

# Disappearance of short chain acids from the preen gland wax of male mallard ducks during eclipse

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**Abstract** Wax esters constitute the major products of the uropygial glands of male mallard ducks (*Anas platyrhynchos*). Combined gas-liquid chromatography and mass spectrometry of the acyl and alcohol portions of the wax ester revealed dramatic seasonal variation. Of the total of nearly 100 compounds detected in the acyl portion, more than 90 were identified as *n*-, monomethyl-, dimethyl-, and trimethyl- C<sub>6</sub> to C<sub>20</sub> acids. The major components were 2-methyl- and 4-methyl C<sub>6</sub> acids which constituted nearly 75% of the total acids during most of the year. As the birds went through the post-nuptial molt into eclipse, these short chain acids decreased to only 4% of the total acids. Fatty acids longer than C<sub>12</sub> became the major components during the summer months when the birds were in eclipse and by fall the characteristic shorter branched acids reappeared as the dominant components. Fatty alcohols did not undergo such dramatic changes. This is the first report of biochemical changes that occur during eclipse. — Kolattukudy, P. E., S. Bohnet, and L. Rogers. Disappearance of short chain acids from the preen gland wax of male mallard ducks during eclipse. *J. Lipid Res.* 1985. 26: 989-994.

**Supplementary key words** methyl-branched fatty acids • post-nuptial molt • fatty alcohols • uropygial gland • sebaceous gland • mass spectrometry • eclipse

Specialized tissues such as the mammary glands of nonruminant animals and the uropygial glands of certain birds contain S-acyl fatty acid synthase thioester hydrolyase which catalyzes the release of short chain acids from fatty acid synthase (1-3). During our studies of this tissue-specific enzyme in the male mallard ducks (4) it was noted that the enzyme activity was hard to detect in the uropygial glands during the summer months during which time the birds were in eclipse. Eclipse is an ecophysiological state that follows immediately after the post-nuptial molt, and during this period the male plumage is virtually indistinguishable from that of the female (5, 6). If S-acyl fatty acid synthase thioesterase of the gland does, in fact, play the suggested role in the production of short chain acids *in vivo*, the uropygial gland lipids produced during eclipse should be much longer than those produced during other times. In this report we show that the

composition of the uropygial gland lipids changes dramatically during eclipse and that longer chain fatty acids become the dominant components during this period.

## EXPERIMENTAL PROCEDURES

### Materials

Mallard ducks (*Anas platyrhynchos*) were purchased from the Ted-Mar licensed game farm, Puyallup, WA, and maintained in outdoor cages on a high-energy breeder ration. The crude lipid was obtained by gently squeezing the uropygial gland and recovering the exudate which was weighed and stored at -20°C until further workup. BF<sub>3</sub>-butanol was produced by gently bubbling BF<sub>3</sub> gas into freshly redistilled *n*-butanol until the final weight increased by 14%.

### Analysis of wax samples

The crude wax samples were subjected to thin-layer chromatography on 1 mm silica gel G with hexane-ethyl ether-formic acid 90:10:1 (v/v) as the developing solvent. Components were visualized under UV light after spraying the plates with a 0.1% ethanolic solution of dichlorofluorescein. The wax ester fraction was recovered from the silica gel by elution with diethyl ether. An aliquot of the purified wax ester sample (2-4 mg) was dissolved in 0.5 ml of toluene; 2-3 ml of 14% BF<sub>3</sub> in butanol was added, and the mixture was refluxed for 3 hr. The reaction mixture was mixed with 10 ml of water and the products were extracted with chloroform. After removing the solvent under reduced pressure, the residue was subjected to thin-layer chromatography as above. The butyl ester and fatty alcohol fractions were recovered from the silica gel by elution with ethyl ether. The alcohol fraction was oxidized to the acid by treatment with CrO<sub>3</sub> in acetic acid at room temperature for 24 hr. The acids recovered from the reaction mixture were treated with BF<sub>3</sub> in butanol and the butyl esters were purified as above.

## Gas-liquid chromatography-mass spectrometry

A Hewlett-Packard model 5840A gas chromatograph attached to an HP 5985 mass spectrometer was used to obtain the electron impact mass spectra. A glass capillary column (25 m × 0.2 mm, OV-1) was used with a 2.5-min isothermal period at 80°C followed by an 80–280°C temperature program at 10°C/min and the spectra were recorded with 70 eV ionizing voltage.

## RESULTS

Thin-layer chromatographic examination of the uropygial gland lipids extruded by gentle squeezing of the gland showed that wax esters constituted the major components. When very large amounts of the lipids were applied to the plates, a minor component which had a  $R_f$  identical to that of tripalmitin was detected; but detailed chemical examination of this material was not conducted. The wax fraction was purified by thin-layer chromatography prior to chemical examination of the acyl and alcohol portions so that the fatty acids and alcohols derived from minor components would not interfere with the results. Since the wax ester region showed two thin-layer chromatographic bands, the two were isolated separately and subjected to transesterification with  $\text{BF}_3$  in butanol. Both fractions yielded fatty alcohols and butyl esters. Gas-liquid chromatographic analysis of the butyl esters obtained from the two wax ester fractions showed that the acyl portions of the slower-moving esters were shorter than those in the faster-moving fraction. On the other hand the fatty alcohols of both wax ester fractions showed nearly identical compositions (data not shown). These results showed that the two wax components differed in the chain length composition of the fatty acids. The relative amounts of the two classes of esters showed dramatic seasonal variation but clear resolution of the two components was not always possible (data not shown). Therefore, for structural studies and proper quantitative comparisons, we resorted to gas-liquid chromatographic separation of the components obtained from the total wax ester fraction which included both types of wax esters.

### Composition of the fatty acyl portion of the wax ester

To minimize possible loss of the short chain acids, transesterification of the wax ester fraction was done with  $\text{BF}_3$  in butanol and the butyl esters were subjected to combined gas-liquid chromatography and mass spectrometry. The results showed that the wax esters were composed of a very complex mixture of up to 100 fatty acids. As indicated above, a short chain group and a long chain group were observed (Fig. 1). Mass spectra of each component were used for identification and the major diagnostic ions used are shown in Table 1. Similar ions

were previously used for identification of methyl-branched acids found in the complex mixtures obtained from the uropygial glands of many species of birds (7). The composition of the fatty acids obtained from the wax esters isolated from the uropygial glands of male mallards at various times during the year is shown in Table 2. Although about 90 different components were identified, only those that constituted 1% or higher of the total fatty acids at some period during the year are included in Table 2. Even after such a simplification, 62 fatty acids had to be included as shown in Fig. 1 and Table 2. Most of these fatty acids had methyl branches at even-numbered carbon atoms. Even though monomethyl branched components with branches at either 2- or 4-position dominated, dimethyl fatty acids were also significant components, and even trimethyl acids were detected. A quantitative analysis of the chain length distribution of the acyl portion of the wax is depicted in Fig. 2 in which  $\text{C}_{12}$  and shorter acids are arbitrarily designated short acids.

The fatty alcohol portion of the wax ester consisted of aliphatic chains much longer than those found in the acyl portion. The major component was *n*-octadecanol which, together with monomethyl  $\text{C}_{18}$ , constituted about 70% of the total alcohols (Table 3). *n*-Fatty alcohols ( $\text{C}_{16}$  to  $\text{C}_{19}$ ) constituted nearly two-thirds of the alcohols, while monomethyl  $\text{C}_{16}$  to  $\text{C}_{19}$  alcohols were also significant components. The fatty alcohol composition did not show significant changes throughout the year, except for an increased level of hexadecanol in May and an increase of 6-methyloctadecanol in the summer.

## DISCUSSION

The novel finding of the present study was that the fatty acid composition of the wax esters changed dramatically during the summer months when the ducks were in eclipse and the composition reverted back when the eclipse was over. In non-eclipse periods, 2-methyl- $\text{C}_6$  and 4-methyl- $\text{C}_6$  were by far the most dominant components and accounted for nearly 75% of the total fatty acids. The proportion of these two components decreased with the approach of eclipse, and by May they constituted only about 10% of the fatty acids (Table 2). During June and July, at which time the birds were at the peak of eclipse, the two monomethyl branched short acids accounted for only 4% of the fatty acids. By the end of August, the birds were coming out of eclipse and the dominance of the monomethyl branched short chain acids began to reappear. As the short chain components decreased, the complexity of the general composition increased as shown by the presence of numerous components, each accounting for only a few percent of the total. Dimethyl branched acids, which accounted for as little as 7% of the fatty acids

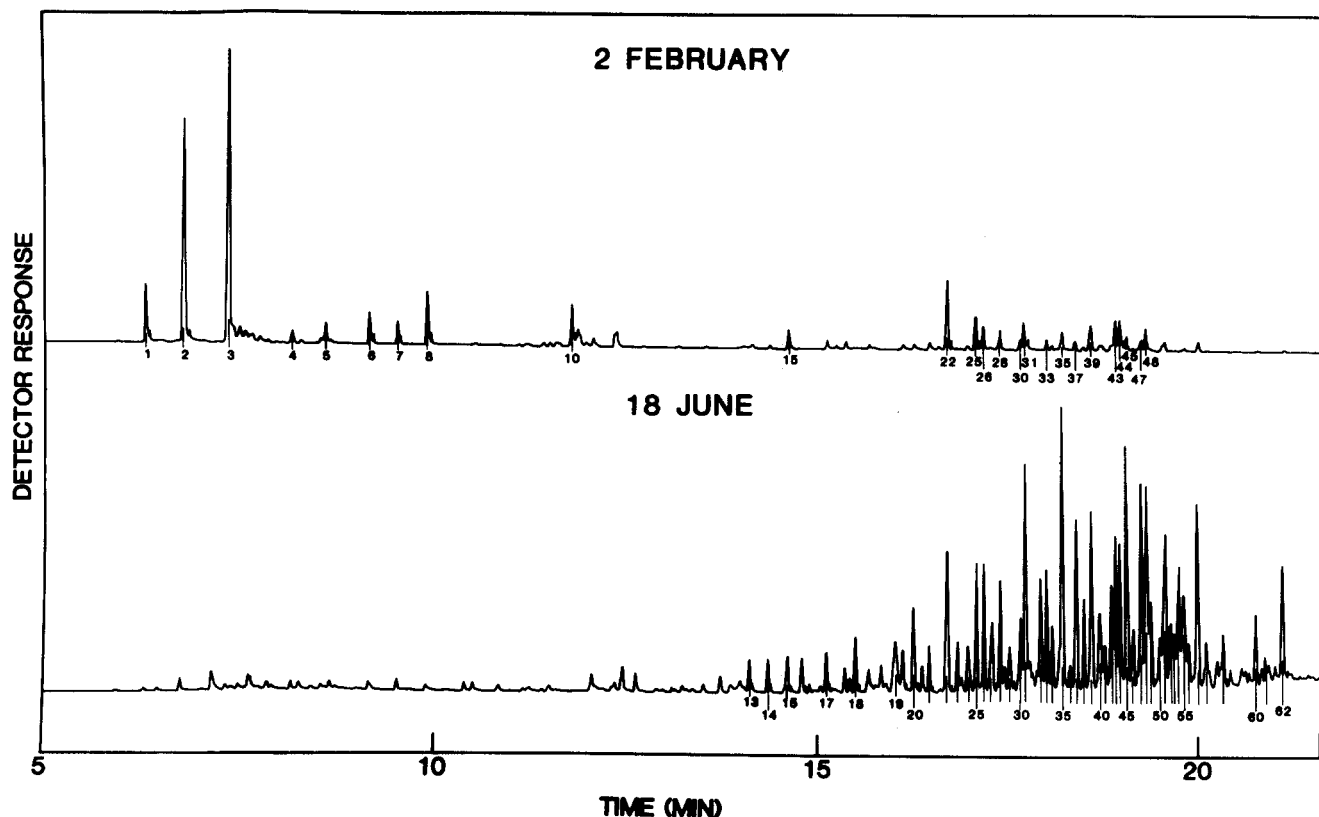


Fig. 1. Capillary gas-liquid chromatogram of the butyl esters prepared from the acyl portions of wax esters from the uropygial gland secretion of male mallards collected on the indicated dates. The chromatographic conditions are described in the text. The identity of each numbered component is indicated in Table 2.

in non-eclipse periods, constituted as much as 75% during eclipse.

In a previous analysis of the uropygial gland wax of ducks, the longer acids found in the present study were not detected because of the low temperature used in the gas-liquid chromatography and the presence of multiple methyl branches was not detected (8). However, a more recent study revealed the presence of substantial amounts of dimethyl branched acids and some trimethyl branched acids (9). Direct comparison of the previously reported composition with that presented here cannot be done without taking into consideration the fact that the composition shows dramatic seasonal variations. The present results strongly suggest that attempts to use the preen gland lipid composition for taxonomic purposes should seriously take into consideration the physiological state of the bird.

The most dramatic change that occurred during eclipse was that the content of short chain acids decreased. Acids shorter than  $C_{12}$ , which constituted more than 70% during non-eclipse periods, accounted for less than 1% of the fatty acids when the birds were at the apex of the eclipse (Fig. 2). We have previously shown that fatty acid synthase of the uropygial glands of mallards is a typical vertebrate fatty acid synthase in all of its properties,

including the chain length of the product generated (3). An S-acyl fatty acid synthase thioester hydrolase present in the gland was suggested to be responsible for the production of the short chain acids. A dramatic decrease in the amount of this activity was observed during eclipse (unpublished results). The changes in composition described in the present report can be explained as being

TABLE 1. The major diagnostic ions in the mass spectra of butyl esters of methyl-branched acids used for identification

Position and Kind of Substitution	Diagnostic Ion
2-Methyl	130, M-55, M-73 <sup>a</sup>
2,6-Dimethyl	130; M-132; 199, 171
2,8-Dimethyl	130; 227, 199
2,10-Dimethyl	130; 255, 227
2,6,8-Trimethyl	130; M-132; 241, 213
2,6,10-Trimethyl	130; M-132; 269, 241
2,4,8-Trimethyl	130, large 143; 241, 213
4-Methyl	129 > 116; 157; M-57
4,6-Dimethyl	129 > 116; M-118, 199; M-57
4,8-Dimethyl	129 > 116; 227, 199; M-57
4,10-Dimethyl	129 > 116; 255, 227; M-57
4,12-Dimethyl	129 > 116; 283, 255; M-57
6-Methyl	129 > 116; M-118; 157, 185

<sup>a</sup>M-55 and M-73 were found in all spectra.

TABLE 2. Composition of the acyl portion of the uropygial gland wax collected from male mallard ducks at different times during the year (% of total acid)<sup>a</sup>

Fatty Acid	2 Feb	17 Mar	2 Apr	17 Apr	4 May	21 May	2 June	18 June	8 July	20 July	4 Aug	17 Aug	8 Sept	28 Sept	12 Oct	3 Nov
1. n-C <sub>6</sub>	4.1	5.7	1.2	1.2	0.14	—	—	—	—	—	—	—	0.94	—	2.2	3.1
2. 2-Methyl-C <sub>6</sub>	20.0	30.0	17.0	18.0	5.7	4.1	1.6	—	1.7	4.4	7.3	14.0	13.0	10.0	16.0	15.0
3. 4-Methyl-C <sub>6</sub>	33.0	44.0	23.0	23.0	7.7	4.7	1.4	—	1.9	5.2	8.7	18.0	21.0	20.0	28.0	21.0
4. 2-Methyl-C <sub>7</sub>	—	—	1.2	1.2	0.52	0.39	0.37	—	0.59	0.83	1.0	1.5	1.6	1.0	1.5	0.90
5. 4-Methyl-C <sub>7</sub>	1.2	—	1.4	1.3	0.53	—	0.20	—	0.23	0.64	0.90	1.4	1.7	1.6	2.3	1.4
6. n-C <sub>8</sub>	1.4	—	1.1	0.75	0.21	—	—	—	—	—	—	—	0.73	—	1.9	3.2
7. 2-Methyl-C <sub>8</sub>	4.5	—	1.7	1.8	0.97	0.73	0.34	—	0.55	1.6	1.6	2.7	3.4	3.0	3.3	3.0
8. 4-Methyl-C <sub>8</sub>	4.0	5.1	3.2	3.5	1.3	0.39	0.36	—	0.56	2.4	3.2	6.4	7.3	8.4	8.0	7.9
9. 4-Methyl-C <sub>9</sub>	—	—	—	—	0.15	—	—	—	—	2.3	1.1	2.4	6.5	3.8	4.3	4.9
10. 2,6-Dimethyl-C <sub>9</sub>	1.2	3.8	0.72	1.1	0.80	—	0.14	—	0.48	0.34	0.29	—	0.53	—	0.40	0.18
11. 2-Methyl-C <sub>10</sub>	—	—	0.50	0.75	0.30	—	0.22	—	0.48	2.1	1.8	2.2	3.5	3.8	2.4	2.2
12. 4-Methyl-C <sub>10</sub>	—	—	0.65	0.67	0.34	—	0.34	—	0.23	1.7	2.0	2.7	4.7	4.7	3.4	3.8
13. 2,6-Dimethyl-C <sub>11</sub>	—	—	—	—	0.07	—	1.7	—	0.32	0.43	0.42	—	0.91	1.8	1.2	1.1
14. 2-Methyl-C <sub>12</sub>	—	—	0.34	0.68	0.10	0.56	0.46	—	1.1	2.6	2.0	3.0	—	—	1.4	1.3
15. 4-Methyl-C <sub>12</sub>	1.1	—	1.3	1.7	0.26	0.96	0.75	—	0.99	2.6	2.4	3.8	2.5	3.2	2.4	2.8
16. 2,6-Dimethyl-C <sub>12</sub>	—	—	—	—	0.74	0.98	0.34	—	0.87	0.67	0.53	—	3.5	5.3	—	—
17. 4,8-Dimethyl-C <sub>12</sub>	—	—	0.71	0.88	0.74	0.88	0.69	—	0.97	1.1	0.79	—	—	—	0.80	1.1
18. 2-Methyl-C <sub>13</sub>	—	—	—	—	0.08	—	0.23	—	0.55	0.83	0.65	—	0.63	1.2	0.40	0.29
19. 2,6,10-Trimethyl-C <sub>12</sub>	—	—	—	—	0.51	0.55	0.73	0.79	1.4	0.95	1.1	—	—	—	0.30	—
20. 4,12-Dimethyl-C <sub>13</sub>	—	—	—	—	0.22	—	0.22	1.5	—	—	—	—	—	—	0.32	—
21. 2-Methyl-C <sub>14</sub>	—	—	0.73	1.2	0.51	1.0	0.90	0.64	2.1	3.2	2.5	2.9	2.6	2.0	0.93	1.2
22. 4-Methyl-C <sub>14</sub>	5.7	8.1	5.3	6.3	3.2	4.2	3.2	2.7	2.5	3.0	2.6	3.9	3.4	5.5	3.0	5.2
23. 2,6-Dimethyl-C <sub>14</sub>	—	—	0.31	0.59	0.44	0.71	0.95	0.74	1.6	1.8	1.2	—	1.9	0.87	0.26	1.1
24. 2,8-Dimethyl-C <sub>14</sub>	—	—	0.56	—	0.44	0.64	0.72	0.87	1.1	1.2	0.88	—	0.86	—	0.82	0.51
25. 4,6-Dimethyl-C <sub>14</sub>	2.0	—	3.0	3.4	3.0	3.2	3.2	2.6	2.7	2.7	2.4	2.8	1.6	2.6	1.7	2.5
26. 4,8-Dimethyl-C <sub>14</sub>	1.5	—	2.0	2.4	2.0	2.6	3.4	2.1	3.1	2.6	2.0	1.8	1.1	1.1	0.98	1.2
27. 2,6,10-Trimethyl-C <sub>14</sub>	—	—	0.38	—	0.67	0.98	0.91	2.1	1.7	1.3	1.0	—	—	0.76	0.38	0.31
28. 4-Methyl-C <sub>15</sub>	1.1	—	1.8	1.5	2.3	2.2	2.6	2.4	1.7	1.1	1.2	—	—	—	—	0.56
29. 2,6,8-Trimethyl-C <sub>14</sub>	—	—	—	—	0.64	0.98	1.1	1.2	1.4	1.0	1.1	—	—	—	—	—
30. 2,4,8-Trimethyl-C <sub>15</sub>	—	—	0.67	0.41	—	1.0	1.2	0.82	1.2	0.95	0.30	—	—	—	—	—
31. 4,6-Dimethyl-C <sub>14</sub>	2.3	—	2.7	3.5	7.5	5.4	7.0	5.2	5.0	3.4	4.3	2.3	6.7	1.4	1.3	—
32. 2,8-Dimethyl-C <sub>15</sub>	—	—	0.54	—	0.21	—	0.39	1.9	0.82	0.84	—	—	—	1.2	—	—
33. 4,8-Dimethyl-C <sub>15</sub>	1.4	—	0.99	0.88	1.9	2.2	2.6	2.9	2.0	1.7	1.8	—	—	—	0.51	1.6
34. 4,12-Dimethyl-C <sub>15</sub>	—	—	0.46	0.48	0.89	0.97	1.2	1.2	1.1	0.80	0.98	—	1.4	—	0.20	0.73
35. n-C <sub>16</sub>	4.4	—	0.84	1.3	1.1	1.8	1.3	7.4	1.2	0.93	1.2	2.1	—	1.9	0.86	—
36. 2,6,8-Trimethyl-C <sub>15</sub>	—	—	—	—	0.29	—	0.47	—	0.36	0.20	—	2.7	1.1	—	—	2.1
37. 2-Methyl-C <sub>16</sub>	—	—	1.4	1.6	2.2	3.8	3.5	3.7	4.4	3.7	3.1	—	—	—	0.32	0.48
38. 4,6-Dimethyl-C <sub>15</sub>	—	—	—	—	1.7	1.7	2.2	1.2	1.6	1.1	1.4	—	—	—	—	—
39. 4,10-Dimethyl-C <sub>15</sub>	2.0	—	0.44	2.0	3.6	4.7	3.7	4.1	2.7	2.2	2.1	2.2	0.80	—	0.86	1.2
40. 2,6-Dimethyl-C <sub>16</sub>	—	—	2.8	1.3	1.5	2.3	2.0	1.8	2.1	2.2	1.7	—	—	—	0.36	0.89
41. 2,8-Dimethyl-C <sub>16</sub>	—	—	1.6	0.50	0.56	0.85	0.74	0.74	0.86	0.89	0.75	—	—	—	—	—
42. 2,10-Dimethyl-C <sub>16</sub>	—	—	0.86	—	1.2	2.1	1.7	2.6	2.3	1.9	1.9	—	—	—	—	—
43. 4,6-Dimethyl-C <sub>16</sub>	1.8	3.2	3.0	3.5	3.6	4.3	4.0	3.7	3.2	3.2	3.2	2.6	1.2	1.6	1.2	1.6
44. 4,8-Dimethyl-C <sub>16</sub>	2.6	—	3.4	3.8	2.9	3.6	2.8	3.2	3.1	2.6	2.8	2.6	0.78	1.2	0.99	1.0
45. 4,10-Dimethyl-C <sub>16</sub>	0.72	—	1.9	1.6	4.1	6.1	5.1	6.4	6.3	4.3	4.6	8.2	—	—	—	0.32
46. 2,6,10-Trimethyl-C <sub>16</sub>	—	—	0.38	—	0.80	1.1	1.1	1.3	1.2	0.89	0.89	—	—	3.3	0.31	0.38
47. 4,12-Dimethyl-C <sub>16</sub>	—	—	1.7	1.0	4.0	5.1	4.8	4.2	4.3	2.3	2.9	1.7	—	—	—	—
48. 4,6-Dimethyl-C <sub>17</sub>	1.4	—	3.0	2.9	5.8	7.3	5.9	5.1	5.8	3.2	4.1	2.5	—	0.70	0.58	0.78
49. 2,6,8-Dimethyl-C <sub>16</sub>	—	—	—	—	0.97	0.63	1.5	1.9	1.9	1.6	1.5	—	—	0.72	—	—
50. 4,8-Dimethyl-C <sub>17</sub>	—	—	1.8	1.8	4.3	4.6	5.4	0.62	0.41	3.3	3.6	3.2	—	—	0.45	0.75
51. 2,6-Dimethyl-C <sub>18</sub>	—	—	0.24	—	1.1	1.2	1.4	4.7	5.1	1.1	1.2	—	—	—	—	—
52. 2,8-Dimethyl-C <sub>18</sub>	—	—	0.34	—	0.25	—	0.32	2.4	1.6	0.23	—	—	—	—	—	—
53. 4,10-Dimethyl-C <sub>17</sub>	—	—	—	—	0.37	—	0.44	—	0.28	—	—	—	—	—	—	—
54. 4,12-Dimethyl-C <sub>17</sub>	—	—	0.33	—	0.89	0.97	1.1	3.3	—	0.80	0.73	—	—	—	—	0.26
55. 4,X-Dimethyl-C <sub>17</sub>	—	—	0.96	—	0.53	0.47	0.56	4.3	1.1	0.32	0.42	—	—	—	—	—
56. n-C <sub>18</sub>	1.3	—	—	—	—	—	0.52	—	0.45	—	0.84	—	—	1.3	0.54	—
57. 4,Y-Dimethyl-C <sub>17</sub>	—	—	—	—	1.6	1.6	1.6	3.4	0.83	0.75	—	—	—	—	—	—
58. 4,6-Dimethyl-C <sub>18</sub>	—	—	—	—	1.2	0.42	1.4	—	0.55	0.35	—	—	—	2.8	—	—
59. 4,Z-Dimethyl-C <sub>17</sub>	—	—	—	—	1.6	1.5	0.95	0.57	1.0	0.19	—	—	—	—	—	—
60. 4,8-Dimethyl-C <sub>18</sub>	—	—	—	—	1.3	1.3	1.1	1.1	1.1	0.71	0.85	—	—	0.80	0.17	—
61. 2,8-Dimethyl-C <sub>19</sub>	—	—	—	—	0.16	—	0.18	—	—	0.29	—	—	—	1.2	—	—
62. 4,6-Dimethyl-C <sub>19</sub>	—	—	—	—	0.48	—	0.07	2.2	—	—	—	—	1.8	—	—	—

<sup>a</sup>The following components were identified but were not more than 1% of the total acids at any time: n-C<sub>7</sub>, 2-methyl-C<sub>9</sub>, n-C<sub>10</sub>, 2,6-dimethyl-C<sub>10</sub>, 2-methyl-C<sub>11</sub>, 4-methyl-C<sub>11</sub>, 4,10-dimethyl-C<sub>12</sub>, 4-methyl-C<sub>13</sub>, 2,6-dimethyl-C<sub>13</sub>, 2,8-dimethyl-C<sub>13</sub>, 4,8-dimethyl-C<sub>13</sub>, 2,6,10-trimethyl-C<sub>13</sub>, n-C<sub>14</sub>, 2-methyl-C<sub>15</sub>, 2,4,10-trimethyl-C<sub>15</sub>, 4,X-dimethyl-C<sub>18</sub>, 4,X-dimethyl-C<sub>19</sub>, 4,X-dimethyl-C<sub>20</sub>, and 2,8-dimethyl-C<sub>18</sub>.

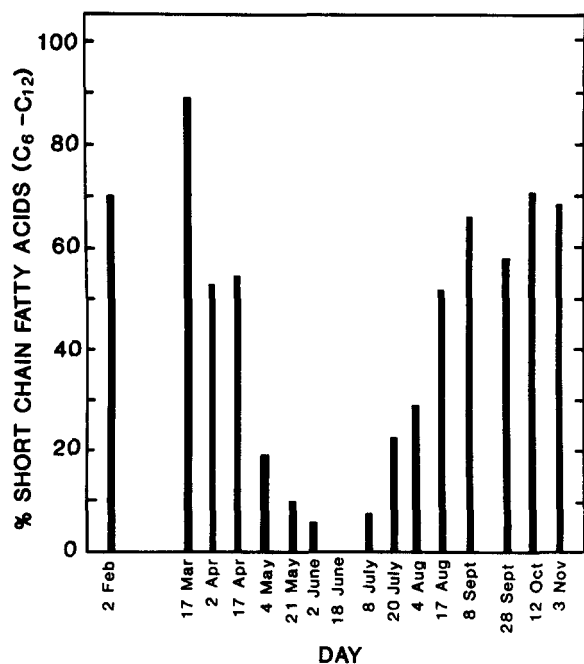


Fig. 2. The short-chain fatty acid content of the wax esters found in the male mallard uropygial glands at the periods indicated.

due to the decrease in the S-acyl fatty acid synthase thioesterase and thus demonstrate the *in vivo* function of this enzyme.

What controls the branching pattern is not known. If the amount of methylmalonyl-CoA available and the level of malonyl-CoA decarboxylase do not change during eclipse, the probability of introducing additional methyl

branches would increase as the chain length of the product increases. Thus, the increased level of dimethyl branched acids found during eclipse could also be a consequence of a decreased level of the S-acyl fatty acid synthase thioesterase and the resulting increase in the chain length.

The fatty alcohol portion of the wax ester does not reflect the changes in chain length observed in the acyl portion during eclipse. In fact, even the mere structure and composition of the alcohol portion are in sharp contrast to those of the acyl portion. Such a contrast is not uncommon in other avian species that produce wax esters as the major product of preen gland metabolism (7). For example, in the wax esters of goose uropygial gland, the major acyl group is 2,4,6,8-tetramethyl decanoic acid whereas the alcohol portion is  $n$ -C<sub>18</sub>. The acids used as the substrate for the acyl-CoA reductase (10, 11) that produces the alcohols must have a different biosynthetic origin than that of the acyl portion of the wax. Since the enzymological studies suggest that the acyl portions are probably generated by the unique enzyme mixtures present in the gland (10), it is possible that the acids used for alcohol synthesis are at least in part imported. Although this possibility is supported by the data on structure of the waxes, no direct evidence is available to support this hypothesis.

Although many studies have shown that thyroid and steroid hormones are involved in the control of molting (12-14), very little is known about the effects of such hormones on the biochemical and molecular biological changes that occur in the uropygial glands. Steroid hormones are known to affect the uropygial glands (15-17)

TABLE 3. The composition of the primary alcohols of the wax esters from the uropygial gland of male mallard ducks

Alcohol	Percent of Total Alcohols			
	April 2	May 21	June 2	August 4
Hexadecanol	3.4	16.5	1.1	3.2
Heptadecanol	7.9	2.9	1.8	3.4
Octadecanol	51.8	50.8	47.9	52.9
Nonadecanol	5.5	7.2	1.0	—
Eicosanol	—	—	3.4	1.9
2-Methyl hexadecanol	0.7	1.0	0.9	1.8
4-Methyl hexadecanol	—	—	0.9	1.9
2-Methyl heptadecanol	1.9	1.8	—	—
4-Methyl heptadecanol	—	—	2.1	1.9
6-Methyl heptadecanol	—	—	0.9	1.4
2-Methyl octadecanol	—	—	0.5	0.6
4-Methyl octadecanol	2.5	3.4	6.5	5.3
6-Methyl octadecanol	2.8	5.6	18.5	15.2
4-Methyl nonadecanol	6.4	2.3	0.6	2.4
6-Methyl nonadecanol	3.3	1.9	0.4	0.4
Unidentified alcohols, C <sub>17</sub> and C <sub>18</sub> range	2.5	1.9	2.5	2.8
Unidentified alcohols, C <sub>19</sub> and C <sub>20</sub> range	11.2	4.5	11.0	4.7



and injection of steroid hormones has been shown to affect uropygial secretion of ducks (18, 19). However, short-term hormonal treatment of ducks failed to show significant compositional changes in the gland's secretion (9). In spite of all of the studies on seasonal variations in the level of the two classes of hormones, little is known about how they affect the biochemistry of the uropygial glands. A comparison of the results of the chemical studies reported in the present paper with the hormonal changes reported previously (14) reveals that the maximal plasma level of thyroid hormones coincides with the minimum testosterone level and the minimum short-chain acids level of the gland (13, 20). Therefore, it is tempting to speculate that thyroid and steroid hormones play a role in suppressing the expression of the gene responsible for synthesis of S-acyl fatty acid synthase thioester hydrolase and thus suppress the production of short-chain acids. If so, the duck uropygial gland may constitute a convenient model for studying the hormonal regulation of gene expression in sebaceous glands. ■

We thank Sally Combelic for maintaining the birds. Scientific Paper No. 7063, Project 2001, College of Agriculture Research Center, Washington State University, Pullman, WA 99164. This work was supported in part by U.S. Public Health Service Grant GM-18278.

Manuscript received 19 February 1985.

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