# Peer Community In Zoology

# Seabird tick diversification and cuticular hydrocarbons

## Felix Sperling based on peer reviews by 2 anonymous reviewers

Marlène Dupraz, Chloe Leroy, Thorkell Lindberg Thórarinsson, Patrizia d'Ettorre, Karen D. McCoy (2022) Within and among population differences in cuticular hydrocarbons in the seabird tick Ixodes uriae. bioRxiv, ver. 5, peer-reviewed and recommended by Peer Community in Zoology. https://doi.org/10.1101/2022.01.21.477272

Submitted: 08 February 2022, Recommended: 26 August 2022

Cite this recommendation as:

Sperling, F. (2022) Seabird tick diversification and cuticular hydrocarbons. *Peer Community in Zoology*, 100014. https://doi.org/10.24072/pci.zool.100014

Published: 26 August 2022

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Ticks are notorious vectors of diseases in humans and other vertebrates. Much effort has been expended to understand tick diversity and ecology with the aim of managing their populations to alleviate the misery they bring. Further, the fundamental question of whether ticks are usually host generalists or host specialists has been debated at length and is important both for understanding the mechanisms of their diversification as well as for focusing control of ticks [1].

One elegant resolution of this question is to consider most tick species to be global generalists but local specialists [1]. This is well illustrated in a series of studies of the seabird tick, *lxodes uriae*, which is comprised of host-specific races that show genetic [2], morphological [3] and host performance [4] differences associated with the seabirds they feed on. Such a pattern has clear ramifications for sympatric speciation; however, the factors that potentially act to drive these differences have remained elusive.

Dupraz et al. [5] have now made intriguing and important steps toward bridging the gap between demonstrating local patterns of tick host association and understanding the physiological mechanisms that may facilitate such divergences. They collected *I. uriae* ticks from the nests of two seabirds – Atlantic puffins and common guillemots – on the north side of Iceland. Four populations of ticks were sampled, with one island providing both puffin ticks and guillemot ticks, to give two tick populations from each of the two seabird host species. They then washed the ticks in solvent and analyzed the dissolved cuticular hydrocarbons (CHCs) using GC mass spectrometry, revealing 22 different hydrocarbon compounds common to most of these samples. CHCs are known to be important across arthropods for a variety of functions ranging from reducing water loss to facilitating communication and recognition between individuals with species.

Dupraz et al. [5] found three hydrocarbons that distinguished puffin ticks most consistently from guillemot ticks. A cross-validation test for host type also assigned 75% of the tick pools to the seabird host of origin.

However, with these limited sample sizes, statistical analysis revealed no significant difference in CHC profiles between the host types, although a tendency was evident. Nonetheless, this study revealed a number of potentially diagnostic CHCs for tick host type, as well as some that may be more diagnostic of locations. This provides a fascinating and actionable foundation for further work using additional sites and host types, as well as an entry point into discerning the mechanisms at play in producing the diversity, complexity and adaptability that make ticks such medical menaces.

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# Reviews

### **Evaluation round #1**

DOI or URL of the preprint: https://doi.org/10.1101/2022.01.21.477272

#### Authors' reply, 30 June 2022

#### Download author's reply

#### Decision by Felix Sperling, posted 27 March 2022

Dear Marlène Dupraz and coauthors,

Two reviews of your manuscript have now been received, and I agree with both of them that this is an interesting and generally well written study. However, they raise some key issues and edits that should be addressed before publication can be considered. In particular, Reviewer 1 recommends the addition of further information on the methods as well as clarification of several items and explicit consideration of factors such as mating status and microclimate. Reviewer 2 suggests that the authors tie in hydrocarbon synthesis pathways,

and include more explicit discussion of how environmental factors may alter the tick hydrocarbon profile. All points raised by the reviewers should be addressed, whether in a revised version of the ms or in rebuttal.

In addition, from my own review of the ms: 1) although this manuscript does a good job of considering the role CHCs may play in reproduction, the discussion seems a bit biased toward the hypothesized involvement of these hydrocarbons in host race formation. Other functions should be considered more fully. For example, Yoder and Domingus (2003) demonstrated that long chain hydrocarbons secreted by Dermacentor variabilis ticks act as a defence against ant predation. Also, 2) this statement needs to be clarified: "For example, aging favors the production of longer hydrocarbon chains and decreased attractiveness in Drosophila melanogaster (Kuo et al., 2012)." It is not fully clear what kind of attractiveness is intended here.

Consequently, this paper still requires revision before it could be recommended. However when the methods and other items listed in the reviews have been clarified, and the discussion has been expanded to include the factors outlined above, this paper should be a valuable addition to the literature on the role of tick cuticular hydrocarbons.

- Felix Sperling

#### Reviewed by anonymous reviewer 2, 16 March 2022

The manuscript by Dupraz et al. outlines and interesting study comparing CHC profiles from among different host and geopgraphic populations of Ixodes uriae ticks. Generally speaking this is a well written study, has been conducted using established techniques and has interesting results. That being said, I have outlined a number of key issues and edits in the manuscript which should be addressed before consideration for publication. Some of the more important of these include:

1. There are key pieces of information in the methods which have been left out of the text and need to be added. Its also unclear why the authors have not quantified their materials using a standard curve or internal standard, and are instead relying on ratios of abnundance?

2. I am concerned about the evaporation and reconstitution method used on the samples. This method will introduce a significant amount of variation in the recovery of solutes in re-adding the solvent. The lack of an internal standard in the samples unfortunately makes it impossible to know what lost in this process.

3. Several of the figure descriptions use acronyms and abbreviations which have not been explained.

4. The amount of variation in the data is discussed, but Figure 2 does not provide a good look at the degree of variation present. I suggest that this figure should be reconfigured as a boxplot, including the individual data points, as well as outliers. I am concerned that the limited replicates used for these samples may be masking other trends that may be present.

5. In the discussion, there are a number of 'suggestions' made by the authors regarding pheromone-based function of these CHC's largely based upon insect literature which has documented such function in other species. In particular, the point is made that the data suggests that these CHCs are important for reproductive function, as these were collected from ticks during the reproductive season. However, the authors collected wild ticks, with no knowledge of mating status. Therefore its difficult to make any strong assumption regarding the behavioral role of these CHCs.

6. While the data support population based differences in CHC profiles, and the authors discuss the possible impact of different habitat and microclimatic differences, theres very little description regarding the discrete differences in environmental variables between these sites (which at least appear to be very similar in geographic distribution).

This manuscript requires revisions addressing the points above and those outlined in the manuscript file before being considered for publication.

#### Download the review

#### Reviewed by anonymous reviewer 1, 18 March 2022

This manuscript is a valuable addition to the literature on tick cuticular hydrocarbons. The experiments attempt to determine the effects of host species and geography on the cuticular hydrocarbons of *Ixodes uriae*. There is great potential in this tick-seabird system to learn about factors that enhance population divergence. The discussion would benefit from a brief account of what is known about hydrocarbon synthesis in insects (e.g. fig. 1 in Howard & Blomquist, 2005). Avian erythrocyte membrane lipids might be quickly mobilized by the tick for conversion to hydrocarbons, and the very long chain hydrocarbons are probably synthesized from pre-existing shorter-chain fatty acids (the *I. scapularis* genome has genes coding for proteins with acyl chain elongase-like sequences). Thus, the host erythrocyte lipid composition might provide a direct pathway for the host to influence the hydrocarbon composition of the tick cuticle. Future studies could benefit from collecting ticks and simultaneously obtaining blood samples of birds likely to have been hosts. It would be interesting to look for correlations between bird erythrocyte membrane lipid acyl chain composition and tick cuticular hydrocarbon composition. The future analysis could also be expanded to include polar cuticular lipids, such as fatty acids and steroids, which have been reported as pheromones in metastriate ticks (I Chem Ecol 11, 1669-1694, 1985; Parasitology 129 Suppl, S405-425, 2004). The manuscript does not present a clear argument for how environmental acquisition would alter the tick cuticular hydrocarbon profile. I do not have expertise in the data analysis methods used by the authors, and I suggest that they post the raw GC-MS areas on the Zenodo site. There is a minor typo on line 365: the authors' names in the reference are given twice.