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Feather Pulp: a Non-destructive Sampling Technique for Electrophoretic Studies of Birds

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Samples of tissue for electrophoretic analysis of avian species are usually obtained from the muscle, heart, kidney, or liver of sacrificed individuals (e.g. Smith and Zimmerman 1976, Barrowclough and Corbin 1978, Avise et al. 1980). Egg-white proteins have also been used (e.g. Sibley 1961). Non-destructive collection of blood is possible but yields less information than that obtained from the muscle or internal organs, because fewer structural gene loci are expressed in blood (e.g. Barrowclough and Corbin 1978, Ryttman and Tegelstrom 1981). Moreover, the loss of comparatively minute quantities of blood in small birds, such as many passerines, or the stress of taking the sample may cause fatal shock. A skilled worker can perform a biopsy of pectoral muscle (Baker 1981), which expresses more loci than blood, but this, again, is not a benign technique.

Because of the need for non-destructive sampling of birds in studies of behavior, ecology, and evolution (e.g. Sherman 1981), we sought to identify another source of tissue that could be taken without harm to the birds. This paper examines the potential of an alternative non-destructive technique for obtaining avian tissue for electrophoresis—the use of the pulp inside the shafts of newly emerging feathers.

A single individual of each of six species of birds was used: turkey (*Meleagris gallopavo*), Red Junglefowl (*Gallus gallus*), Common Quail (*Coturnix coturnix*), domestic duck (derived from *Anas platyrhynchos*), Rock Dove (*Columba livia*), and Bobolink (*Dolichonyx oryzivorus*). All birds except the Bobolink, which was frozen intact for 2 weeks, were fully dissected within 15 min of sacrifice. A 0.5-cc sample was taken of kidney, liver, heart, and pectoral muscle; whole blood was collected from the jugular vein or heart. Developing feathers with pink, living tissue showing through the quill were collected from each bird, regardless of stage of development. For each gel sample, approximately 0.05 ml of homogenate permits an examination of up to seven enzyme systems, if a 0.95-cm-thick gel is used. In the larger birds (turkey, duck, Red Junglefowl and Rock Dove), even body feathers had useable quantities of tissue in their quills; we collected tissue from a variety of wing, tail, and body feathers. In the Bobolink, the 5th and 6th primaries on each wing were approximately half emerged, and all four were collected to obtain sufficient tissue. Fortunately, as molt of flight feathers is symmetrical and often overlaps tail molt, 2-4 large feathers are available throughout most of the molting period. The quail had only superficial head molt, and the 5 feathers that were collected were homogenized whole (see below). In addition, flight feathers in three distinct stages of molt—quill just emerged, feather half grown, and fully emerged—were collected from the turkey in order to assess possible changes in enzyme expression during feather development.

During sampling, care was taken to grasp the feather quill next to the skin and to pull straight outward in order to avoid breaking the quill. This is especially important in living birds, as a broken quill left in the skin inhibits the growth of a new feather and may cause infection. If a bird is not in molt, feather growth may be induced by plucking a feather

TABLE 1. Enzyme expression in six tissues of six species of birds: B = Bobolink, D = domestic duck, J = Red Junglefowl, Q = Common Quail, R = Rock Dove, T = turkey. ( ) indicates faint expression of the locus.

Locus[a]	Kidney	Heart	Liver	Muscle	Feather pulp	Blood
Aat-1	BDJQRT	BDJQRT	BDJQRT	BDJQRT	(bdjrt)	BDJQRT
Aat-2	BDJQRT	BDJQRT	BJQRT	BDJQRT	B(t)	B(t)
Acp	DJQRT	D(jqr)	DJQRT	D(q)	DR(jqt)	
Ada	BDJRT(q)	DJR(bqt)	DJR	BDJR(qt)	D(bjr)	DJQRT(b)
Agp	DJQR	RT(q)	BDJQRT	BDJQRT		
Cpk	BDJQRT	BDJQRT	(jrt)	(bdj)	DJQRT(b)	(bj)
Est	(bt)	(bjt)	(bt)	(b)	T(bj)	BT(j)
Gpi	BDJQRT	BDJQRT	BDJQRT	BDJQRT	BDJRT	B(djqrt)
Gr	T(bdjr)	(bdjqrt)	T(bdjqr)	(jrt)	(jqt)	R
Idh	BJ	BQRT(j)	BJ	R(bjq)	(j)	
Ldh-1	JQR(bdt)	J(bdqr)	DJQRT(b)	BDJQRT	BDJRT(q)	(dj)
Ldh-2	BDJRT	BDJRT	BDJRT	J(bdqt)	DRT(j)	BJT(q)
Mdh-1	DJQRT(b)	BDJQRT	DJQRT(b)	DJQRT(b)	DJRT(bq)	(b)
Mdh-2	BDJQRT	BDJQRT	BDJQRT	BDJQRT	BDJQRT	BDJQRT
Me	BDJQRT	BDJQRT	BDJQRT	BDJQRT	D(jrt)	(b)
Np		BDJRT(q)	BDJRT(q)	(dr)	(t)	
Pep-gl	BDJQRT	BDJQRT	BDJQRT	BDJQRT	BDJQRT	BDJQR(t)
Pep-pap	BDQRT	DQRT(bj)	R(bdjqt)	BJQRT(d)	R(bdjqt)	Q(bdjt)
Pgd-1	BQ(rt)	BQRT	BQT(r)	BQR(t)		(b)
Pgd-2	BDQT	DQT	BDQT	DQT(b)	DQ(bt)	T
Pgd-3	BJRT	BJRT	BJRT	BJR	BJR(t)	BJR(t)
Pgk	DJQRT	DJQRT	DJQRT	DJQRT	J(d)	
Sdh	BDJQ(r)		B	DJQT(r)		
Sod	DJQR(t)	BDJQRT	BDJQRT	BDJQR(t)	(j)	BDJQR
Totals[b]	215	214	211	201	131	102

[a] Aat = aspartate aminotransferase; Acp = acid phosphatase; Ada = adenosine deaminase; Agp = alphaglycerophosphate dehydrogenase; Cpk = creatine phosphokinase; Est = esterase; Gpi = glucosephosphate isomerase; Gr = glucathione reductase; Idh = isocitrate dehydrogenase; Ldh = lactate dehydrogenase; Mdh = malate dehydrogenase; Me = malic enzyme; Np = nucleoside phosphorylase; Pep-gl = peptidase with glycyl-leucine; Pep-pap = peptidase with phenyl-alanyl-proline; Pgd = phosphogluconate dehydrogenase; Pgk = phosphoglycerate kinase; Sdh = sorbitol dehydrogenase; Sod = superoxide dismutase.

[b] Good expression received a score of 2, and faint expression received a score of 1.

and waiting 11–14 days for a new feather to emerge (Mengden and Stock 1976).

All tissues were immediately put on dry ice for up to 12 h before being placed in storage at −80°C for 7–30 days until electrophoresis could be performed; feathers were frozen whole. All tissues, except for feather pulp from small feathers, were homogenized by hand in an equivalent volume of 0.05 M Tris-HCl extraction buffer (pH 7.1) in 12 × 75-mm test tubes and then centrifuged for 5 min at 3,000 rpm. We slit large feathers along the shaft and scraped out the tissue with a spatula, whereas we placed small feathers, with the barbed portion of the shaft removed, into test tubes and homogenized them whole. Alternatively, the pulp may be squeezed out of the shaft into a ceramic depression well and homogenized therein; gel samples then may be taken directly from the well.

Horizontal starch-gel electrophoretic procedures were followed as described in May et al. (1979). Staining methods were adapted from Harris and Hopkinson (1976). Previous screenings in our laboratory of White-fronted Bee-eaters (*Merops bullockoides*), Rock Doves, and Leach's Storm-Petrels (*Oceanodroma leucorhoa*) identified 24 loci that could readily be resolved in birds. We used these in this study (see Table 1).

In all the species and enzyme systems examined, feather-pulp proteins migrated at the same rate as those from the other tissues. For the majority of loci examined, the turkey feathers in different stages of emergence gave identical results. In three cases (Ada, Pgd-1, and Pgd-2), however, the fully emerged feather gave poorer expression than those at earlier stages of development. Because more fully developed feathers will be in the process of resorbing the pulp, this result is not surprising. This suggests that, when possible, feathers that are less than fully emerged should be selected.

Results of the enzyme staining are presented in Table 1. In general, the feather pulp yielded fewer loci than the internal organs but was better in most cases than blood. For example, in Red Junglefowl, 18 loci were scoreable in feather pulp, compared to 12 in blood, 19 in kidney and muscle, and 20 in liver and heart. Only in the Bobolink did more loci appear in blood than in feather pulp (15 versus 13 loci). Note that the specific expression of particular enzymes in a given tissue and species is not relevant here; a greater availability of feathers, the development of

new buffer systems, or better staining regimes may reveal the expression of enzymes not seen in our study. Rather, the total score for each tissue over all the taxa we examined is more informative. Therefore, a quantitative comparison was made by assigning a 1 to all tissues with faint expression and a 2 to those with good expression and then totalling these numbers for all enzymes in each tissue (see bottom of Table 1). Again, feather pulp was more informative than blood but less informative than internal organs.

There are only two serious limitations to using feather pulp for electrophoretic analysis. First, the birds must either be molting when sampled or be subjected to plucking to stimulate feather regrowth and then recaptured at a later date. Second, in small birds such as Bobolinks, warblers (Parulidae), or chickadees (*Parus* spp.), only tiny amounts of tissue can be obtained from the flight and tail feathers, necessitating the use of two or more feathers. Mengden and Stock (1976) point out, however, that small quantities of tissue may be put into cell culture to increase the amount available; such tissue cultures may also be stored for indefinite periods of time. Alternatively, other forms of electrophoresis that require smaller quantities of tissue (e.g. cellulose acetate) may be performed when only a few loci need to be examined.

Especially useful to field workers, the tissue is conveniently "pre-packaged," and may be placed, whole or with the upper shaft removed, directly onto dry ice or into a portable liquid nitrogen flask. In terms of ease of sample collection and minimization of stress to birds, we suggest that feather pulp is a viable alternative tissue for non-destructive sampling in avian electrophoretic studies.

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Food-niche Relationships Between Great Horned Owls and Common Barn-Owls in Eastern Washington

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Great Horned Owls (*Bubo virginianus*) and Common Barn-Owls (*Tyto alba*) have recently become sympatric in the Pacific Northwest (Stewart 1980, Smith and Knight 1981) and provide an opportunity for examining resource partitioning between two members of the same feeding guild. We quantified diet relationships from our analysis of 622 Common Barn-Owl pellets, from 6 nesting and 2 roosting sites, and 234 Great Horned Owl pellets, from 4 nesting and 3 roosting sites, collected between October 1977-June 1979 in Esquatzel Coulee, Franklin County, Washington. The study area and its raptor populations are