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AMINO ACID COMPOSITION OF THE CALAMUS, RACHIS, AND BARBS OF WHITE-CROWNED SPARROW FEATHERS[1]

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The amino acid composition of the plumage of White-crowned Sparrows (*Zonotrichia leucophrys gambelii*) in general resembles that of plumage, feathers, or feather parts of the handful of other species for which data are available (Schroeder and Kay 1955, Harrap and Woods 1967, Fisher et al. 1981, Nitsan et al. 1981), but includes substantially more cystine (Murphy and King 1982). Cystine concentration (μmoles/g dry mass) in White-crowned Sparrow plumage averaged 894, compared with averages of 756 in feather parts of domestic chickens, geese, ducks, and turkeys, 753 in the Silver Gull (*Larus novaehollandiae*), and 480 in the Emu (*Dromaius novaehollandiae*). The samples, however, consisted of the homogenized entire plumage in the case of the sparrow, but of feather parts (calamus, rachis, barbs) in the case of the other species. To provide a more reliable basis for the comparison of the amino acid composition of feather parts between White-crowned Sparrows and other species we undertook the analysis reported herein.

MATERIALS AND METHODS

We washed (Harrap and Woods 1967) separate samples of primary remiges obtained from each of five *Z. l. gambelii* that had recently completed the postnuptial molt. We cut the calamus from the rachis at the superior umbilicus and trimmed the vanes (barbs) from the rachis. We were not able to remove the medulla from the calamus and rachis, although other investigators using larger feathers have sometimes done so (e.g., Schroeder and Kay 1955, Harrap and Woods 1964).

We measured the nitrogen content of duplicate desiccator-dried samples of pooled feather parts by the micro-Kjeldahl method (Horwitz 1980). To prepare subsamples for amino acid analysis we hydrolyzed ca. 10-mg portions of each feather part from each bird in 6 *N* HCl for 24 hr, vacuum-dried the hydrolysates, redissolved them in sodium citrate buffer (pH 2.2), and analyzed the solutions (Beckman model 121 MB) in the Bioanalytical Laboratory, Washington State University. The concentration of cyst(e)ine was measured in parallel as cysteic acid after oxidation with performic acid (Schram et al. 1954), and is reported in this paper as cystine/2.

Finally, we weighed samples of calami, rachises, and vanes (barbs) from White-crowned Sparrow remiges and rectrices (ca. 100 mg, 10 to 12 feathers each), and dorsal and ventral contour feathers (ca. 20 to 30 mg, 20 to 30 feathers each). Combined with estimates of the proportions of remiges, rectrices, and body feathers in the total plumage (Murphy and King 1984), these data enabled us to estimate the amino acid composition of the entire plumage from that of feather parts and to assess the differentiation of feather parts with respect to amino acid composition.

TABLE 1. Percentages of calamus, rachis, and barbs in feather classes and weighted percentages for the entire plumage of White-crowned Sparrows.

Class of feathers	% of plumage[a]	% of feather in		
		Calamus	Rachis	Barbs
Body	77	2.5	17.8	79.7
Rectrices	8	11.3	43.4	45.3
Remiges	15	18.3	50.9	30.8
Weighted % parts[b]:		5.57	24.82	69.61
Proportionate parts:		1.00	4.46	12.50

[a] Murphy and King (1984).
[b] For each part (calamus, rachis, barbs), weighted % part = $\sum$[% part × (% plumage/100)].

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TABLE 2. Amino acid composition of White-crowned Sparrow feather parts and whole plumage.

Amino acid	Parts, μmoles/g dry mass[a]			Plumage, mole %		Estim./ actual
	Calamus	Rachis	Barbs	Actual	Estim.	
Alanine	541 ± 4.0	517 ± 11.7	437 ± 7.5	5.85	6.22	1.06
Arginine	332 ± 3.9	322 ± 9.8	265 ± 18.5	4.23	3.79	0.90
Aspartic acid	594 ± 10.1	546 ± 12.1	503 ± 4.7	6.89	6.97	1.01
Cystine/2	613 ± 13.6	697 ± 29.2	1,164 ± 34.7	12.95	13.68	1.06
Glutamic acid	660 ± 8.0	626 ± 18.1	633 ± 8.6	9.31	8.51	0.91
Glycine	1,868 ± 99.7	1,465 ± 39.7	955 ± 16.0	12.83	15.21	1.19
Histidine	46 ± 2.5	30 ± 1.4	67 ± 2.6	0.94	0.77	—
Isoleucine	356 ± 9.5	369 ± 16.2	374 ± 6.8	5.11	5.00	0.98
Leucine	709 ± 15.0	699 ± 21.0	547 ± 6.9	7.50	7.98	1.06
Lysine	90 ± 5.7	50 ± 3.2	109 ± 3.7	2.17	1.25	—
Methionine	75 ± 5.1	74 ± 6.1	37 ± 1.5	0.74	0.65	—
Phenylalanine	304 ± 4.4	292 ± 7.6	162 ± 2.9	2.62	2.71	1.03
Proline	781 ± 9.5	841 ± 19.0	972 ± 17.6	13.06	12.48	0.96
Serine	326 ± 14.3	472 ± 34.8	495 ± 14.2	—[c]	—	—
Threonine	325 ± 5.0	380 ± 17.8	300 ± 5.9	5.23	4.31	0.82
Tyrosine	300 ± 3.6	293 ± 7.3	151 ± 2.7	2.29	2.62	1.14
Tryptophan	nd[b]	nd[b]	nd[b]	nd[b]	—	—
Valine	423 ± 14.4	451 ± 13.4	643 ± 30.5	8.27	7.83	0.95
NH_3 released[d]	1,307 ± 90.6	1,245 ± 39.7	1,584 ± 57.5	—	—	—
% Accounted for:						
Nitrogen	95	94	97	93	96	1.03
Dry mass	86	84	83	82	83	0.99

[a] For calamus, rachis, and barbs, mean ± SE, $n = 5$; for actual plumage, $n = 6$ (for SE, see Murphy and King 1982). Values uncorrected for destruction during hydrolysis.
[b] Not determined.
[c] Omitted because of differences in destruction during hydrolysis between this investigation and an earlier one (see text).
[d] Under conditions specified in the text.

RESULTS AND DISCUSSION

The mean nitrogen contents of calamus, rachis, and barbs were 16.0, 15.5, and 15.1% of dry mass, respectively. When apportioned to the entire plumage by appropriate weighting factors (Table 1) these averages combine to yield 15.25%, compared with an average of 15.22% actually measured in the homogenized plumage (Murphy and King 1982). This concordance cannot be construed as a verification of the weighting factors, since the barbs dominate these factors, and the nitrogen content of the barbs is close to that of the homogenized plumage. The use, for example, of 75% instead of 69.61% (Table 1) as the weighted proportion of barbs in the plumage would reduce the estimated nitrogen content by only 0.03%.

The amino acid analyses accounted for 94 to 97% of the Kjeldahl nitrogen and 83 to 86% of the dry mass of feather parts. These are equivalent to weighted averages of 96% and 83%, respectively (Table 2), and may be compared with 93% and 82%, respectively, measured previously in the homogenized entire plumage (Table 2). A greater NH_3 recovery in the analysis of feather parts compared with whole plumage explains 2.9% of the 3% difference in nitrogen accounted for (Table 2). By current standards these are very good recoveries (Williams 1981, p. 145). We have, however, omitted serine from further discussion because of its sensitivity to destruction during hydrolysis (Ambler 1981) and because of the large difference between the serine concentrations found in feather parts in this investigation and in homogenized plumage analyzed previously (Table 2). To explore the basis of this discrepancy we hydrolyzed duplicate samples of rachis in 6 *N* HCl for 12, 16, and 24 hr. The loss of serine was apparently linear between 12 and 24 hr (ca. 16 μmoles/g per hr) and, assuming linearity between zero and 12 hr, extrapolated to 890 μmoles/g dry mass at zero time. This minimum estimate resembles the 920 μmoles/g found in whole plumage of White-crowned Sparrows (Murphy and King 1982) and conforms approximately with the relative concentrations of serine in feather parts of other birds (Harrap and Woods 1967). We cannot explain the large loss of serine in the present analyses compared with earlier analyses (Murphy and King 1982) made under ostensibly identical conditions.

As in chickens and turkeys (Harrap and Woods 1967), the amino acid profiles of the calamus, rachis, and barbs in White-crowned Sparrows are approximately congruent; i.e., with a few exceptions, the parts have similar ratios of amino acid concentrations. In White-crowned Sparrows the amino acid profile of the calamus resembles that of the rachis more closely than either resembles that of the barbs (Table 2), as might be expected from the similar structure and function of calamus and rachis. The coefficient of determination (r^2) computed by one-way analysis of variance for 16 amino acids (Table 2, serine omitted) is 0.97 for calamus vs. rachis and 0.70 for calamus vs. barbs. This mode of comparison (which, incidentally, we prefer to

TABLE 3. Amino acid profiles of feather barbs.[a]

Amino acid	Mole % concentration				
	Chicken[b]	Turkey[b]	Goose[c]	Emu[b]	Sparrow
Alanine	5.97	6.38	6.37	5.98	5.97
Arginine	5.14	5.24	6.28	5.05	3.62
Aspartic acid	6.94	6.93	7.16	4.00	6.87
Cystine/2	10.27	10.18	11.61	8.49	15.90
Glutamic acid	9.18	8.69	7.68	7.77	8.65
Glycine	12.97	13.60	16.21	13.43	13.05
Histidine	0.23	0.35	0.35	0.18	0.92
Isoleucine	5.12	5.35	4.47	4.92	5.11
Leucine	8.09	7.79	7.49	10.99	7.47
Lysine	0.95	1.18	1.12	1.13	1.49
Methionine	0.20	0.34	0.21	tr[d]	0.51
Phenylalanine	3.96	4.22	3.03	3.76	2.21
Proline	14.13	12.84	11.42	14.28	13.28
Threonine	5.85	5.53	5.39	5.89	4.10
Tyrosine	1.58	1.80	3.02	4.00	2.06
Valine	9.43	9.54	8.17	10.12	8.79

[a] Excluding serine and tryptophan.
[b] Data from Harrap and Woods (1967).
[c] Data from Schroeder and Kay (1955).
[d] Trace.

the "difference index" of Metzger et al. [1968]), expresses the degree but not the kind of difference. Percentage difference of amino acid concentration between feather parts calculated as [100(part − calamus)/calamus] reveals some substantial deviations. Cystine/2 (+14%) and threonine (+17%) are more concentrated in rachis than in calamus, and glycine (−22%) is less concentrated (Table 2). All other differences are 8% or less, and hence within the range of individual variation (Murphy and King 1982) and (or) analytical error (Williams 1981). Barbs differ from calamus most conspicuously by their greater cystine/2 content (+89%), but also by large differences in valine (+52%), proline (+24%), glycine (−49%), tyrosine (−48%), phenylalanine (−47%), leucine (−23%), arginine (−20%), alanine (−19%), and aspartic acid (−15%). Cystine/2 and proline are of special interest because of their known roles in the structural or physical properties of keratins (Fraser et al. 1972). We excluded histidine, lysine, and methionine from the foregoing percentage comparisons because their low concentrations in keratin make measurements sensitive to contamination by free amino acids, and hence susceptible to a strong magnification of error when their concentrations are expressed as percentages or ratios. In spite of the moderate differences in amino acid composition between calamus and rachis and the larger differences between calamus/rachis and barbs, all three parts have practically the same total essential amino acid content: 33, 35, and 34 mole % in calamus, rachis, and barbs, respectively (100 mole % includes all amino acids in Table 2 except tryptophan and serine; essential amino acids = arg, his, ile, leu, lys, met, phe, thr, and val).

Amino acid concentrations in the whole plumage can be estimated with fair accuracy ($r^2 = 0.96$, actual vs. estimated values) for White-crowned Sparrows from measurements of amino acid concentrations in feather parts (Table 2) and estimates of the proportions of these parts in the plumage (Table 1). For reasons already stated, serine was omitted from the concentrations, and histidine, lysine, and methionine were omitted from the comparisons (right-hand column in Table 2). Amino acid concentrations were expressed as mole % to account for the omission of serine and NH_3 and the small difference in actual and estimated nitrogen recoveries. Eight amino acid pairs differed by 6% or less of the actual value, whereas five others deviated by 9% or more: glycine, +19%; threonine, −18%; tyrosine, +14%; arginine, −10%; and glutamic acid, −9%. We suspect that the deviations of threonine and glycine may be explained by destruction or conversion during hydrolysis. It is possible that glycine was formed as a by-product of serine destruction, which was greater in the current analysis of feather parts than in our earlier analysis of whole plumage (see above). Destruction of threonine during hydrolysis tends to parallel serine destruction, although at a much lower rate, and may account for the underestimate of threonine in whole plumage. We have no hypothesis about the overestimate of tyrosine in whole plumage.

Comparisons of amino acid composition of feather parts between White-crowned Sparrows and other species (the latter summarized by Harrap and Woods 1967; see also Schroeder and Kay 1955) are complicated by the fact that most other investigators removed the medulla from the calamus and rachis but we did not. This would be unimportant if the compositions of medulla and enclosing structures were alike, but this is apparently not always so. For instance, amino acid concentrations (excluding histidine, lysine, methionine, and serine) in the calamus and its medulla in chickens differed by only 9% or less in all but alanine, which was 17% more concentrated in calamus (Schroeder and Kay 1955). In contrast, comparisons of rachis and medulla from this same report yielded larger differences: alanine, +25%; leucine, +11%; arginine,

−11%; tyrosine, −29%. Before computing these percentages we adjusted the amino acid concentrations to a common basis of nitrogen recovery (calamus or rachis vs. medulla). To the extent that these results can be generalized it is evident that it is not appropriate to compare the compositions of demedullated and intact feather parts. From the perspective of nutritional or physiological questions about molt we strongly recommend that future investigators of plumage composition refrain from excising the medulla.

It is not possible to demedullate barbs and their appendages, even in very large feathers. Interspecific comparisons of barb composition are therefore appropriate. Comparative data (Table 3) are available for only an odd assemblage of species: chickens, turkeys, and the Emu (Harrap and Woods 1967), domestic goose (Schroeder and Kay 1955), and the White-crowned Sparrow (this report). The amino acid profiles of chicken and turkey barbs are nearly alike ($r^2 = 0.99$). Only proline and tyrosine (−9.1% and +13.9%, respectively, in the turkey) differ by more than about 6% between the two species. White-crowned Sparrows differ more extensively than this from chickens, turkeys, and geese (identical $r^2 = 0.87$ for each pair) and from Emus ($r^2 = 0.73$). Compared with chicken barbs, White-crowned Sparrow barbs contain much more cystine/2 (+54.8%) and more tyrosine (+30.4%), but less phenylalanine (−44.2%), threonine (−29.9%), and arginine (−29.6%). All other differences are less than 8%. The Emu differs substantially from the other species. Except between chickens and turkeys, there are no obvious pairs or sets of amino acids that consistently vary together. Barbs contain similar proportions of essential amino acids (34 to 42%) in all five species. We assume that these interspecific differences, as well as differences among feather parts, result from variable assemblages of the several heterogeneous monomers that constitute avian keratins (Brush 1978, Busch and Brush 1979). It remains to be shown whether these variants reflect selection for particular structural properties (as is probably true among feather parts), or whether they are selectively neutral epiphenomena (as may be true among species).

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