# Temperature shapes oviposition site selection and post-oviposition egg 1 transport in an insect with parental care (Tourneur et al)

# **PCI Zoology**

This manuscript explores the effect of temperature on the fitness and oviposition site selection behaviour of two different populations of the European earwig. For this purpose, the authors collect adult females from the two different populations in the wild. These females are then provided with 3 (partially overlapping) temperature gradients and their behaviour is quantified.

## **General** appreciation

Although the model is fascinating and the question worth exploring, there are in my opinion several substantial issues that need to be addressed.

The paper has two aims. The first aim is to demonstrate that earwig females select oviposition sites and move eggs around according to the environmental temperature. Their results are interesting in that they clearly show that females move their eggs towards the highest temperature available. However, despite the paper hinging on the potential benefits of egg transport as a strategy for avoiding cold temperatures, the experimental design does not allow a full assessment of the costs of benefits of moving eggs around. Although Figure 5b shows that eggs in the higher temperature gradient do better, I feel that more could have been done to explore this issue further (eg comparing the hatching rate of eggs that are moved around vs eggs that are kept at the highest optimal temperature at each range throughout their development, or comparing gradients with a different distance between the lowest and the highest temperature).

The second aim of the experiments is to establish differences in the behaviour of the two different earwig populations. The large majority of the paper is dedicated to exploring and interpreting these differences. However, very little context is initially given as to why these two particular populations are compared, and what biological insights may eventually stem from the comparison. Both populations experience very similar mean temperatures in the wild (Figure 1) and no further information about other environmental differences between these two populations is provided. When we get to the discussion, we learn that these two populations correspond in fact to two different, previously described, cryptic species (named Species A and Species B). I am not convinced that any meaningful conclusions can be obtained from the comparison of a single population of each species (or subspecies, the authors use both terms alternatively) as genetic and environmental factors will be confounded. In addition, to what extent these differences may reflect differences in female condition at collection (nutrition, phenology, infection status etc) is not known, or discussed.

More background about the species biology would help to understand the logic behind the experimental design. For instance, these females are expected to be virgin at collection and then mated to the males in the lab (there is an unexplained reference to male collection in the m&m)? Or are they expected to be mated in the field? Can females lay unfertilized eggs? How many eggs does an average female lay? Were there any differences in weight between females of the two populations?

The experimental design is complex and I found it quite confusing: 36 females are initially collected, then there's a second part of the experiment (why?) with a further 24 females, which are kept in petridishes instead of aluminum rails. Experiments are climatic cabinets and thermal bridges (why these two different equipments?) and rails of different lengths. All this needs great clarification before the experimental design can be fully appraised.

Below I detail these and other issues in a little bit more detail. I hope the authors find these useful.

### Introduction

**Q1 -** L102-104 – Shorter than what? (or do you mean 'short exposure'?). Fast hatching and accelerated development are not necessarily negative traits, unless they are correlated with eg smaller size and lower lifetime fecundity. Please expand on what is meant by 'alters the immune system' : what is altered and in what way? Is this alteration correlated with an increased risk of infection. This is key as the whole paper hinges on the potential benefits of avoiding cold temperatures.

Q2 - L96 - As the term "population" is used interchangeably with that of species and subspecies across the paper, it is not obvious what this sentence refers to.

Q3 - L111 – Please provide context by explaining whether you expect these behaviours to be population specific. Judging by Figure 1 these two populations seem to be very similar temperature-wise.

#### Materials and Methods

Q4 - L122 – Why collect males?

Q5 - L126 – Please provide background about the species biology. Females are collected from the field and directly used for the experiments. Are these females fertilized? Can unfertilized females lay eggs and would you expect the same type of behaviour in fertilized and unfertilized females? (presumably the latter should not go into any lengths to protect eggs that will never hatch?)

Q6 - L137 – Please clarify what 'second part of the experiment' means and why a second batch of females was collected

Q7 - L139 – These females were not placed in a temperature gradient?

Q8 - L145-146 – At this point it would be useful to understand why some females are maintained in aluminum rails and some in petri-dishes

**Q9** - L160-165 – Sorry I got lost here. What is the difference between a climatic cabinet and a thermal bridge and why are two different equipments used? How is the temperature distributed in a thermal cabinet as opposed to a thermal bridge? What are the practical implications of some rails being shorter than others ?

**Q10** - L163 – Confused here too: you transfer a 'random subset' of 60 females, but 60 is the total number of females in the experiment (L140). Please clarify

**Q11 -** L168 – Why not count the total number of eggs hatched (L208 makes a reference to the number of eggs produced)? How many eggs does a female lay?

**Q12** - L187 – The manuscript uses the term population, subspecies and species interchangeably. A background to the genetic structuring of this species with reference to Species A and Species B needs to be made in the Introduction.

#### Results

**Q13** - L257-260 – This is a very short and not surprisingly brief account of the genetic differences found between these two populations. Please provide more detail. Context must be given in the Introduction.

**Q14** - The results are interpreted as being the result of intrinsic differences between the two "populations". However, these experiments were carried out with females collected from the field, so to what extent the differences observed are the result of differences in e.g female condition, or physiological status at collection, or to differences in the reproductive phenology between the two sites??

**Q15** - In the introduction it is mentioned that in some "populations" eggs need to be exposed to nearzero temperatures to trigger embryo development. Figure 4 shows a dip towards 0 at around weeks 4-5 (in the intermediate and cold range) – could this be it?

**Q16** – Figure 5 – It would be interesting to add a figure of the nb of weeks until hatching by gradient (cold, intermediate, warm)

### Grammar and style suggestions

The first paragraph of the Introduction is very long and could be shortened to make it more focused.

L27 – Reword sentence. I would not expect oviposition site selection and egg transport to be mutually exclusive?

L42 - habitats (instead of habitat)

L76 – predation (instead of predations)

L82 – Take off "by holding them in their mouth": it references a very specific form of egg transport while, presumably, the statement may also apply to other species with other transport strategies

L87 – until they hatch

L89 - ,the application of chemical compounds....and fierce protection....

L101 – egg exposure

L111 – egg age

L116 – juvenile production

L129 – I found the term thermal "bridge" confusing, I looked for the reference in the manufacturer's website and this is described as a thermoelectric plate. Wouldn't it make more sense to call it like that?

L138-144 – This should be said earlier (L125)

L152 – 'with eggs deposited' replace with new sentence 'Eggs were deposited in the middle of the range corresponding to 5.22°C (warm)....'

L215 – interaction

L257 – Reword 'each population contained a different member of...' is not what you want to say here.

L242 – week

L247 - 'new nest in which the eggs moved' - please reword

Figure 4 – The \*\*\* for the p values (comparing each point to the reference point) clutter the figure and are not helpful: the standard error bars already do a pretty good job of showing us which values are significant. Set up reference horizontal lines for min and max temperatures within the thermal range, this will provide a useful visual image of the range of temperatures explored within the range provided.

Figure 5 legend – 'Effect of population (A) and thermal constraint (B) on the percentage of females with at least one hatched. Effect of population on the location of the eggs at the time of hatching (C) and on the number of weeks between ovipositon and egg hatching (D)'

Figure S2 – Please provide axis labels