Peer Community In Ecology

Breaking barriers in butterfly tracking: how drone technology and image analysis could boost movement ecology of butterflies

Nicolas Schtickzelle *based on peer reviews by 3 anonymous reviewers*

Emmanuel de Margerie, Kyra Monmasson (2024) Tracking butterfly flight in the field from an unmanned aerial vehicle (UAV): a methodological proof of principle. bioRxiv, ver. 5, peer-reviewed and recommended by Peer Community in Ecology.
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Understanding how animals move within and across landscapes is fundamental to behavioural ecology, conservation biology, and movement ecology. Tracking movement provides insights into migration and dispersal patterns, habitat preferences, intra- and interspecific interactions, etc. For long, movement recording was limited to indirect methods, such as Capture-Mark-Recapture. Despite being at the basis of an incredible amount of knowledge and developments in ecology, these methods do not inform on the movement path itself, just its beginning and end. Tracking individuals during their movement was really needed.

Over the years, researchers have developed a range of tracking methodologies, with technological innovations continually improving precision and efficiency (Trappes, 2023). While tracking large terrestrial and marine animals and birds is now well-established using GPS telemetry and biologging, monitoring small flying insects remains a significant challenge due to their size, erratic flight patterns, and sensitivity to environmental disturbances. It is especially the case for butterflies due to their lightweight bodies and relative low flight power. Given the role butterflies play as model organisms in diverse areas of ecology, research to allow tracking their movement path is of prime interest.

I remember the many hours I spent, in a time (early 2000s) GPS technology was still quite imprecise, following butterflies for a distance, placing sticks at turning points and reconstructing afterwards the movement path by triangulating the distances of sticks to know location marks (Schtickzelle et al., 2007). It was quite effective

but prohibitive in terms of resources. Later came GPS devices precise enough for an individual to run in the footsteps of a butterfly to record its path. Still, methods have been highly desirable that could track butterflies with some level of automation and from a distance. Experiments were performed with harmonic radar (attaching a passive transponder that reflects radar signals; Cant et al., 2005) but were never largely adopted given they required acquiring and positioning costly and heavy equipment and maintaining at all time a direct line of sight with the tracked butterfly.

Here comes this pioneering study by de Margerie and Monmasson (Margerie & Monmasson, 2025) who introduce an innovative approach using a consumer-level commercial drone to track butterfly flight, offering a promising solution for long-duration, high-resolution flight trajectory analysis in natural habitats. Their study is a proof of principle that a drone, hovering in a fixed position, can be used as a flying platform to capture high-resolution vertical imagery to precisely record butterfly flight movements. Images are then analysed to reconstruct the flight path, a point on which they developed innovative approaches in the study.

The study therefore represents a significant leap forward in butterfly flight tracking methodology, with technology that many labs could acquire and operate. Further research is needed to alleviate some of the current limitations before large-scale adoption to track butterfly movements in the field is within reach: e.g. the need for a very high contrast between the butterfly and the vegetation above which it flies (here white *Pieris* butterflies over a relatively homogeneous green crop field were filmed), the limits in spatiotemporal scale due to the fixed drone position and its short battery life, and some difficulties in image processing to reconstruct movement paths, in particular when several individuals would cross paths. Considering the fast progress in both the drone technology and image analysis techniques, such progress could however come faster than we might anticipate.

References:

Cant E. T., Smith A. D., Reynolds D. R. & Osborne J. L. (2005). Tracking butterfly flight paths across the landscape with harmonic radar. Proceedings of the Royal Society of London B 272, 785–790. https://doi.org/10.1098/rspb.2004.3002

de Margerie E. & Monmasson K. (2025) Tracking butterfly flight in the field from an unmanned aerial vehicle (UAV): a methodological proof of principle. bioRxiv, ver.5 peer-reviewed and recommended by PCI Ecology https://doi.org/10.1101/2024.07.17.603869

Schtickzelle N., Joiris A., Van Dyck H., & Baguette M. (2007). Quantitative analysis of changes in movement behaviour within and outside habitat in a specialist butterfly. BMC Evolutionary Biology 7, 4. https://doi.org/10.1186/1471-2148-7-4

Trappes R. (2023). How tracking technology is transforming animal ecology: Epistemic values, interdisciplinarity, and technology-driven scientific change. Synthese 201, 128. https://doi.org/10.1007/s11229-023-04122-5

Reviews

Evaluation round #2

DOI or URL of the preprint: https://doi.org/10.1101/2024.07.17.603869
Version of the preprint: 4

Authors' reply, 24 April 2025

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Decision by Nicolas Schtickzelle, posted 08 April 2025, validated 08 April 2025

Thanks for your revised manuscript. Despite changes made were not so profound, I found the ms clearly improved in terms of readability and ecological background related to movement, dispersal and migration.

I have a few minor suggestions before I can recommend the ms:

· The 5 min limitation recording justified by the UAV software might be perceived as a stronger limitation than what it is in reality. Indeed, if the video files could be put end-to end without frames being dropped in-between, analysis would be nearly as easy as if a single video file was produced. I suggest indicating that the key point is not the potential splitting of the video file but the ability to avoid some non-recorded period in-between consecutive videos, so readers can consider this when choosing an UAV to record longer movements.

· Track straightness is also known in part of the movement literature as Net To Gross Displacement Ratio (NGDR); maybe worth mentioning it.

· Fig 4: at first read, it was unclear what the small vertical bars are on the bottom track. I had to zoom quite a lot to see these are + signs indicating individual positions. I'd advise to make that explicit. Also, I do not really see the benefit of duplicating the track; I'd keep only the bottom depiction, showing both the general track, individual positions (hence relative speed) and the comparison with the position that could be recorded using SHR.

· Uncertainty about flight altitude: is the video resolution small enough to use the butterfly (bob) size as a measure of flight altitude, assuming individual real size is not too variable? I guess not because blob size is even not precise enough to discriminate the two species. But the reader might wonder themselves about that before reading the section on blob size for species discrimination, so maybe a sentence on that in the flight altitude section is beneficial. Also discussing this in relation to the benefits of using a higher camera resolution could be interesting, especially for cases where flight altitude would not be useful only to correct for recorded distance but also as a response variable of biological interest.

· Fig 9, caption: it would be clearer to remind there that the Raleigh test null hypothesis is a uniform distribution, i.e. that a red vector indicates that there is a significant directional preference. Also specify exactly what arrow length represents.

· Discussion about "repeat the measurement of the reference scale on every video frame": what about using reference marks that can be automatically thresholded on every frame (e.g. use a specific colour channel), allowing for an automatized measure of UAV drift?

That being said, I still believe that the ms would be clearer and more focussed with less details on the biological interpretation (and the many associated tests) of the Pieris example. I fear that trying to reach two goals (methodological advance in tracking using UAV and biological interpretation of Pieris tracks) may decrease the impact of the methodological advancements. Some results are of secondary importance to me and contribute to make a longer ms with many figures. This might also be a constraint if you want to try and publish this ms into a journal. To be clear, I let you to decide how you want to deal with that; I'll recommend the ms whatever the choice you make.

Evaluation round #1

DOI or URL of the preprint: https://doi.org/10.1101/2024.07.17.603869
Version of the preprint: 3

Authors' reply, 03 April 2025

Download author's reply
Download tracked changes file

Decision by Nicolas Schtickzelle, posted 13 March 2025, validated 13 March 2025

I have now received three reviews of your preprint. I'm sorry it took some time but as you may know, many researchers decline review requests.

As you will read from their review, the three reviewers found great methodological merit in your study as a proof of principle that drone imaging could be used as a new tool to track flying butterflies (and potentially other species). They also found that the application to Pieris butterflies shows several biological limitations. I share their views.

Given the manuscript is relatively long and complex in its current form, I would suggest to focus much more on the methodological aspects, using the case study only to demonstrate the strength and weaknesses of the method, largely reducing the biological interpretation. Indeed, the choice of tracking Pieris butterflies is very appropriate as a first test because the species is indeed easily distinguishable from the vegetation background. But at such a limited spatial scale at which they were tracked, their movement is not appropriate to give strong answers to specific questions about their movement ecology.

This should make the manuscript much more focussed, while avoiding the critics on limited/poor treatment of the movement ecology aspects.

The saved space might, at authors' discretion, be used to test or discuss in more detail alternative tracking algorithms that could be used to identify butterfly tracks from the video, in particular for the case when several butterflies would be observed on the same video and potentially cross paths, likely to happen in some applications that could be made from this methodology. In recent years, several video tracking algorithms have been published, and testing/comparing/mentioning them might be useful to the reader. Examples are:

- trackdem: https://besjournals.onlinelibrary.wiley.com/doi/full/10.1111/2041-210X.12975
- IDL: http://www.physics.emory.edu/faculty/weeks//idl/
- Traktor: https://besjournals.onlinelibrary.wiley.com/doi/full/10.1111/2041-210X.13166
- DeepLabCut for Multi-Animal Projects : https://deeplabcut.github.io/DeepLabCut/docs/maDLC_UserGuide.html
- TRex: https://elifesciences.org/articles/64000

Reviewed by anonymous reviewer 2, 03 March 2025

This study deals with a methodological/technical proof of principle of using an unmanned aerial vehicle to track butterfly movements in the field. Movement ecologists working with insects (or entomologist interested in movement behaviour of their study animals in the field) usually have less access to tracking technology and devices giving the small size and body mass of their study organisms compared to vertebrates (e.g. birds). Hence, any form of progress in this field is therefore welcomed. The current study aimed making a contribution to this challenging kind of research. The value of this manuscript is in showing a proof of principle with Pieris butterflies for tracking relatively short trajectories of on average 40 m or so. I do not expect this contribution to revolutionize immediately the field of butterfly (or broader insect) mobility research, but it is certainly an interesting first, cautious step, I would argue. I may indeed inspire other people in the field to apply it as well.

I understand why the authors tested their application with white Pieris butterflies as they provide a sharp visual contrast with the background (at least when their wings are (partially) open during cyclic wing claps). However, as these Pieris species of show a unique track of flight path during their adult life, the representative of only a 40 m section is arguably of lower significance than it would be for a butterfly that would more frequently adopt a more limited home range pattern of movement. The sample sizes presented are also very limited to derive any sound ecological significance. Since, the work is clearly presented as a methodological test case, that issue is not necessarily a point of major concern, but at several places in the manuscript, the authors should remain within this restricted methodological application range and hence not really speculate too much on the biological relevance of the test study system, I would argue. I would suggest making several adjustments in that sense. I have listed a number of specific comments that may help the authors further improve their manuscript and really focus on the methodological proof of principle and not really on the biological significance already at this early stage.

Specific comments:

1) L. 1: In line with the general issue about a more explicit focus on the proof of principle rather than immediate on the biological significance of the recorded information per se, I suggest modifying the title. Suggestion: "Tracking butterfly flight in the field with an unmanned aerial vehicle: a methodological proof of principle"

2) L. 44: Why do you mention "movement ecology" between quotes?

3) L. 47: Add a more recent reference as well since this field is moving rapidly.

4) L. 59: Not a very recent example. For a paper on methodological progress, I would expect to read about recent developments, unless nothing significant would have happened…

5) L. 121 and next: Please indicate to which extent there were larval and adult ecological resources present (host plants, nectar, etc.) as the presence/absence of these resources is relevant for the type of movements you are likely to observe. (routine, foraging vs dispersal or eventually also migration as these behavioural moods can change during the same flight trajectory in Pieris butterflies with consequences for the degree of straightness of the flight trajectory).

6) L. 405: servoing?

7) L. 461: Provide details or a reference how wing length was precisely measured.

8) L. 528: Explain why this matters (here or already in Material & Methods)

9) L. 535: Not significant at alfa-level 0.05 but close, so given the small sample size this would require some caution here.

10) L. 541: The significance of wind direction would not be independent from wind speed; was this also measured? Probably there will be threshold-effects here.

11) L. 554: Add reference(s) about insectaries.

12) L. 574: In this paragraph or elsewhere in the Discussion, I would like to see a more elaborated part on the relevance of the method relative to the type of movement.

13) L. 583: Why? Not clear to the reader.

14) L. 593-594: Would be interesting if you could estimate/indicate the potential order of magnitude of this overestimation of the flight speed.

15) L. 599 and next: The advice of the authors remains somewhat vague on the necessity to combine this UAV-method with behavioural observation fieldwork on the ground. Suggestion to elaborate this more and to give clear guidelines. Here again the type of movement will be of significance, I would argue.

16) L. 627-629: This sounds like a strong limitation on its use under natural conditions for several (the majority) of butterfly species? Moreover, there will be a unavoidable bias towards species from open habitats. It would make sense to elaborate in a critical sense on this aspect.

17) L. 649-650: That is indeed a concern, which I share.

18) L. 656 and next: I agree but comparing your novel method with tracking the butterflies by GPS on a following researcher would be a great test or comparison, at least for those cases where both techniques

would be applicable.

19) L. 680: “distracted” is not the best way to express their behaviour and this actually refers to my other point about the significance of the context of the type of movement.

20) L. 691: It would make sense to place the number of 600 somewhat in perspective, particularly for those readers who are less familiar with this taxonomic group and the studies on butterfly movement ecology.

21) L. 693: Although spectacular cases like the Monarch and the painted lady attracted much attention, they may not be representative for the average migratory butterfly species.

22) L. 698: For mass migrations you refer only to a couple of older papers, but here there is also work using radar methods (e.g. Bauer, S., Tielens, E. K., & Haest, B. (2024). Monitoring aerial insect biodiversity: a radar perspective. Philosophical Transactions of the Royal Society B, 379(1904), 20230113). It would be relevant to integrate this in the Discussion.

23) L. 726: It would be interesting to assess the time investment of your method; not only for collecting data, but also for all the work on the screen to derive movement data.

L. 737: “perhaps reveal information”: that does not sound really promising as it is phrased that way.

Reviewed by anonymous reviewer 3, 13 March 2025

The idea of using UAVs to record the movement of insects - in this case, butterflies - is interesting because this technique would help fill a knowledge gap. As the authors point out, capture-mark-recapture methods provide information on the first (departure) and third (arrival) stages of a movement, but they are not informative about what happens in between. In this respect, the data capture and analysis methods presented in this manuscript are clear, convincing, and will undoubtedly be useful.

On the other hand, the theoretical development of movements is poor, ignoring the differences between resource-searching, dispersal, and migration movements. Similarly, the spatial use strategies separating sedentary species (with home ranges or territories) from those that forage widely could be usefully detailed. The presentation of the emerging field of movement ecology is, to say the least, weak.

My biggest criticism concerns the treatment of their animal models: Peiris brassicae and P. rapae are two species with very different ecological requirements, and combining them into a single batch makes little, if any biological sense.

My opinion is that at this stage, this is more of a technical report than a scientific article. I suggest the authors explore deeper the literature on movements, and, above all, develop a more biologically sound demonstration of their interesting and useful technique by working on clearly identified species.

Reviewed by anonymous reviewer 1, 10 February 2025

This manuscript presents the use of the unmanned aerial vehicle (UAV) to record movement of butterflies. The authors have developed a pipeline to record the movements of such butterflies as Pieris brassicae and P. rapae in the field conditions, at high temporal resolution of about 30 positions per second. They have developed an appropriate image-filtering algorithm to process the recorded videos and to analyse the resulting movement tracks. The authors have carefully investigated possible biases in the recorded movement data due to some movement of UAV (non-stationarity) and different heights at which butterflies are flying. They have also explored the possibility of automatic species assignment using the videos. As a demonstration of one possible application of this method to learn more about biology of butterflies, the authors show that the flight azimuth of Pieris butterflies had changed across seasons, with majority of the butterflies heading towards north-east in June while south being the predominant direction in September. Overall, I found this paper a very interesting read with an exciting application of quickly developing devices to record movement of small invertebrates. The paper is well thought-through and clearly written. I have only a few minor remarks that are

detailed below. I would like to congratulate the authors on such a great job in applying new tools in movement ecology!

Minor comments

My only major concern is regarding the use of "migratory" in association with the flights that the authors had studied. I know the authors have used a very specific definition of migratory flights, as specified on LL304-305. More generally, though, migration is defined as "Bidirectional movements between distinct breeding and non-breeding sites, which are often long-distance in relation to body size" (Schlägel et al., 2020). When talking about migratory movements we thus mean large-scale movements. Since no inference can be made on the exact character of the observed movements based on a rather small spatial scale of the observation, I would suggest that the authors were more careful when calling them "migratory". One possibility would be to call these movements "persistent directed" flights (or similar) throughout the manuscript. And then in the discussion highlight that the recorded trajectories may in fact be migratory movements as their azimuth correspond to the expected azimuth of migrating Pieris butterflies in different seasons in this region.

L125: suggest revising to '...situated 1.7 km away'.

L179: 'automatically' instead?

L206: the use of 'translated' here is somewhat strange, perhaps better "relocated" or "moved"?

L211: 'battery' should be in plural, i.e. 'batteries'.

References

Schlägel, U. E., Grimm, V., Blaum, N., Colangeli, P., Dammhahn, M., Eccard, J. A., Hausmann, S. L., Herde, A., Hofer, H., Joshi, J., Kramer-Schadt, S., Litwin, M., Lozada-Gobilard, S. D., Müller, M. E. H., Müller, T., Nathan, R., Petermann, J. S., Pirhofer-Walzl, K., Radchuk, V., ... Jeltsch, F. (2020). Movement-mediated community assembly and coexistence. Biological Reviews, 95, 1073–1096. https://doi.org/10.1111/brv.12600

Title and abstract

Does the title clearly reflect the content of the article? [X] Yes, [ ] No (please explain), [ ] I don't know

Does the abstract present the main findings of the study? [X] Yes, [ ] No (please explain), [ ] I don't know

Introduction

Are the research questions/hypotheses/predictions clearly presented? [X] Yes, [ ] No (please explain), [ ] I don't know

Does the introduction build on relevant research in the field? [X] Yes, [ ] No (please explain), [ ] I don't know

Materials and methods

Are the methods and analyses sufficiently detailed to allow replication by other researchers? [X] Yes, [ ] No (please explain), [ ] I don't know

Are the methods and statistical analyses appropriate and well described? [X] Yes, [ ] No (please explain), [ ] I don't know

Results

In the case of negative results, is there a statistical power analysis (or an adequate Bayesian analysis or equivalence testing)? [ ] Yes, [ ] No (please explain), [ ] I don't know

Are the results described and interpreted correctly? [X] Yes, [ ] No (please explain), [ ] I don't know

Discussion

Have the authors appropriately emphasized the strengths and limitations of their study/theory/methods/argument? [X] Yes, [ ] No (please explain), [ ] I don't know

Are the conclusions adequately supported by the results (without overstating the implications of the findings)? [X] Yes, [ ] No (please explain), [ ] I don't know