodal and internodal morphology are best examined at 200 X, 500 X, and 1,500 X. The type of material (and practice) will establish appropriate magnifications.

We use Polaroids (4" X 5" type 54), negatives (Ko-
dak 4” × 5” type 4162), and thermoprints (Sony 110 mm × 20 mm) to document plumulaceous structures. We use an alphanumeric generator to label the face of photomicrographs using species codes from Edwards (1982, 1986). Each photomicrograph is labelled with species, tract, vane, vanule, and position of the barbules along the rachilla or ramus. We include technical information, such as the type of SEM, working distance, and magnification (if not shown on the face of the photograph). The photomicrographs are stored in file-card boxes or notebooks (in systematic order) following alphanumeric codes developed by Edwards.

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**Sexual Selection and the Evolution of Extravagant Traits in Birds: Problems with Testing Good-genes Models of Sexual Selection**

IAN L. JONES

*Department of Zoology, University of Cambridge, Cambridge CB2 3EJ, United Kingdom*

The evolution of extravagant traits that may be favored by sexual selection has received much attention in recent literature. Empirical studies have focused on attempts to test alternative sexual-selection mechanisms, using ornaments of some birds as examples of such elaborate traits. However, the interpretation of empirical evidence has been controversial, and recent papers have pointed out numerous difficulties in testing these models (Read 1990, Kirkpatrick and Ryan 1991). Here, I reevaluate some findings of a recent paper on ornaments of curassows (Buchholz 1991) to point out some pitfalls to consider in inter- and intraspecific tests of sexual-selection hypotheses. Buchholz (1991) pointed to a correlation between knob-ornament size and age of Yellow-knobbed Curassows (Crax daubentoni) as evidence for “good-genes” models of sexual selection. The interpretations presented in his study illustrate several perceptions of sexual selection in general, and “good-genes,” “runaway” and “direct-benefits” models (references in Kirkpatrick and Ryan 1991) in particular, that merit further discussion.

Buchholz (1991) suggested that direct-benefits models to explain the evolution of mating preferences do not apply to Yellow-knobbed Curassows, because males “do not appear to defend territories or care for chicks.” Even if this were true, it should not eliminate this model from consideration, because direct benefits (e.g. involving parasite, predator, or harassment avoidance; Reynolds and Gross 1990) could favor evolution of female preferences for extravagant male traits in lekking species, or others where males provide no care. Ornaments could be favored by sexual selection if they reflect nongenetic phenotypic differences among males that involve these mating advantages to females. Tests of the direct-benefits hypothesis seem