

Review report on revision by Gilles et al. - No sex difference in preen oil chemical composition during incubation in Kentish plovers

I am happy with the careful and extensive way all my comments have been addressed by the authors.

However, I still have a few remaining comments that I hope the authors can address.

I notice that the authors stress that there should be a function of preen oil during incubation. They use two final sentences in the abstract on this, but these are just speculations and do not follow from the results from their study. Could it not be possible that the preen oil has no special function during incubation? I would argue that based on the findings in my studies in shorebirds, that it is not so likely that there is a special function of preen oil in Kentish Plovers, given that if they show seasonal changes in preen oil composition, they must be rather subtle and do not involve large changes in volatility. I have liked to see that possibility discussed in the manuscript too.

If these last points can be addressed, I would recommend accepting this manuscript.

Minor comments:

L 65 “(more volatile)” ... “(less volatile)”. This is also mentioned in L 68-69 so can probably be deleted here?

L72 “three passerine species”. I wondered whether it would be useful to mention whether both sexes incubate in these species?

L74-75 Why would sex discrimination by use of smell have evolved? One may assume that birds know the (opposite) sex of their partner, and I assume that song, territorial behaviour and sexual dimorph plumage or other traits differ between sexes. I have never really understood what the additional function of smell, in discriminating sexes, would be.

L199-200 “Number of days after laying”. You never explained how you determined laying date. I presume by egg flotation? Please mention this in your methods too.

L275 “found only monoesters in the preen oil of incubating Kentish Plovers”. This is of course correct, but more important to mention is that I found no seasonal change from mono- to diesters in Kentish Plovers (and the two other species).

L280-289 I agree that a simple classification into mono- or diesters comes with limitations, but I visually compared the chromatograms and they are (more or less) identical. I would argue that, to play a role in volatility (olfactory crypsis), drastic changes in retention time (which is an indicator of volatility) should be visible.

L287-289 “ This warrants ...throughout the year”. This would only identify the substances, but would not lead to insights into the function of the preen oil mixture?

L291-292 Everything is possible, but given your results I do not see why you expect a function in olfactory crypsis in Kentish Plovers?

L298-302 Why do you still hypothesize this, if I have shown in my thesis that such seasonal changes do not take place? I realise that my simple visual inspection is limited, and that subtle seasonal changes in composition could easily have been missed, but I doubt that such subtle seasonal changes would make a large difference in volatility and detectability of birds.

L298 “measure its volatility”. Maybe you can explain how you would measure that?

L341-342 Exactly, there may not be a incubation-related function at all, right? This contradicts your previous paragraphs somewhat?

L345-347. You may perhaps consider to add Reneerkens et al. 2008 here? We found anti-bacterial effects of preen oil, but -importantly- no differences between mono- and diesters. How likely is it then that you would find such a difference in Kentish plovers?

L356-358 “..may have a function..” Or no incubation-related function at all??

Thank you for your interesting work and for making me think of my old studies on seasonal changes in preen oil composition. I am happy to see that this work has been followed up!

Jeroen Reneerkens