PCI ZOOLOGY Revisions

**Round #1**

*by Michael Lattorff, 2021-05-12 09:06*
Manuscript: [**https://doi.org/10.5281/zenodo.4489067**](https://doi.org/10.5281/zenodo.4489067)

**revision requested for your manuscript**

Dear authors,

your submission was evaluated by four experts in the field. All of them were very convinced about your study and enjoyed reading your ´well written manuscript. However, they also raised some concerns about methodology and data analysis. I think these issues need to be addressed to improve your manuscript. Please, read all reviewer reports carefully and respond to the points raised in a point-by-point response document incl. how the points raised affected your manuscript.

Many thanks for submitting your work to PCI Zoology, we are looking forward to receiving you revision.

– Dear Dr. Lattorff, thank you for handling our manuscript. We have addressed all the experts’ comments and modified the text accordingly. We have provided precise replies to each comment here below. We have uploaded the revised version of the manuscript, supporting information and R scripts on Zenodo.

**Reviews**

***Reviewed by Patrick Lhomme, 2021-03-14 20:08***

Dear authors, I have enjoyed reading your ms entitled “Cuckoo bumblebees perform slower and longer flower visits than true bumblebees”, it is a well written MS that is pleasant to read.

Males are generally considered to visit fewer flowers than workers, to be slower paced and to have a lower flower fidelity however most of the pollen they carry is freely available for pollination so I believe that their contribution to pollination services is often overlooked.

One strength of the study is to highlight this contribution and show that what we generally think about bumblebee male foraging is maybe not always true and probably more context-dependent.

Among the weakness, I feel that the authors are stretching their data a bit too much in trying to compare foraging behavior of cuckoo bumble bees and free-living bumble bees using only cuckoo male versus host worker/male foraging data. Including data from Bombus queens and Psithyrus females would have been a plus.

Unlike Bombus queens, Psithyrus females do not forage to feed their colony but only for themselves, their incentive to forage is thus very different although both have also offspring to produce, the comparison would thus have been very interesting. I noticed that females of certain species of cuckoo bumble bees like B. vestalis are regularly observed foraging while others like B. rupestris females are rarely encountered foraging on flowers although both species population density seem pretty similar (interestingly it’s opposite for males, as you show in your dataset). I believe females of B. rupestris feed directly on the resources gathered by their hosts and don’tbother much foraging, so the parasitic strategy can I’m sure explain differences in foraging behaviours between cuckoo and free-living bumble bees but also between the different cuckoo species.

– We agree that having data on queens and Psithyrus females would have been very interesting. The fact is that our observations rely on the flowering period of *G. lutea*, which blooms too late in the season to have both types of bumblebees. It would have required a specific study earlier in the season focused on finding bumblebee queens and females independently of the plants they visited. It could actually be a good idea for a future study.

We always tried to keep the three bumblebee groups (Psithyrus, female and male free-living bumblebees) separate to avoid too general comparisons, that we agree would have been imprecise given the absence of queens and female Psithyrus. To specify the fact that we are referring to male Psithyrus we have added the term “male” in the text where it was previously missing.

The comparison of both cuckoo and free-living males makes sense too, although they might have very similar physiological requirements, the parasitic strategy of the females could probably explain some the minor differences between cuckoo and free-living male foraging behaviors, as discussed by the authors. I also noticed that mating durations between cuckoo and host bumblebees were drastically different (see Lhomme et al 2013) for reasons that I can’t really explain, but this could somehow impact male foraging behavior. As mating duration is almost 10 times shorter in cuckoos their energetic needs might also be much lower I don’t know.

– This is a very interesting suggestion. We agree that such great differences in mating duration can contribute in the observed foraging behaviors. We have added this hypothesis (and reference) at the end of the paragraph. We also added that potential differences in the metabolism of cuckoo and free-living males can further increase such differences, as suggested by another reviewer – while we think that differences in size might play a much smaller role.

I’m also a bit frustrated not to have more qualitative data to interpret the results. You measured visiting durations but it would have been useful to measure foraging durations, it’s not clear to me if the males are feeding or just resting on the flowers, quantifying resting / nectar foraging / pollen foraging would have also been a plus.

– Females always visited flowers to forage for pollen or nectar, so for them visiting duration equals foraging duration. For males, it was sometimes complicated to understand what individuals were doing during their visits. Because *G. lutea* has several flowers per whorl on all sides of the plant, and because we had multiple plants per observation, it was sometimes difficult to follow the complete behavior on all flowers. In general males showed all behaviors mentioned by the reviewer: they walked on flowers, then they foraged for some time, then rested, etc. It was complicated to quantify or to attribute single behavioral types to individuals as they basically showed all of them together, so we understand the reviewer’s frustration as we share the same feelings. From the plant perspective, all of these behaviors (with the exception of resting) would lead to the same result, that is self-pollination (within and/or among flowers of the same individual), likely resulting in increased selfing rate. However, we understand that this is a shortcoming of the paper and that it would have been very interesting to discriminate all these differences to better characterize bumblebee behavior.

Here are some minor comments:

Title : This title is misleading, you can’t really state this based on your study. You are comparing bumble bee workers with cuckoo males. It would have made sense if you were comparing Bombus queens and Psithyrus female foraging behaviors, but it’s not the case here. Please provide a more accurate title or at least specify that cuckoo bumblebees are males and that true bumblebees are workers

– We understand this point and have changed the title to reflect that cuckoo bumblebees were males and free-living bumblebees were males and workers.

L 26-28 :  Many studies have investigated differences between cuckoo bumble bees and their hosts in terms of social behaviors, pre-mating behaviors etc... You should be more specific on the behavior (flower visiting behavior) you talk about.

– Yes, thank you for the suggestion. We have modified the text.

Keywords : In general, avoid using words that are already in the title.. I would for example replace “cuckoo bumble bees” by Psithyrus, “behavior” is also too vague, use flower visiting behavior

– We agree, thank you for the suggestions. We have changed the keywords.

L 52 : Williams 1998 actually cites 29 species, it was before Lecocq et al. (2011) show conspecificity between B. barbutellus and B. maxillosus. I don’t like doing that (self citation) but we also confirmed in a recent paper (in press) that B. flavidus and B. fernaldae are conspecific, so the number of species is now 27. You can just simply stick to 28, but if you wish to specify 27 and if you’re allowed to cite papers in press with DOI then feel free to cite “Lhomme P, Williams SD, Ghisbain G, Martinet B, Gérard M, Hines HH (2021) Diversification pattern of the widespread Holarctic cuckoo bumble bee Bombus flavidus (Hymenoptera : Apidae) : The East Side Story. Insect Systematics and Diversity, in press, doi: 10.1093/isd/ixab007”

– Thanks for catching our mistake. We don’t see why we cannot cite your more recent paper, and in fact we think it would be the best choice. We see that it is now published, so it’s even better. We have modified the text and added the reference to the Reference section.

L. 56 :  To be honest I’m not a big fan of the adjective ”true” to refer to non-parasitic bumblebees, Psithyrus species are also true bumble bees.. I would rather use the terms “cuckoo vs free-living” or “cuckoo vs host”, but it’s just a personal preference feel free to keep “true” if you wish.

– We agree,following your and other reviewers’ suggestions we changed “true” into “free-living” throughout the paper.

L. 211-214 :I agree but it doesn’t really make sense here. If you were comparing bumblebee queens and cuckoo females I would agree but here you compare workers versus males, the parasitic behavior of females has nothing to do with male flower visiting behavior.

– We agree that the parasitic behavior of females is not the correct explanation here. We actually wanted to highlight the fact that these differences are mainly related to sex, i.e. males vs workers, rather than to parasitic vs. free-living. While the explanation is similar when comparing free-living males and workers, we separated this paragraph from the following one to highlight the differences observed in this study between the groups and to discuss the previous knowledge on free living males, while little is known on Psithyrus males.

L. 271 : were cuckoo bumblebee male**s**.

– Yes, we modified the text

Best wishes,

Patrick Lhomme

***Reviewed by anonymous reviewer, 2021-04-06 13:47***

Fisogni and co-authors present an interesting study comparing behavior of cuckoo and free-living bumblebees. They studied floral visit of different individuals on patches of Gentiana lutea and they show that behaviors are different. We need more empirical studies like this one to better understand the ecology of the pollinators, and their conservation. I recommend the publication of this article but I present here different propositions to make the presentation of this study more accurate.

Change everywhere in the manuscript the term “true bumblebees”, including in the title. As you know, Psithyrus are now included in the genus Bombus. I recommend to use the term “free-living bumblebees” or “non-parasitic” if you want to distinguish the cuckoo bumblebees with the other bumblebees.

– Yes we agree, following your and other reviewers’ suggestions we changed “true” into “free-living” throughout the paper.

You have to include in the abstract and in the introduction the interest of your study. Why is it important to compare the two groups? Why is it important to compare their behavior? What’s your hypothesis? You should indicate, in the abstract and in the introduction, that the evaluation of the behavior during the floral visit is important to estimate the efficiency of a pollinator and its fitness. We might think that the cuckoo bees are not good pollinators because they don’t forage on pollen.

– Thank you for the suggestion. We have extended the importance of the study at the end of the introduction and modified the last sentence of the abstract.

However, we have refrained from making explicit hypotheses. There are no studies to date on cuckoo flower visiting behavior nor on differences with their hosts, so this limits our expectations. Moreover, the fact that cuckoo bumblebees do not forage on pollen should not decrease their pollination efficiency. In fact, pollen available for pollination is not the one directly collected in the corbiculae as the reviewer correctly points out in another comment, which on the contrary becomes unavailable for pollination, but rather the one that inadvertently remains attached to their bodies during flower visits. As we discuss, the fact that cuckoo bumblebees visit several flowers in a “messy” way could actually potentially increase their pollination efficiency because they contact anthers from several flowers, but with negative consequences for the plant because of geitonogamous selfing.

Line 34: Significant differences?

– Yes, we added this in the text

Line 37; Lines 239-241: I did not understand this mechanistic hypothesis to explain the different behavior by the difference in colony development. Where are the data on the timing of colony development?What about other traits like the size? The physiological needs? The flight metabolism?

– Our hypothesis is based on the extensive literature on the timing of colony development of cuckoo and host bumblebees, rather than direct observations of wild colonies in the field (unfortunately very complicated to implement). Our main point here is that these differences can lead to significant gaps in the period of appearance of females and males of the two groups, and especially to the lack of queens and cuckoo females. In turn, this would lead to different levels of activity for males that should (free-living males) or not (cuckoo males) actively look for partners during our observation periods. Based on our observations and in accordance with the other reviewers we think this could be a solid and interesting hypothesis.

As highlighted by Reviewer 1 and discussed by us, physiological needs between cuckoo and free-living males can be expected to be similar and therefore not significantly influence their behavior. We also do not think that size plays a significant role with regard to this aspect, as they seem quite comparable. However, we agree that metabolism could influence different behaviors between males of the two groups. We added a sentence in the Discussion to acknowledge this possibility. We also included an additional hypothesis based on very significant differences in mating duration between cuckoo and free-living bumblebees that could lead to different energetic requirements by males, as suggested by Rev 1.

Lines 51-53: evolution of inquilinism in 3 subgenera (see Lhomme& Hines 2019)

– Because here we are more interested in highlighting the presence of obligate parasites within the subgenus Psithyrus we prefer to avoid going into too much detail on the evolution of inquilinism in groups outside this one. Following the comment, we now cite Lhomme & Hines 2019 because it should be more appropriate here and includes a wider overview on the subject for the interested readers.

Lines 65-67: to be developed. What makes a pollinator efficient or not? Cleaning behavior for example should be introduced. Pollen on corbiculae is not available for pollination.

– Here we are introducing the fact that females can be efficient pollinators by transferring large amounts of conspecific pollen because of high flower constancy. While actively collected pollen is no longer available, it does not exclude the fact that pollen attached to their body can be transported among flowers. We agree that the cleaning behavior to collect pollen from the body in the corbiculae could increase the amount of pollen lost for the bumblebee, but it is likely not the main driver of an efficient pollination. As for the previous comment, we would like to avoid going into too much detail about the mechanisms of pollination efficiency, as this is not the focus of the article.

Line 90: size of the flower?

– We have checked herbarium specimens collected in the study site and measured flowers of around 4 cm diameter. We have added this information as personal observation in the text. This size easily allows bumblebees to enter flowers to feed on nectar (anthers are exposed and easily accessible for pollen collection).

Line 94: Rossi et al. 2014 did not explore the present question?

– No. Rossi et al. 2014 estimated pollinator performance based on insect abundance, behavior (sedentary *vs* dynamic), stigma contact, pollen identity carried on their bodies and frequency of visits. Bumblebees were clumped in the same category (*Bombus* spp.). The authors in that article did not discriminate specific flower visiting behavior of different groups based on the time spent on flowers nor on the number of flowers visited within whorl or plant.

Line 108: How did you determine the species in the field?

– Following this and Reviewer’s 4 comment we added details in the text to clarify how we identified species in the field. It was relatively easy given our previous knowledge of the few species present in the study area. We also sampled some individuals to confirm our field IDs.

Line 128: why you don’t consider species level? Difference among free-living bumblebees species might be more important than between cuckoo versus free-living forms. I would test first differences at species level, and if you don’t have differences, you can pool the data. Aggregation of data has to be justified.

– This is a good point. We should have mentioned in the text that we have made a preliminary analysis to check for an effect of species on flower visiting behavior. As also found by Reviewer #4 after running the analyses, we did not find any significant differences between species. Therefore, we decided to aggregate data to focus on differences between groups and sexes, the latest being probably more important even within species than differences between species. It is also to note that we found large sample sizes for only few of the observed species, which could lead to unreliable analyses or generalizations.

Line 156. Table 1: The subgenus system presented here is outdated. Please follow Williams et al. 2008. All cuckoo species listed here are included in the subgenus Psithyrus.

Williams P.H., Cameron S.A., Hines H.M., Cederberg B. &Rasmont P. (2008) A simplified subgeneric classification of the bumblebees (genus Bombus). Apidologie, 39, 46-74

– Thanks for pointing this out. We decided to exclude the subgenus classification from the table.

Line 161. There is no justification to lump the data from different species. In other words, are the differences related to species or to “trait” (cuckoo versus free-living)?

– As discussed above, we did not find significant differences between species. We have added this detail in the text.

Line 167. The accuracy to study these metrics was not introduced.

– We have added in the M&M the reference to the fact that we recorded times of visit for each flower within whorl, from which we could then extrapolate the time spent on the whole plant.

Lines 204-205; lines 216-217. If it’s not significant, don’t mention it. It does not make sense.

– After re-running some analyses, as suggested by Reviewer #4, the difference in the number of visited flowers became significant, which makes sense looking at data distribution and SE. Time spent on flowers remained non-significant. However, we do not think that in this case “statistically non-significant” means “not worth mentioning”. As we discussed, we believe that although these differences are not statistically significant they can be biologically relevant, especially looking at the high differences between the average values and the high inter-individual variability. We therefore would like to maintain our very short discussion about this result.

Line 247: Please clarify. How?

– We have added in the text that more efficient dispersal would occur between plants of a same species to justify the increased intraspecific pollen flow. We rather wouldn’t enter in too much detail about the general mechanisms behind pollen transfer and pollination efficiency, and we think that the text with the cited reference could be enough to give the appropriate context to the reader.

***Reviewed by anonymous reviewer, 2021-02-28 16:38***

This is a nice example of rare studies asking the relevance and importance of bumble bee males for pollination and plant reproductive success. In particular socially parasitic cuckoo bumble bees are completely neglected in this context. Here, the authors found that cuckoo males stay much longer on flowers and behave slower than host bumble bee workers and males, most likely related to the different foraging strategies and colony development.

The study design, data analysis and presentation is clear and easy to follow. The authors use all relevant literature to discuss and explain their data. However, a major downside of the study is the very limited observation time with only 3 days per year (6 in total). It is well known that climate (temperature, hours of sunshine, rain, etc.) significantly impact flower nectar production and bumble bee foraging behaviour. It is needed to include climate data during observation to discuss the pros and cons of the data. Slower behaviour and resulting longer flower visits might also be explained by the climate data, this discussion is missing so far.

– We understand the Reviewer’s concern. Unfortunately given the relatively short flowering duration of *G. lutea* and the difficult logistic conditions (high mountain, steep slope) we could not manage more than 6 days of field observations over two years. Although more observation points would have certainly improved the dataset, we think that our sample size should capture the temporal variability in the visiting behavior of bumblebees.

About differences in climate data, we do not think that it should be a major concern. The main reason is that we observed both free-living and cuckoo bumblebees at the same exact time in the same place, therefore both groups were subject to the same meteorological conditions. We do not expect climate to affect the two groups in a different way. Regarding more detailed measures, we recorded temperature during our observations and we did not find marked differences between the sampling periods. Temperature in 2013 was on average 20.8 ± 2.3 °C, while in 2015 it was 24.0 ± 2.8 °C, therefore they had overlapping values. Moreover, in our analyses we did not find a significant effect of year on bumblebee behavior, indicating that any differences in climatic conditions did not lead to inter-annual variations in the observed patterns.

It might also be worth separating data for the host bumble bee species, having a huge difference between sexes for B. terrestris, B. lapidarius and B. pratorum. For each species, colony life cycles differ strongly among months and might be included in the discussion of the data.

– This is a good point. We should have mentioned in the text that we have made a preliminary analysis to check for an effect of species on flower visiting behavior. As also found by Reviewer #4 after running the analyses, we did not find any significant differences between species. Therefore, we decided to aggregate data to focus on differences between groups and sexes, the latest being probably more important even within species than differences between species. It is also to note that we found large sample sizes for only few of the observed species, which could lead to unreliable analyses or generalizations.

The efficiency of pollination success can be easily supported by having pollen data of the foraging bees. Did the authors count pollen grains per cuckoo and host bumble bee males and workers while foraging?

– We did not directly count conspecific pollen grains on bumblebee bodies. However, Rossi et al. 2014 showed that in the same community both free-living and cuckoo bumblebees present large amount of pollen grains on their body and contact receptive stigmas during their floral visits, and can potentially be efficient pollinators.

Table 2 and 3 might move to the suppl. material.

–Tables were moved to the Supplemental Material

Line 208-214: Is there anything known on nectar and pollen quality, nutritional values, of G. lutea. If yes, this would be relevant for the discussion on energy intake and foraging behaviour strategies.

– We recently published a paper detailing *G. lutea* nectar sugar (glucose and fructose dominant) and amino acid composition (Bogo et al. 2021 Oikos 130:1180-1192), but we do not have information on pollen. However, we don’t think reward quality could explain the observed differences. Males of both free-living and cuckoo bumblebees clearly visited flowers only for nectar, while females visited flowers for both nectar and pollen. As nectar is the same for all groups, we don’t expect it to drive different behaviors. We discuss differences between females and males in the following paragraph. As a consequence, although an interesting subject, we would avoid further speculation on this complicated issue.

Line 216-223: Seems to be redundant compared to previous sections and should be shortened. Generally, the authors should double-check for redundancy between the introduction and discussion.

– We slightly modified this paragraph after the new results found from re-running some of the models, as suggested by Reviewer #4. In this paragraph we discuss differences between free-living female and male bumblebees, which we did not discuss elsewhere. While we understand that the previous paragraph (workers vs cuckoo males) mainly has the same underlying hypothesis, we would like to keep them separated to highlight the differences observed in their behaviors, and to discuss the previous knowledge on free-living males while little is known on Psithyrus males

The authors highlighted several times that only males of cuckoo bumble bees have been found, but no queens. Do they have any data that at the same time queens were foraging on other plants in the same vicinity and that young queens already emerged? Otherwise I would reduce the discussion on this fact.

– We did not observe any queen in the area. At that point in the season all queens/females would have been new/young individuals for both bumblebees and Psithyrus. We hypothesized in the following paragraph that queens of free-living bumblebees had not yetemerged, while cuckoo females had probably already emerged, mated and dispersed (or even hibernated given the high elevation of the study population ~1400). Because this is the only point in the discussion where we discuss this subject, we think it would be interesting for the reader without being redundant with the introduction.

***Reviewed by anonymous reviewer, 2021-04-06 13:25***

In general, it’s a well written and interesting manuscript. I do see a few areas where the ms could be improved and clarified.

# Methods

Line 110: the authors verify visual identification by capturing and keying out species after visual surveying the visitations. My concern here is that this approach will validate the experimenter’s eye for identifying species/sex but not whether they were successful in the instance of observation. IDing the bees while visiting plants is absolutely achievable by experienced observers but it’s not always trivial and especially when they’re moving around and have their head buried in a flower etc. For example, B. terrestris males look largely similar to females but have a different ‘face’. Being confident in sexing can require a close look.

– We agree that identification of bees in the field can be tricky (and often just plainly impossible depending on species/genus), and while we agree with the comment on experimenter’s eye validation in this case we had a few advantages. For species IDs, there were very few bumblebee species in the areathat were relatively easy to distinguish, and we already had some knowledge on their IDs from previous studies and samplings (we have done a few years of field work on pollinators in the same site). Moreover we sampled individuals to confirm our IDs. Discrimination between females and males was probably the easiest part, as females have corbiculae! This trait was basically always visible during observations, especially when they visited flowers for pollen collection and they had full corbiculae. In addition, other traits as the length of antennae and especially the shape of the last tergite, which was visible even when individuals were fully into the flower, helped in case there were any doubts. Two of the co-authors (GB and LB) are especially good at sexing individuals because of years of lab experiments with *B. terrestris* and *B. pascuorum* where this technique was required.

We have added more detail in the M&M section to clarify how we identified bumblebees.

Although 100% certainty does not exist –and we certainly don’t claim it– we are quite confident that our identifications could be sufficiently reliable for the purpose of this study. Also, the fact that we aggregated data between species in all the analyses would make any imprecision of little significance in this context.

Stats: I think that some mention of species variation in foraging is warranted. I have gone through their well annotated code and included bumblebee species. In none of the models I tried did bumblebee species significantly explain the response variables. This then makes it sensible to ignore, but it should be mentioned explicitly in text that they did not vary by species (unless I missed something).

–Thank you for running the code and for checking for effect of species! We did a preliminary analysis including species as response variable, but since the effect was never significant we chose to focus on differences between groups (cuckoo *vs* free-living). We should have clarified it in the text. Following your and other Reviewers’ comments we have added this information in the M&M section.

I do find, however, some issue with two things. First, I suspect that a generalized linear model would be more appropriate for the number of flowers/whorls visited with a Poisson error distribution (or quasi if overdispersed). Second, some of these counts seem to have quite a bit of missing data (NAs) in the data file. More explanation of these missing data should be provided.

– Thank you for this suggestion. We think you are right and a Poisson error distribution is more appropriate as this is count data. We have re-run the models using a Poisson distribution. Results now show significant differences in the number of flowers visited, which makes sense looking at the distribution of the data.

Yes, unfortunately some data are missing regarding the number of visited flowers. These usually occurred when bumblebees visited flowers in a blind-spot for the observer (e.g. on the opposite side of the plant) or when the bumblebee visited several flowers in a row and was difficult to keep track of the exact number of flowers visited. We added some explanation in a readme file on Zenodo about the missing data.

How well do these correspond with individual identifications. I find it a bit hard to connect the two. When recording visits on a plant, one has to make spot judgments. The sampling afterwards would help confirm your confidence of identification in general but won’t actually validate your decision at the time. For the species here, it is pretty straightforward to distinguish. But, for identifying sex it can be trickier. For eg. B. terrestris males look very similar to females. People with experience can vey reliably identify which are males and females (and I’m sure you are) but it relies on a clear view of the bee, and in particular, their head (which can be obscured if it’s deep in a flower).

– Please see our previous comment on bumblebee identification. We would like to highlight again that distinguishing between males and females was probably the easiest part, as traits like corbiculae can be easily spotted even from a distance. Also, we were pretty close during our observations.

# Discussion.

L235 is an interesting suggestion, that phenology determines foraging activity. This is intuitive. A correlate of this is that the bees differ in age. If sexuals are produced earlier for cuckoo bumblebees then we would expect them to be older on average than the other bumblebees. This could be estimated by wing wear from the collected samples.

– This is an interesting point. We checked the wings of Psithyrus individuals collected during the days of observations, and they tend to be more worn than those of free-living males. Because we sampled only Because we only caught a limited number of individuals to reduce the impact on local populations, we do not have a robust sample size to carry out a quantitative alanysis. However, this is a really good suggestion that could be useful for future studies.

L241 this would be supported by phenology data.

– Yes, and it would have been extremely interesting. Unfortunately this was not possible in the field, therefore we relied on previous knowledge to formulate this hypothesis.