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LETTER TO THE EDITOR

Comments on Recent Work by Zhang and Colleagues: ''Uropygial Gland-Secreted Alkanols Contribute to Olfactory Sex Signals in Budgerigars''

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In a study recently published in *Chemical Senses*, Zhang and coworkers explored the chemical content of the uropygial secretions from budgerigars (*Melopsittacus undulatus*) with a particular focus on their role as precursors of olfactory sex signals (Zhang et al. 2010). The authors also behaviorally tested the response of female budgerigars to either living birds, body odors, or glandular secretions from both sexes, as well as to a mixture of components from these secretions. The work presented, carried out in 2007–2008 and already partially published in a non peer-reviewed journal (Zhang et al. 2008), contains valuable results for the study of avian chemical communication. However, we disagree with the authors' claim that their study ''robustly demonstrates that a blend of three long-chain alkanols synergistically acts as a male pheromone in budgerigars'' as several results of their chemical and behavioral investigation are weakened by major analytical shortcomings. As related shortcomings also affected a previous study by the same authors, on a different avian species (Zhang et al. 2009), we explain here our methodological concerns so they can be openly discussed for the benefit of the field.

First, some of the authors' decisions regarding the processing of their chromatographic data are questionable. For example, the apparently arbitrary selection of the 23 compounds retained for quantitative analyses among the complex chemical profiles of budgerigars is unexplained. In addition, the authors assume, probably based on evidence from mammals, that avian social information is coded through the relative abundance of compounds. This assumption, however, does not require the conversion of absolute abundances to percentages as applied in the study; an approach particularly flawed by the restriction of the analysis to a subset of a priori chosen compounds. Instead, the use of the whole chromatogram area to calculate percentages or, even better, the standardization of quantitative data using a unique internal standard would be more appropriate. The latter approach in particular would have prevented the major conceptual flaw of this study discussed below.

The amounts of the 3 alkanols involved in the ''male pheromonal blend'' (octadecanol-18OH, nonadecanol-19OH, and eicosanol-20OH), in 1 mg of uropygial secretion, are found to be, respectively, 3.58 ± 3.06, 2.78 ± 2.67, and 5.32 ± 3.10 μg in males (note the huge interindividual variation) but are not indicated for females. This is unfortunate because this information would clearly show that females' secretions have a similar (if not higher) content of these alkanols than males (as indicated by the GC areas from Table 1 and unlike what is suggested by the chromatograms of Figure 3). Nevertheless, once converted into relative abundances (in percent, using the total area of the 23 subjectively selected compounds), the contribution of the alkanols becomes around 4 times more important in males than females; a result that the authors used as a basis for subsequent behavioral bioassays. The authors, however, overlooked 2 important aspects of their data: 1) the wide and overlapping spread of the alkanols' absolute abundances in males and females and 2) the fact that the higher relative contribution of alkanols in males exclusively results from the presence of additional highly abundant compounds, that is, pentanoates, in the secretions of

females (a fact briefly mentioned in the legend of Figure 3). Therefore, we think the chromatographic data of Zhang and coworkers could equally suggest, if not more, the presence of a female signal in the uropygial secretions of budgerigars.

The authors have propagated their misinterpretation further by converting the 4-fold ratio in the relative abundance of alkanols into a 4-fold ratio of absolute abundances for their bioassays. Namely, they mimicked a ''male'' odor by preparing a blend of the 3 alkanols 4 times more concentrated than the one supposed to mimic a ''female'' odor, despite the fact that the actual absolute quantities of these compounds are similar in both sexes (if not higher in females). Consequently, outcomes from the bioassays presented are ambiguous as, in a majority of cases, they could result simply from a preference of the birds for the strongest stimulus (the most concentrated blend which, as argued above, does not correspond to the reality of chemical sex differences).

Other behavioral results reported by Zhang and coworkers show that female budgerigars explored the body and ''uropygial'' odors of males more than their female counterparts. This indicates that budgerigars have olfactory capabilities of sexual discrimination, a novel and important finding. The protocol presented by the authors cannot resolve, however, whether the choice made by the females originate from the attraction of females to some male sex pheromones, from the avoidance of female-associated odors, or from simple habituation. As indicated in the methods, each sex-mixed group was indeed separated into male or female subgroups for 12 h prior to trial, meaning that tested females were habituated to only female odors for 12 h before being presented with a choice including a novel male odor in the maze. More generally, the design of this study suffers from the bias of the authors toward a male sexual signal (possibly originating from their mammalian research background). Indeed, only females were tested in behavioral assays despite the ambiguity of chemical results. In contrast, studies on birds' chemosignals have to date only reported female-biased chemical signals in birds (Jacob et al. 1979; Balthazart and Taziaux 2009; Hirao et al. 2009; Mardon et al. 2010). Looking at the response of males to females' chemical emissions could therefore be a useful addition to the work presented.

Finally, some methodological aspects of this study, although not as critical as the analytical ones already discussed, appear suboptimal. For instance, the starting temperature of 70 °C of the gas chromatograph program is not ideal for studying the volatile fraction of samples. The ad hoc statistical comparison between the 2 sexes, of the abundances of compounds a priori selected for being sexually dimorphic, is also conceptually problematic.

Overall, the work of Zhang and coworkers discussed above support the current realization of avian chemical communication and extend the range of species investigated. The comments developed in this letter do not aim at refuting these intriguing findings but advocate, instead, for higher methodological and analytical standards in the investigation of avian chemical communication. We look forward to future discussions on these questions with Zhang and colleagues and others working in the field.

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