Peer Community In Zoology

New insights into maternal egg care in insects: egg transport as an adaptive behavior to extreme temperatures in the European earwig

Anna Cohuet *based on peer reviews by* ***Nicolas Sauvion***, ***Ana Rivero*** *and Wolf U. Blanckenhorn ?*

Jean-Claude Tourneur, Claire Cole, Jess Vickruck, Simon Dupont, Joel Meunier (2022) Pre- and post-oviposition behavioural strategies to protect eggs against extreme winter cold in an insect with maternal care. bioRxiv, ver. 3, peer-reviewed and recommended by Peer Community in Zoology. https://doi.org/10.1101/2021.11.23.469705

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Because of the inability of eggs to move, the fitness of oviparous organisms is particularly dependent on the oviposition site. The choice of oviposition site by mothers is therefore the result of trade-offs between exposure to risk factors or favorable conditions such as the presence/absence of predators, the threat of extreme temperatures, the risk of desiccation, the presence and quality of nutritional resources... In addition to these trade-offs between different biotic and abiotic factors that determine oviposition site selection, the ability of mothers to move their eggs after oviposition is a game-changer in insect strategies to optimize egg development and survival [1]. Oviposition site selection combined with egg transport has been explored in insects in relation to the risk of exposure to egg parasitoids [2] or needs for oxygenation [3] but surprisingly has not been investigated in regards to temperatures. Considering egg transport in the ability of insects to adapt their behavior to environmental conditions and in particular to potential extreme temperatures is yet inherent in providing a complete picture of the diversity of behaviors that shape adaptation to temperature and potential tolerance to climate change. In this sense, the study presented by Tourneur et al. [4], explores whether insects capable of egg-care might use egg transport as an adaptive behavior to protect them from suboptimal or extreme temperatures. The study was conducted in the European earwig, *Forficula auricularia* Linnaeus, 1758, which is known to practice egg-care in a variety of ways, that presumably includes egg-transportation, for

several weeks or months during winter until hatching. The authors characterized different life-history traits related to egg-laying, egg-transport, and egg-development in two device systems with three experimental temperature regimes in two populations of European earwigs from Canada. The inclusion of two populations, which turned out to belong to two clades, allowed the identification of a diversity of behaviors although this did not allow to attribute the differences between the two populations to specific population differences, genetic differences, or to their geographical origins. Interestingly, the study showed that oviposition site selection in the European earwig is driven by temperature and that in winter temperatures, female earwigs may move their eggs to warmer temperatures that are adequate for hatching. These results are original in the sense that they highlight new adaptive strategies in female insects used during the post-oviposition stage to protect their eggs from temperature changes.

In the current context of climate change and potential changes in selective pressures, the study contributes to the understanding of the wide range of strategies deployed by insects to adapt to the temperature. This appears essential to predict and anticipate the consequences of global instability, it also describes from an academic point of view a new and fascinating adaptive strategy in an overlooked biological system.

References:

[1] Machado G, Trumbo ST (2018) Parental care. In: Insect Behavior, pp. 203–218. Oxford University Press, Oxford. https://doi.org/10.1093/oso/9780198797500.003.0014

[2] Carrasco D, Kaitala A (2009) Egg-laying tactic in *Phyllomorpha laciniata* in the presence of parasitoids. Entomologia Experimentalis et Applicata, 131, 300–307. https://doi.org/10.1111/j.1570-7458.2009.00857.x

[3] Smith RL (1997) Evolution of paternal care in the giant water bugs (Heteroptera: Belostomatidae). In: The Evolution of Social Behaviour in Insects and Arachnids (eds Crespi BJ, Choe JC), pp. 116–149. Cambridge University Press, Cambridge. https://doi.org/10.1017/CBO9780511721953.007

[4] Tourneur J-C, Cole C, Vickruck J, Dupont S, Meunier J (2022) Pre- and post-oviposition behavioural strategies to protect eggs against extreme winter cold in an insect with maternal care. bioRxiv, 2021.11.23.469705, ver. 3 peer-reviewed and recommended by Peer Community in Zoology. https://doi.org/10.1101/2021.11.23.469705

Reviews

Evaluation round #1

DOI or URL of the preprint: https://doi.org/10.1101/2021.11.23.469705
Version of the preprint: 1

Authors' reply, 09 March 2022

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Decision by **Anna Cohuet**, posted 16 February 2022

Invitation to revise the manuscript

This manuscript explores the strategies of oviposition site selection and egg care modulated by temperatures in two different populations of the European earwig. The reviewers reported the interest of the model and the questions but also raised some limitations. Especially, the status of the 2 cryptic species, the biological relevance of their comparison and the significance of the experiements with only one population per species should be better addresses. Besides, the reviewers found the manuscript in general well written but provided detailed suggestions for improving the understanding. I invite the authors to revise the manuscript according to reviewer's comments and to re-submit.

Reviewed by Wolf U. Blanckenhorn, 22 December 2021

Review of PCI Zoology MS#120

This MS reports a single-species (??) investigation extending the study of thermal preferences of animals to the tending of laid eggs by earwig females during winter. Two eastern Canadian populations of the European earwig – is the invasiveness of this species an issue worth mentioning here?? – with slightly differing thermal regimes (Fig. 1) are being compared, an absolutely minimal comparison in terms of geographic variation that constitutes not much more than a simple population replicate. (In addition, there seems to be a possibility of cryptic species, which complicates the comparison even further and better should not be discussed here, else there is no population replication whatsoever.)

In total, the study reports interesting and apparently novel data (which does not become completely clear from the Introduction) on the egg-tending behaviour of earwig mothers that are worth publishing in a zoological, entomological, behavioral, or thermal journal (such as J. thermal Biology). The MS is overall well written, though I have added a few (text) edits and comments here and there (using Acrobat).

Specific comments:

1) I would de-emphasize the (presumably) latitudinal comparison of merely two populations (or even cryptic species) in lieu of a mere population replicate. Else the study leaves much to be desired.

2) A figure of the thermal apparatus should be part of the MS (as Fig. 1).

3) Why are EU earwigs studies in Canada?

4) There are some unclarities left regarding the Methods, especially regarding the transfers (see my comments in the MS).

5) The genetic analyses in Methods and Results really don't fit here and are not necessary (cf. comment 1 above). Remove.

6) The statistics used are also not super-clear. In particular, I can only hope that the most fitting error-distributions were used in the models. Or were all simple non-parametric analyses after all?

7) Figures 2 & 3 could be combined (underneath), as the panels are identical.

Wolf.blanckenhorn@uzh.ch

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Reviewed by Nicolas Sauvion, 27 January 2022

I was very interested in this study on earwigs. It's a biological model that I didn't know about, so I would have liked the authors to give a little more detail on the biology of these insects. These details are sometimes lacking to fully understand the observations made. I will clarify these points later.

Overall, I find this article clear, very well written, well structured (although I also make some suggestions below). The problematic is well posed, but it seems to me that the authors do not emphasise the biological question enough (what kind of strategies earwigs adopt to minimise the risks to their eggs during winter). Yet, it would be more interesting to present the study from this angle (as it is very original!!) rather than 'simply' presenting another study on the effect of temperature on the biology of an insect. This is only my point of view!

Overall, I have no major substantive comments to make: I note that the authors are very familiar with their biological model and the concepts of evolutionary biology on which they argue, especially in the discussion

On the other hand, I have many detailed remarks to make. In particular, I would like to insist on the formalism to be respected for the names of the species, even if I know that the editors are pushing for concision.

I will be happy to proofread a revised version of this manuscript

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For the first sentence line 32 add in reference :

Meunier J, Körner M and Kramer J (2022) Chapter 17: Parental care. In Reproductive strategies in insects (ed. Omkar & Geetanjali Mishra). CRC Press, Taylor and Francis. pp 337-359 [pdf] - Book chapter https://doi.org/10.1201/9781003043195

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In all rigor and in accordance with the international code of zoological nomenclature (https://code.iczn.org/ see Article 51. Citation of names of authors ; Article 22. Citation of date) : 'It is strongly recommended that the date of publication (and the authorship; see Article 50) of a name be cited at least once in a work which deals with a taxon. This is particularly important for homonyms and for species-group names not in their original combinations'

I have thus searched for the 'correct' names of the species mentioned in the manuscript. I list them below, with the sources.

• You will notice that the name of the Gerris species mentioned is ambiguous. I am not a specialist of this group. I found references on this question but without being really affirmative on the correct name of species that should be retained [Gerris paludum insularis Miyamoto, 1859 ?]

• You will notice that Hyla versicolor is a synonym of Dryophytes versicolor (LeConte, 1825)

in this case I suggest to write : Hyla versicolor (LeConte, 1825), formally Dryophytes versicolor (LeConte, 1825)

because Hyla versicolor takes the name mentioned in the reference cited by the authors (here Takahashi, 2007)

• the same for the flat rock spider : Hemicloea major L. Koch, 1875 synonym of

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(Walckenaer, 1837)

Aquarius paludum insularis ?= Aquarius paludum insularis (Motschulsky)?

=> ? *Gerris (macrogerris) insularis* (Motschulsky, 1866) [cf. Damgaard & Cognato 2005 Syst. Entomol. : https://onlinelibrary.wiley.com/doi/10.1111/j.1365-3113.2005.00302.x]

=> Gerris paludum insularis : Miyamoto (1859 : 118 ; non Motschulsky, 1866).

https://www.gbif.org/fr/species/2020415/treatments

see also : https://research.amnh.org/pbi/library/4039.pdf

Anthocharis cardamines (Linnaeus, 1758)

https://inpn.mnhn.fr/espece/cd_nom/54451/tab/sources

Orange-tip (Anglais)

Culiseta longiareolata (Macquart, 1838) [Culicoidea]

https://inpn.mnhn.fr/espece/cd_nom/225143

Anopheles punctipennis (Say, 1823)

https://wrbu.si.edu/vectorspecies/mosquitoes/punctipennis

Lechriodus fletcher (Boulenger, 1890)

https://fr.wikipedia.org/wiki/Lechriodus_fletcheri

Hydroporus incognitus Sharp, 1869
https://inpn.mnhn.fr/espece/cd_nom/9490

Hydroporus nigrita (Fabricius, 1792)
https://inpn.mnhn.fr/espece/cd_nom/223388

Lestes macrostigma (Eversmann, 1836)
https://inpn.mnhn.fr/espece/cd_nom/65205

Hyla versicolor = Hyla versicolor LeConte, 1825 => Dryophytes versicolor (LeConte, 1825)
https://amphibiansoftheworld.amnh.org/Amphibia/Anura/Hylidae/Hylinae/Dryophytes/Dryophytes-versicolor

Phrynocephalus przewalskii Strauch, 1876
Przewalski's toadhead agama
The agamid genus Phrynocephalus, known as toad-headed agama
https://reptile-database.reptarium.cz/species?genus=Phrynocephalus&species=przewalskii
https://en.wikipedia.org/wiki/Phrynocephalus_przewalskii

Osmia bicornis (Linnaeus, 1758)
https://inpn.mnhn.fr/espece/cd_nom/816756

Morebilus plagusius (Walckenaer, 1837)
Syn. Hemicloea major L. Koch, 1875
https://wsc.nmbe.ch/species/42887

Phyllomorpha laciniata (Villers, 1789)
https://inpn.mnhn.fr/espece/cd_nom/829029

Abedus (Deinostoma) herberti Hidalgo, 1935
https://www.gbif.org/species/2007558

Forficula auricularia Linnaeus, 1758
https://inpn.mnhn.fr/espece/cd_nom/65991
*****
Line 34
In their introduction the authors mention benefits to (1) egg-laying females, (2) their current eggs, and (3) their future 34 juveniles. This chronological order is biologically logical. Next, the authors expand on point 1 (lines 37-41), point 3 (lines 41-48) and point 2 (lines 48-57). I suggest restructuring the paragraph and detailing point 2 before point 3.
Line 103
'shorter exposure to cold speeds up egg hatching' taken out of context this sentence is counter-intuitive. Indeed, one can wonder if it is really the duration of exposure to cold that affects the speed of hatching, or rather the intensity of the cold. Intuitively, we imagine that the regions with the longest winters are also those where the temperatures are the coldest.

Reading the article by Körner et al. 2018, I understand that the observations were made under experimental conditions, and that the minimum temperatures were identical between the two conditions tested (long winter versus short winter). so I do agree with the conclusions and statement of the authors. However, I suggest that the sentence be qualified as follows: 'under experimental conditions, by exposing insects for varying lengths of time to the same minimum temperatures (in this case 5°C), it is observed that shorter exposure...'

Sampling & experimental process

Line 122 : Explain why these locations were chosen in relation to the biological question; why the authors would expect females from these two populations to behave differently.

Quote figure 1 here to explain this choice

Specify gps coordinates :

Harvey station Lat. 45° 44.556'N ; Lon. 67° 0.944'W

St John's Lat. 47° 33.691'N, Lon. 52° 42.755'W

Line 31

'insulated with thick foam to ensure complete darkness.'

For a naive reader who does not know this biological model, it would be useful to specify here how this device imitates the conditions encountered in natural conditions by the earwig

Line 126

Please add that the females were transferred 'individually'.

This is important, because otherwise (e.g. batch tests of 6 individuals), one could suspect interaction effects between females, and this should be taken into account in the choice of statistical tests (tests on paired data)

I assume that you did not use 36 thermal bridges, but N1 bridges on which you placed N2 rails.

Are the temperatures perfectly identical laterally on the bridges? Otherwise, there is no risk of edge effect? I think it is necessary to precise this point

Line 134

'To limit stress on the females due to rail handling, we divided each rail into 12 zones of 60 mm length and defined the distance between a female and the coldest edge as the centre of the zone she was in.'

I understand the trick but I have some questions:

- Have you been confronted with the situation of a female straddling two zones? If so, how did you then estimate the distance?
- I don't know the behaviour of this insect: was it very mobile? or rather placid? in other words, was it very sensitive to disturbances and therefore very reactive at the time of the counts? and therefore could this behaviour have biased the distance estimates?

Lines 132-134

The expressions 'before egg-laying' and 'until they laid eggs' lead me to wonder about one point, because - once again - I am not familiar with this biological model: the choice was made for this study to work on wild populations and not on reared populations. Is egg production relatively well synchronised in time in natural populations, or is there a large variability in individual behaviour, which could bias the results of the study? Females could have responded less well to the temperature stimulus simply because they were not yet in an active period of searching for an egg-laying site. For the biological models of hemipterans that I am familiar with, we know that the periods of sexual reproduction/laying are highly synchronised in time and that the photoperiod plays an essential role in this synchronisation.

I also have several questions:

- did all the females lay eggs (I understand that they did)?
- Did they lay eggs almost simultaneously or can the behaviour of finding the laying site be (very) variable? (range of duration? min/max?)
- how do we know that the females have laid eggs? is the laying site very well identifiable?

After reading the rest of the manuscript, I realise that you did not specify that the observations of the females were made during the 15 days prior to egg laying. It is here in this sentence (line 132-134) that it should be specified

Line 149

'Three days after oviposition', I don't understand what exactly the authors mean. :

- all the females were left for X days (i.e. same durations for all females) to lay eggs, and three days later they were transferred ?

- or, as soon as oviposition was observed (i.e. variable duration depending on the females), you waited for 3 days and then transferred them individually ?

Lines 150-152

You should specify what type of cabinet you used. I found it difficult to understand that you used a cabinet that allows to have such an accurate temperature gradient. I did not know that this type of climatic cabinet existed. I am rather familiar with climatic cabinet in which the temperature can be varied by programming different cycles of variable duration and temperature.

The temperatures are surprisingly accurate (100th of a degree, really?). Moreover, it seems to me astonishing that no imprecision is given on these values. From a metrological point of view, there is at least some imprecision on the device that produces/maintains the cold, and on the device that measures the temperature.

There is no legend for the axes of the graphs in figure S2.

I assume that the x-axis refers to the length of the rail

Choose to express lengths in cm or mm but use the same unit throughout (currently mm in the text and cm in figures S1 and S2).

Why not choose whole numbers such as 1°C to 7°C form warm range, etc ? as for Figure S1.

I suggest to put the 3 curves together in one graph. This would make it easier to visualize the temperature differences tested between the 3 conditions.

Conditions B and C do not seem to be very different (less than A and B anyway). Why not have chosen conditions C a little colder, i.e. sharper compared to condition B (technical limits?)

Line 153-155

'These temperature ranges… during the natural period of egg care'

For readers who are naive about this biological model, specify here the known spawning period for this species. I assume 'autumn' which would explain the choice of populations and the consistency of the results. One locality has a harsher winter (HNB) than the other (SJNL), with a population that seems to have adapted to this climate.

Line 155-160.

I suggest :

To test whether and how mothers transported their clutch throughout egg development, we then measured the distance (in cm) between the centre of the pile of eggs and the coldest edge of the rail once a week during the 15 following weeks. Because rail handling occurred only weekly in this part of the experiment, we measured the distances between the (center of the pile of) eggs and cold edge directly in cm.

Again, no risk of disturbing the females too much during the rail handling?

Lines 163-165

I think this sentence is incorrect.

For each population, the authors tested 10 females per modality (warm, intermediate, cold ; lines 151-152). I understand that they took each of these 10 females and transferred them to the thermal bridges used before oviposition (0°C => 20°C).

However, the authors probably transferred a random subset of eggs (how many ? variable/fixed number).

Line 166

'to record the date of egg hatching'

I guess there were several eggs transferred/observed, and that they did not hatch at the same time, so it would be more correct to write 'the hatching dates of the eggs'

Line 173-174

'1.5, 22.5, 43.5 173 and 64.5 cm'

these figures appear to be consistent with Figure S2

'2, 25.5, 49 and 72 cm'

these figures do not appear to be consistent with Figure S1

the first point is 0

the second point is below 25

the third is close to 45

the third one seems correct

Line 175

'every hours' 'four aluminium rails'

Line 177

'six aluminium rails'

I suppose that the temperatures measured were a little different from one rail to another, from one hour to the next. This remark is in line with the one I made above (lines 150-152) on the imprecision of the measurements.

Thus, I understand that the point on the graphs in Figures S1 and S3 are average values and that the 'precision' to the 100th is just a choice of the authors to give a value with two decimal places.

*****

Genetic analyses

This paragraph seems perfect

*****

Statistical analyses

Lines 204-209

Exact Mann-Whitney Rank Sum tests is a non-parametric test. It is a modification of the exact test of Wilcoxon Rank-Sum Exact Test to provide an exact test for (classical) Mann-Whitney test. It would be more correct to call this test 'Exact Wilcoxon-Mann-Whitney Test'.

A condition for its application is the independence between the samples (here two populations) to be compared.

As the protocol is described, the (individual) data are all independent: each female was tested separately. The test is therefore applicable. In fact, I do not understand the precision in line 204 'correcting for tied observations'. There is no need to correct for the observations (they are not tied) and intrinsically this type of test is not intended to correct for this effect if it were the case. The 'exact test' simply calculates an 'exact p-value'

Thus, I suggest the sentence :

'We used Exact Wilcoxon-Mann-Whitney Test to test the effect of population on…'

To be consistent with the logic of the chronological order of presentation of the figures, I suggest restructuring the rest of the sentence:

'…to test the effect of population on the amplitude of temperatures at which females were observed before oviposition, the warmest and coldest temperatures reached by females before oviposition, location of females at oviposition, the date of oviposition, the number of eggs produced, the number of weeks until egg hatching and the temperature of the area of egg hatching.

Line 218-219

I think I understand the idea of the multiple comparison test: the principle is to compare the value of one week to the initial temperature, knowing that the position of the nest at that moment is dependent on the position of the nests of the previous weeks. Right ? the test really allows to answer this comparison objective ? To my knowledge, multiple comparison tests take into account all possible pairwise comparisons.

Line 223-226.

I suggest putting the following sentence at the beginning of the 'Statistical analysis' paragraph: 'All the analyses were conducted with the software R v4.1.1 (R Core Team, 2017).'

And then specify the package used for each type of analysis. It might even be useful to specify the function used

e.g. package car, Boxplot function?

Complete the reference for car (https://rdrr.io/cran/car/)

J. Fox and S. Weisberg. An R Companion to Applied Regression. Sage, Thousand Oaks CA, 3rd edition, 2019. URL http://z.umn.edu/carbook.

https://rdrr.io/cran/car/

e.g. package exactRankTests, wilcox.exact function?

https://www.rdocumentation.org/packages/exactRankTests/versions/0.8-34/topics/wilcox.exact

I understand that the authors have used the function wilcox.exact, and probably not the argument 'paired' (contrary to what they write line 204), if not we would see the letter V and not the letter W line 228 for example (Fig. 2A; W = 53,...)'

Line 228

Not sure if it is useful to specify the value of the test statistic (e.g. 53) with a letter describing it (here W for classical Wilcoxon test).

Replace 'P' by 'p-value' to be more explicit

Line 227-231

It would be interesting to give the observed temperature differences (differences between median values) (respectively approx. 5°C and 8°C / Fig 2A and 2B)

Line 232

Higher ? again, give the temperature differences between the two populations: approx. 7°C ?

Line 234

Earlier ? approx. 50 days in average ?

More eggs ? approx. 23 eggs in average ?

Lines 236-247

On each graph in Figure 4, it would be useful to show a horizontal line at the maximum rail temperature (6.96°C, 2.73°C, 1.61°C). This would allow a better visualization of the females that have reached the end of the rail.

Line 245

Add : '....this move started six weeks earlier in SJNL...'

Line 246

I don't understand the meaning of this remark 'Interestingly, egg transport was always associated..' Because it is possible that this is not always the case?

If the females always progress (without turning back) towards the hottest end of the rail (as the graphs show), this implies that each time they build a new nest. Perhaps this is what the authors meant.

Line 249

Add : '...was overall significantly higher in HNB compared to SJNL females (Figure 5A; 52% versus 24%; Likelihood ratio $\chi 2$= 8.52, p-value = 0.004)

Line 252

Add : '...overall significantly higher in females previously maintained under the warmest range of temperature compared to the two others ranges (Figure 5B, 60% versus 25%; Likelihood ratio $\chi 2 = 8.09$, $P = 0.018$).

Line 257-260

I think that the populations were not chosen randomly, and that the results of the genetic analyses were expected. As I wrote earlier, this choice of populations should be made explicit.

It would also seem more logical to present the results of the genetic analyses before the results of the experiments themselves.

The authors mention a species A and a species B ? does this refer to previous work describing the existence of two species ?

Discussion

The discussion is really very interesting.

But reading the lines 321-342, I think that all or part of the information given (existence of two species, hypothesis of local adaptation, etc.) should be taken up again to better contextualise the study in the introduction. This would also help to better understand the generic question (local adaptation versus novel species-specific traits) and make the study described even more interesting. In fact, this is a key question of the study. However, in the presentation of the objectives (lines 109-112), I realise on rereading that this question is somewhat drowned in a series of observations (whether earwig females select an oviposition site etc) which are in fact only the 'how' the authors tried to answer their biological question.

To my mind, the biological question is what kind of strategy earwigs adopt to minimise the risks to their eggs during winter. So, this title would seem more catchy to me:

'Pre-and post-oviposition behavioural strategies in an insect with parental care to protect eggs against extreme winter cold'

DATA ACCESSIBILITY

Unfortunately I did not take the time to go through the files provided in the Zenodo archive in detail. On the other hand, I underline this effort to open up the data!

REFERENCES

Please review carefully the list of reference.

There are many corrections to be made

Line 364 : <scp> Forficula auricularia </scp> ,

Line 369 : Hydroporus spp. , species name to be italicised etc

In the spirit of data open access and interoperability, I encourage authors to add DOI links or links to download PDFs, where possible

Reviewed by **Ana Rivero**, 10 February 2022

Download the review