



## **In Defense of Chemical Defense: Quantification of Volatile Chemicals in Feathers is Challenging**

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**In defense of chemical defense: Quantification of volatile chemicals in feathers is challenging.**—As part of recent reviews, Hagelin (2007) and Hagelin and Jones (2007) evaluated evidence that the citrus-like odorant of the Crested Auklet (*Aethia cristatella*) functions as a chemical defense against ectoparasites, as proposed by Douglas et al. (2001, 2004, 2005). The authors incorrectly applied extrapolations in these reviews that underestimated the concentrations of the Crested Auklet's odorant in nature. This resulted in a misleading characterization of previously published bioassays (Douglas et al. 2004, 2005) and, as a result, evidence for chemical defense was largely dismissed. The reviewers based their extrapolations on just six samples, each consisting of 10 Crested Auklet feathers, and this was but a small percentage of the samples that they reportedly collected at Buldir Island, Alaska, in 2001. More recently, it has been shown that these estimates fall two to three orders of magnitude below the average concentrations that can be attained on head and neck plumage of Crested Auklets during the breeding season (Douglas 2006, 2008). It has also been shown that the Crested Auklet odorant can function as a chemical defense against ectoparasites and that previously published bioassays were relevant to this question (Douglas 2006, 2008).

Hagelin and Jones (2007) may have had problems with sample degradation, and this may have led their analysis astray. In support of their arguments, Hagelin and Jones (2007) cited in their data (from Hagelin et al. 2003) an odor profile that appears to have lost constituents of the Crested Auklet's species-specific chemical signature and acquired chemical compounds attributable to oxidation. Six aliphatic aldehydes occur in the Crested Auklet odorant during the breeding season: *n*-hexanal, *n*-octanal, *n*-decanal, (*Z*)-4-decenal, (*Z*)-4-dodecenal, and (*Z*)-6-dodecenal (Douglas et al. 2001, 2004; Douglas 2006, 2008). These compounds have been consistently detected in both feathers and secretory tissues at more than one colony and in more than one year (Douglas et al. 2001, 2004; Douglas 2006, 2008). Quantifying two of these compounds, Hagelin et al. (2003) found that median values for octanal and (*Z*)-4-decenal were 2.98 and 1.2  $\mu\text{g g}^{-1}$ , whereas Douglas (2006, 2008) measured these compounds in head and neck feathers at mean values of 590 and 344  $\mu\text{g g}^{-1}$ , respectively, and other odorant constituents at similar levels; for example, hexanal was 528  $\mu\text{g g}^{-1}$ . Hagelin and Jones did not report hexanal or three other odorant constituents and, in place of these, they published a list of compounds in trace amounts (table 1 in Hagelin et al. 2003) that could have arisen from oxidation of lipids. Some readers could perhaps better understand that these same oxidation processes can occur with foods in a refrigerator, where they can give rise to these same compounds (e.g., controls in table 3, Chung-Wang et al. 1997).

I believe that the methods employed by Hagelin et al. (2003) did not ensure accurate quantitative or qualitative measurements of the Crested Auklet odorant as it occurs in nature, and I suggested this to Hagelin in September 2002. My concern, then and now, is that feather samples stored in vials, in air, at temperatures well above 0°C, would be subject to oxidation. Aliphatic aldehydes like those that constitute the Crested Auklet's odorant are inherently unstable and readily oxidize in the presence of air

(Mann 1994, Loudon 2002). The large surface area of bird feathers provides ample reaction sites for oxidation of feather lipids. As a result, constituents of the Crested Auklet odorant could diminish or disappear, and other chemical compounds could arise. Some differences in results can be expected because of differences in methods (Raguso and Pellmyr 1998) or natural variability; however, the common and widespread problem of lipid oxidation is a more parsimonious explanation in this case. Until Hagelin and Jones produce estimates of sample degradation for their storage methods, they cannot make meaningful quantitative extrapolations from their data to other studies. Furthermore, to evaluate hypotheses regarding odorant function, it is important to have an accurate qualitative characterization of its composition as it occurs in nature. The point here is not to disparage previous work that went forward when less was known but to update and clarify the literature and point out a potential pitfall. Scientists researching avian odorants must be cognizant of problems with lipid oxidation, and they can increase confidence in their results by giving attention to methods development and validation.—HECTOR D. DOUGLAS III, *Department of Biology, Kuskokwim Campus—CRCD, University of Alaska Fairbanks, Bethel, Alaska 99559. E-mail: hddouglas@yahoo.com*

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