Dear recommender and reviewers,

We are very grateful for the positive and constructive comments we received from the reviewers and the recommender on our manuscript entitled "**Intra- and interspecific variations in flight performance of oak-associated Agrilinae (Coleoptera: Buprestidae) using computerised flight mills**". Multiple points were raised that needed clarification or could be improved. We have addressed these issues to the best of our ability when comments were hard to reconcile, but most concerns were shared by all reviewers and we gave them high priority.

This included shortening the manuscript by removing several figures (Figures 4, 5 and 8 in the previous version, as well as Figure S2) and some of the text that was not necessary in the main body (e.g., the section on "Correlations among variables"). When we thought some of this material could be useful to readers or allow further understanding of the methodological choices or results, we proposed transferring the information into electronic supplementary material. To ease reading the *Discussion*, we also subdivided it into separate sections with titles and we reorganised the text.

We believe this round of reviews has significantly improved and strengthened the manuscript. We hope that this revised version will adequately answer your remarks and that you will consider it suitable for recommendation by *PCI Zoology*. Our detailed reply to the comments and the details of the revisions are reported below.

Best regards,
The authors

Review a15763

This study presents comprehensive data on the flight behavior of Agrilinae beetles. I was particularly impressed by the extensive dataset, which has significant value for pest management. The methods are scientifically sound. Introduction is easy to follow as well and no need to be revised. However, the manuscript contains some redundancy and is difficult to follow especially Results and Discussion part. I recommend reconsidering the presentation and summary of the results. My specific comments are as follows:

We have lightened the manuscript to remove the redundancy, and we have reorganized both the *Results* and the *Discussion* part to increase the comprehension. Subtitles were also added in the Discussion

Tables and Figures: I believe that Table 2, which highlights the main findings, is essential. However, Figures 2-8 include a lot of unnecessary information. I suggest removing these figures or moving them to the Supplementary Materials unless they are crucial for discussing key aspects of beetle biology and management.

You are absolutely right that not all results shown between figures 2 and 8 were exploited to their full extent, and doing so would significantly increase the length of the manuscript or drift from its main scope. Therefore, we moved the figures 4, 5 and 8 to supplementary materials. Further explanation for figures 3, 6 and 7 can be found below and is now better detailed in the text. We have also reduced figure 2 by removing the Total flight duration and the Mean flight bout duration because of their strong correlation with Total flight distance and Mean flight bout distance, respectively, while the methodological choices consecutive to correlations are now detailed in the supplementary material (Document S2) instead of the main text.

For instance, is the latency to the first flight bout depicted in Fig. 2 critical? If so, please clarify its importance.

We agree that the latency to the first flight is by no means critical, but we believe its interspecific variation is not meaningless as it can serve as a proxy to infer the prevalence and motivation to take-off. Insects taking-off quickly after installation are the most active but this does not necessarily correlate with flight distances or durations equally among species or individuals, and as such can be a parameter of flight behaviour that is worth comparing at both interindividual and interspecific focal scales.

Our intent with Figure 2 is to show the overall dataset and the distributions among and within species for the measurable variables without *a priori*. However, we fully agree that this figure in its original form was too big and complex, and we removed two variables in this version, which was made easier by the other suggested changes in the *Results* (*i.e.,* moving the methodology and results regarding correlated variables to the supplementary material, and thereby allowing to show only a subset of variables even at the beginning of the *Results*).

Fig. 3 is quite difficult to read, and its relevance to the discussion is unclear.

Figure 3 shows the characteristic distribution of flight data from flight mills, with the typically high interindividual variability. This spans from no active flight or very short distances for the vast majority

of individuals to few good flyers, or even sometimes extreme individuals that, alone, can play a central role in the species- or population-level expansion and colonization events. We believe it is a relevant feature of flight mill data that needs being acknowledged, and which may to some extent reflect similar inter-individual variations in nature.

Besides putting emphasis on the scarcity of very good flyers, this is also the only result in the manuscript showing how the initial 8-hour trials and the consecutive 4-hour trials look alike in terms of distribution, despite the difference in duration and the putatively accumulated fatigue in the most long-lived insects.

Fig. 4 either needs revision or should be removed. What key message are you trying to convey here? Consider focusing on biologically significant correlations and using scatterplots to represent them. For example, the strong correlations between total flight duration, total flight distance, and the number of flight bouts are fairly obvious and may not need detailed description in the main text with figures.

We moved the whole part about the correlation between variables into the supplementary materials, to lighten the text. We explained in the *Material and Methods*, in the *Data analysis* section, that we removed some variables because of their high correlation with others and that the detailed results and methodological decisions can be found in the Supplementary materials.

See L245-247 "*However, several measures were correlated and we chose to not include the mean flight bout duration and the total flight duration in analyses. The selection method with the correlation tests are available as supplementary material (Document S2)*".

Figure 5: Some results in Fig. 5 are intriguing, but it's unnecessary to display all parameters for each species. Instead, select one or two representative parameters, such as the total flight distance for species with sufficient sample sizes. Scatterplots would be more informative than bar plots in this case.

We agree that some parameters appear less meaningful in some species, but this was the main goal of this figure: displaying links with pre-flight body mass for all other variables with no *a priori*, and sorting species by increasing coefficient to make the important relationships and their interspecific variations stand out from the dataset, instead of only selecting the most positive results. We believe the low correlations or even orthogonal interactions can also give insight on how flight parameters interact, especially when species rank differently for each pair of variables. This figure, however, was moved to the supplementary material with the other materials on analyzing and interpreting correlations since it was out of the main scope of an already lengthy paper.

Figure 6: The results in Fig. 6 should not be described as "Evolution" (line 415) since they reflect intra-generational changes. Consider revising the terminology.

We have changed it as recommended, for "Changes in" instead of "Evolution of", see L378.

Figures 7 and 8: It would be better to remove these figures unless you can clearly articulate their relevance to the discussion.

We moved figure 8 to the supplementary materials. See the first comment of the reviewer 2 (Review 968e25) for a more complete answer.

The sentences in lines 495-497 are unclear. I don't understand the logical connection between "the high correlation between the number of flight bouts and total distance flown" and "spreading by performing a series of short flights". I suggest emphasizing the importance of repeated short flight bouts leading to long distances covered, rather than the correlation between the number of flight bouts and total distance flown.

We modified that sentence accordingly.

See lines L447-449: *"In fact, our study showed that Agrilus species could spread by performing a series of short flights ultimately resulting in relatively long total distances covered, instead of single sustained flights (e.g., Ávalos et al., 2014)"*.

Additional Comment:

The sentences in lines 466-468 appear to rely on the outdated concept of "naïve group selection" (e.g., traits evolve for the benefit of the species). I recommend revising or removing these sentences to align with current evolutionary theory.

We remove that sentence from the manuscript.

Review 968e25

This paper assesses intra- and interspecific variability in flight properties, as well as sexual differences and the effect of mass on the flight properties of 12 beetle species using flight mills. Valuable data are presented, and the paper is worth publishing if unnecessary analyses are removed and the study's limitations are properly explained.

1. Consecutive flight patterns

The study aimed to assess: 1) intra- and interspecific variability in beetle flight, and 2) sexual differences and the effect of mass on beetle flight properties. The analyses of the consecutive flight patterns presented in Figures 6, 7, and 8 are not the main focus of this study. Moreover, Figures 7 and 8 are not effective in demonstrating the consecutive flight patterns. I believe these analyses are not essential for this study.

We would like to keep the analyses about the consecutive flight patterns because we believe it is a part of the global flight performance of these beetles. Figures 7 and 8 highlight the strong inter-individual heterogeneity in flight distance, during the first and the subsequent flights. In fact, individuals that are able to sustain flights for several days could search longer and more far away for available sexual partners or hosts, while individuals that can sustain long flight only for one day could be limited in their search, especially if no available host or partner are nearby. However, we have lightened that part, by removing the former figure 8 and some text.

2. Limitations of the study

It is important to describe the limitations of the study, such as the differences between tethered and natural flight. If the authors wish to argue for the validity of this study despite these limitations, they should explain why it remains valid. See specific comments for details.

See below in *Specific comments* for the answer.

Specific comments

Figure 4: The correlation network figures are not effective in showing the correlations among variables. A correlation table would suffice for this purpose.

A correlation table would suffice to highlight highly correlated variables, but the network plot representation carries more theoretical information to depict how variables are intertwined or dissimilar because it also scales them multidimensionally using the principles of a principal coordinates analysis so that variables can be displayed on a plane to highlight not only the sign or coefficient of the correlations, but also clusters of variables. While it is true that this information is under-discussed in the manuscript given its initial scope, we believe that this can be useful to a reader who may want more details on the interrelations of the flight variables, and could be moved to the supplementary material in a section dedicated to the methods and results on variable correlations (see Document S2).

L465-468: The strategy helps individual beetles, not beetle species, to increase their fitness.

We removed that sentence from the *Discussion*, in accordance with the comment of the other reviewer on it.

L549-565: I also believe that the data obtained from flight mills should be discussed carefully. Both overestimation and underestimation can occur, and this may vary by species. The discussion should conclude here, as further discussion regarding the validity of the method (Lines 560-563) is difficult without presenting a basis. Alternatively, the authors should describe other possible methods.

We have added some basis about the different methods that could be used to estimate the flight capacities of insects in the *Introduction* (see L102-117). We have also re-organised the end of the *Discussion* by first talking about the limitation of the interception traps, then by those of the rearing method and then by finishing with those of the flight mills (subsection *Limitations of the study and interpretation caveats*).

L595-622: The limitations of the experimental design are discussed here, particularly regarding the rearing environment. Similar to the point mentioned above, further discussion on the validity of the method (Lines 610-612) is difficult without presenting a basis. Alternatively, the authors should suggest how to address this issue in future research.

The rearing method that we used is the traditional method set up for the maintenance of Agrilinae in the literature. We have added a sentence to explain that in the manuscript, see L546-547 "*Our method was nevertheless the same one used successfully by other studies of flight performance of Agrilus spp. (Taylor et al., 2010; Lopez et al., 2014)*".

L625: The phrase "Despite experimental limitations," should be deleted for the same reason mentioned above. L627-628: The phrase "several flight behavioural patterns" is ambiguous.

We have modified the conclusion accordingly.

See L572-582 "*This work provided a first comparative approach on the flight behaviour and performance in several Agrilinae species associated with oak forests. It showed several common traits among the focal species, namely (1) the considerable inter-individual variation, (2) the low average flight performance compared to other insect species using similar experimental designs, and (3) the relative homogeneity of this pattern among most of the species investigated, despite the probable*

existence of different flight behaviour among species as seen with long sustained flights in C. undatus. This provides insights into the poorly understood dispersal ecology of oak-associated Agrilinae, suggesting a generally low average dispersal propensity and the importance of scarce events carried by a few extreme individuals in shaping the ultimate colonization and spread patterns at the population and species levels."