We thank the 3 anonymous reviewers and the editor for their useful comments, which we addressed point by point below (our replies written in blue).

Line numbers refer to the revised preprint PDF.

We also provide another PDF with all changes tracked (unfortunately without line numbering, as MS Word is not able to display consistent line numbering with changes tracked).

Revision round #1

Decision for round #1 : *Revision needed*

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.

We agree that ~40 m is an unsatisfactory spatial scale to assess long-range movements such as migration. Still, our flight azimuth data remains a significant improvement compared to the latest published data in *Pieris* (e.g. take-off azimuth in 2 m flight cages: Spieth & Cordes 2012, Larranaga et al. 2013), so we have prefered to not remove the whole azimuth analysis from the manuscript. However, to try and shorten the migratory interpretation, we have reduced the corresponding discussion section by 12 lines (L727-757), and we have emphasised the spatial scale limitation of the study at multiple places in the manuscript (L31, 591, 753, 766).

We made two main changes to our manuscript: First, as suggested by reviewers 1 and 2, we enriched the text with consideration/discussion of movement types and ecological context (routine, foraging, dispersal etc., see multiple changes below in our replies to comments). Second, we have abandoned the idea of presenting the recorded flights as *a priori* migratory. As suggested by referee 3, we present the flights as simply « directed », and only in the discussion we suggest that these directed flights are probably segments of migratory movements, as their azimuths change over the season.

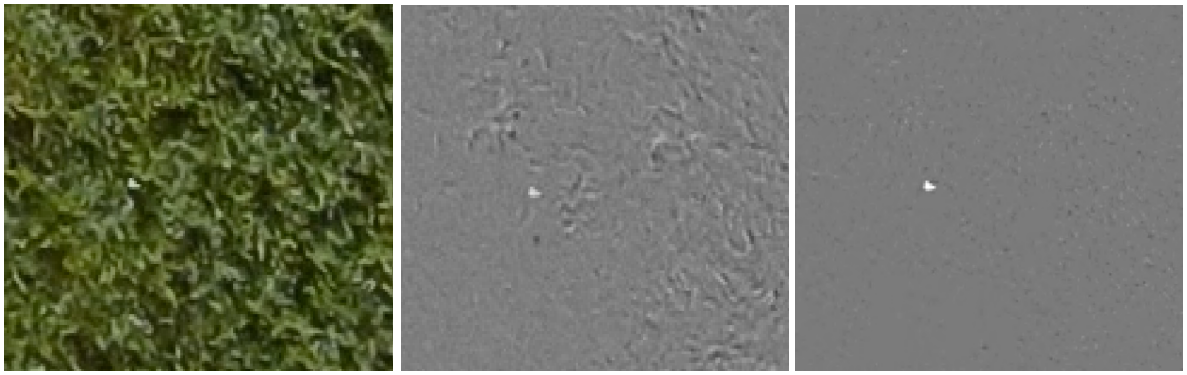
The saved space might, at authors' discretion, be used to test or discuss in more detail alternative tracking algorithms that could be used to identify butterfly tracks from the video, in particular for

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

Four tracking steps can be discussed here : blob identification / blob tracking / AI / multi-tracking.

The *blink* video filter, as presented in the manuscript (L226-277), is a simple solution to blob identification, over a textured and moving background (due to UAV drift + wind on vegetation). It is not a tracking tool *per se*, rather a pre-processing step that yields videos with a more uniform background. Many existing softwares (e.g. trackdem, IDL, TRex), initially rather adapted to lab settings and/or static backgrounds, rely on background substraction to identify blobs or « particles », which unfortunately does not work well on field UAV imagery (as stated on L232). For example, see below a comparison between original UAV frame (left), BG substraction (center) and blink filter (right).



Blob tracking through successive video frames is the next step. We chose DLTdv because it tracks blobs using 2D cross-correlation between unsegmented frames, rather than distance between blobs in binarized images (which adds thresholding parameters in the process). Also, DLTdv uses a Kalman filter to predict the location in the next frame, which has proven well adapted to track actively locomoting animals (e.g. Ruaux et al. 2023). However, we agree that we could have used other tracking softwares to derive the tracks, but benchmarking third-party tracking softwares was not our goal in this paper, and we prefered to focus on our original contribution (i.e. the preliminary blink filter step). Still, we have added 3 references to the latest alternative tracking softwares, and a mention about the benefit of starting with a blink-filter step (L285-288).

Several softwares (notably DeepLabCut) propose to use AI to help the tracking, which can be very powerful, but comes with specific hardware requirements (GCUs), which cannot always be met. DLTdv8 also proposes a tracking mode based on deep-learning. We did not have to use AI, as 2D cross-correlation gave good results (leveraging the low background noise from the blink filter), while keeping the process as simple and replicable as possible.

Multi-tracking: in our data, multiple butterflies flying in the same video happened frequently, but did not cause any problem as the Kalman filter favours a next location that is congruent with the previous location series, so that if the butterflies remain distant enough from each-other, track swapping is unlikely to occur. Path-crossing on the other hand was rare in our data (and most of the time trigerred movement interactions, which was an exclusion criterion: L322), but we agree this could be an issue in some applications of UAV tracking We anticipate that Kalman-filter predictions could significantly help reconstruct crossing (or pursuing) paths with low mis-identification, but in our experience some level of manual monitoring will certainly be necessary (regardless of the software used). Unfortunately we did not have the opportunity yet to measure if the amount of manual corrections in a frequent path-crossing scenario would remain acceptable.

Review by anonymous reviewer 1, 03 Mar 2025 09:22

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”

We have changed the title accordingly.

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

Quotes were removed (L45)

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

We added 2 references (Wilmers et al 2015, Kays et al. 2015, L49-51), which together with Joo et al 2022 (already cited L46), give a good overview of recent GPS-based tracking developments.

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…

Here (L61), Ovaskainen et al 2008 is not cited as a recent SHR example, but rather as a reference paper specifying the range and temporal resolution of SHR. More recent examples of SHR tracking were given on L58-59 (Lihoreau et al 2012, Maggiora et al 2019), and to our knowledge report no improvement in range or sampling frequency.

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).

We have added further details on L148-149. We also presented our choice on (low) ecological ressources and movement types in the introduction, with additional references (L120-123).

6) L. 405: servoing?

In robotics, « servoing » refers to a closed-loop control where inputs from sensors are used to regulate motion. We rephrased to avoid this specialised term (L417)

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

Sorry for this oversight. Details and reference were added on L473.

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

We added an introductory explanation on L540-542.

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

We had acknowledged that this p value is close to 0.05 (L549), which is the reason why we performed a supplementary verification on azimuths in stronger winds (>2 m/s, L550-555). We tried to make the logic clearer in the text, by merging both paragraphs (L550)

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.

We added a mention to the median measured wind speed on L540.

About an effect of wind speed, we approached this point with the sub-analysis focused on tracks recorded in stronger winds (fig. 10 C-D, L550-555). As explained in the manuscript (L554, 603, 708), our aim here was only to check that wind did not cause a strong bias on observed flight azimuths, and once this was confirmed, we voluntarily refrained from going deeper in wind analysis in the present manuscript. We are currently exploring a refined analysis of butterflies' air speed, compared to wind speed and direction, in order to assess whether the tracked butterflies compensated wind variations (in speed and direction) along their tracks. Threshold-effects on wind speed are indeed something to consider, thank you for pointing this out.

Unfortunately, a more in-depth « wind compensation » study would be a far too long and detailed analysis (involving 30 Hz positional data and 1 Hz wind data, compared through vector analysis, see e.g. Srygley & Oliveira 2001) to be included in the present manuscript, which was already perceived as relatively long and complex (see editor comment).

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

We added several references about insectaries and more detailled data on recorded areas and track length (L569-573)

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.

We added sentences discussing types of movements, associated ecological functions in multiple places in the revised manuscript (L114, 121, 585, 607, 630, 774).

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

We gave a more detailled explanation on L603, congruent with our reply to your point 10) above.

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

We added a formula to compute the overestimation factor on L613, as well as an example value for a 15 m butterfly flight height. The overestimation can virtually be infinite if the butterfly flew just below the UAV camera, but our results and simulations (Fig.6) rather suggest that most butterflies flew close to the ground.

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.

Preliminary behavioural observations are indeed necessary. We modified this paragraph to be clear about this (L627-638)

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.

The fact that the method can detect both light and dark-colored species is not a limitation, but rather a strength, extending the number of species that can be studied. Light species will appear as white blobs in filtered frames, while dark species will appear as black blobs (Fig. 2F), so that there cannot be confusion between these 2 colour groups at least. We modified the sentence to be clearer (L659-660). However, a species confusion problem arises when there are two or more light-coloured species of similar sizes flying simultaneously (or 2 or more dark-color species), as we discussed at length in results and discussion (L468-501 and L666-695). We added mentions about the limitation of UAV imagery to open landscapes (L664-665, 773).

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

OK, we thought it was important to warn about this logical trap.

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.

We agree that this would be an interesting comparison. This was unfortunately not possible, as we did not have the authorization from land owners to walk in crop fields. In appropriate landscapes, GPS tracking can yield longer tracks than our UAV method (e.g. Schtickzelle et al. 2007, Fernandez et al. 2016), but we can foresee a few limitations of human GPS tracking: 1/ a max temporal resolution near 1 Hz (e.g. Fernandez et al. 2016), hence as for SHR, GPS would not resolve very fine movements along the flight path (see effects of location depletion in Fig. 4). 2/ fast flying butterflies might be hard to follow by foot and 3/ the presence of a walking/running human a few meters behind the animal might be slightly more prone to influence the animal's flight than a distant, static UAV. We added a few comparison points about human-held GPS in the revised discussion (L572, 596).

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.

« distracted » was written in echo to the « undistraced » migratory flight definition adopted by Chowdhury et al 2021, given a few lines above. We replaced it with « attracted » (L715), and also modified the paragraph to better discuss the observed patterns of movement (directed vs. tortuous).

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.

We agree, but this sentence was removed from the revised manuscript, in an effort to shorten sections on biological interpretation (see editor comment).

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.

This was also removed for concision, but we agree that these species' migratory behaviours might be special cases.

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.

Here we had limited references to documented *Pieris* mass migrations, and we are not aware of radar data for this taxon. However, thank you for this reference, which adds the distinction between weather and biological radar. It was integrated in the introduction and discussion (L80-83 and 733).

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.

We mentioned the time investment in image processing on L285.

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

Sorry for the inappropriate phrasing. We replaced « perhaps » with « has the potential to » (L764)

Review by anonymous reviewer 2, 13 Mar 2025 09:16

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.

Thank you for this encouraging comment.

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.

In line with this comment and reviewer 1 comments, we added sentences discussing types of movements and associated ecological functions in multiple places in the revised manuscript (L114, 121, 585, 607, 630, 774).

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.

We agree that this is a strong limitation, that was not a choice but an inconvenient caused by the simultaneous phenology of these species in our area. We have acknowledged, explored ways to lift (fig. 7 and L468-496) and discussed this limitation at length and transparently in the manuscript (L32, L666-695, L766). Also we note that this sort of confusion between species and individuals is not specific to our UAV imagery method, but well-known in most passive sensing, tag-free tracking methods (as discussed by Schlagel et al 2020). We agree that *P. brassicae* and *P. rapae* have differences in their ecology, however concerning the discussed migratory behaviours, both species happen to have congruent seasonal variation of flight azimuth (initially northward, and southward later in the season ; Baker 1968, Gilbert & Raworth 2005, Spieth & Cordes 2012). Thus, although we are the first to deplore the fact that we cannot yet distinguish between the two species in our data, we hope that on this particular point (flight azimuths in a migratory context), a pooled analysis retains some validity.

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.

As replied to reviewer 1 and reviewer 2 comments above, we now have included more detailed considerations on the types of movements and their ecological significance, which we agree were missing in the original manuscript. We certainly hope to collect new data on a more clearly identified species, and inspire others in the field to do the same.

Review by anonymous reviewer 3, 10 Feb 2025 09:30

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!

Thank you for this very encouraging comment on our work.

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.

We are aware of the difficulties with the definition of migration in insects, and butterflies in particular. There is a lot of classic litterature on the problem of the « return flight » (e.g. Baker 1968). There is also a possible overlap with dispersal movements (discussed in Chowdhury et al 2021). Dispersal itself has various definitions (as discussed in Schlägel et al. 2020).

The behaviour-based (persistent directed, undistracted movement) definition of migration as adopted by Chowdhury et al. (2021, Biol. Rev.) for butterflies, is *widely use*d according to the authors, and congruent with classic entomology litterature (e.g. Southwood 1962, *Migration of terrestrial Arthropods in relation to habitat*, Biol. Rev. 37), but might indeed appear too broad compared to migration as known in vertebrates. On the other hand, the definition given in Schlagel et al. 2020 might appear too tight for butterflies, as the « breeding/non breeding site » dichotomy does not really fit multi-generational migratory patterns.

We decided not to get into the migration definition conundrum in our manuscript. As suggested by reviewer 3, we accepted to not consider migratory movements as a starting hypothesis, but rather as a possible deduction form the congruence of our results on directed azimuths with the literature (see L24, 712, 721, 751-754). Chowdhury et al's definition was removed from the methods (L318) and now only appears in the discussion (L712). The term migration/migratory was also removed in several places (L72, 74, 713).

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

The text was revised as suggested (L142)

L179: 'automatically' instead?

The text was revised as suggested (L189)

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

The text was revised as suggested (L216)

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

The typo was corrected (L221).