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Temperature shapes oviposition site selection and post-oviposition egg transport in an insect with parental care

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ABSTRACT

Depositing eggs in an area with adequate temperature is often crucial for mothers and their offspring, as the eggs are immobile and therefore cannot avoid exposure to sub-optimal temperatures. However, the importance of temperature on oviposition site selection is less clear when mothers have the capability to avoid these potential adverse effects by both moving their eggs after oviposition and providing other forms of egg care. In this study, we addressed this question in the European earwig, an insect in which mothers care for the eggs during several months in winter and often move them during this period. Using 60 females from two Canadian populations (St John's and Harvey station) set up under controlled thermal gradients, we demonstrated that earwig females both select oviposition sites according to temperature and move their eggs after oviposition to reach warmer environmental temperatures. While this set of behavioural thermoregulation is present in the two studied populations, its modality of expression was population-specific: St John's females explored greater ranges of temperatures before oviposition, laid their eggs in warmer areas, and moved their eggs quicker toward warm locations. Overall, our study reveals that earwig females have evolved both pre-and post-oviposition behavioural strategies to mitigate the risks inherent to tending eggs during winter. More generally, it also reveals that egg care and egg transport do not prevent behavioural thermoregulation via oviposition site selection and highlights the diversity of behaviours that insects can adopt to enhance their tolerance to global climate change.

Keywords: Dermaptera, Egg brooding, Overwinter, Parental care, Thermal preferences

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INTRODUCTION

Oviposition site selection shapes the fitness of most oviparous species (Thompson 1988, Refsnider and Janzen 2010). This is because choosing the right place to deposit eggs typically provides direct and indirect benefits to egg-laying females, their current eggs, and their future juveniles. This behaviour may first limit the high risk of predation on adult females that is inherent to their lack of mobility during oviposition, as well as favour their direct access to specific food sources necessary for oviposition. For instance, females of the water strider *Aquarius paludum insularis* avoid ovipositing in sites containing a predator attacking adults only (Hirayama and Kasuya 2013), while females of the orange tip butterfly *Anthocharis cardamines* deposit eggs on plants that have high nutritional value for adults but poor nutritional value for their larvae (Courtney 1981). Oviposition site selection can also help future offspring by favouring proximity to suitable habitat and providing direct access to resources necessary for juveniles. This is the case in the mosquitoes *Culiseta longiareolata* and *Anopheles punctipennis*, where females avoid laying eggs in pounds containing predators or competitors of their future larvae (Petranka and Fakhoury 1991, Spencer et al. 2002, Silberbush and Blaustein 2011) and in the sandpaper frog *Lechriodus fletcher* and several lady beetle species, where females choose oviposition sites that contain the largest quantities of nutritive resources for their future larvae (Sicsú et al. 2020, Gould et al. 2021). Finally, oviposition site selection can provide direct benefits to eggs by limiting the risks of predators finding the eggs, eggs drying out or egg exposure to toxic pollutants. In the aquatic beetles *Hydroporus incognitus* and *H. nigrita*, for example, females select waters where no fish can predate on their eggs (Brodin et al. 2006), while in the damselfly *Lestes macrostigmata*, females prefer to lay their eggs on plants growing in the deeper parts of temporary ponds to minimize the risk of egg desiccation at the end of the drought season (Lambret et al. 2018).

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Similarly in the gray treefrog *Hyla versicolor* and two mosquito species, females avoid ponds contaminated with pesticides and chemical pollutants that could be toxic to their eggs (Takahashi 2007, Muturi et al. 2014).

The effect of temperature on egg development and survival is another potential driver of oviposition site selection by females. Exposure to extreme temperatures can indeed damage living organisms of all ages, through alterations in their physiology, immunity and behaviour, which may overall reduce their fitness and/or lead to premature death (Hance et al. 2007, Dillon et al. 2009, Fey et al. 2015, Filazzola et al. 2021)**.** These effects can be particularly strong in eggs because they are immobile and thus unable to escape from environmental temperatures, their shell provides limited thermal protection, and the development and survival of embryos (contained in eggs) are generally sensitive to subtle changes in surrounding temperatures (Wang et al. 2010, Nicolai et al. 2013, Mortola and Gaonac'h-Lovejoy 2016, Cordero et al. 2018, Yang et al. 2018). As a result, females of many species select oviposition sites according to optimal temperatures for their eggs, such as in the toad-headed agama lizard *Phrynocephalus przewalskii* (Li et al. 2018), the solitary red mason bee *Osmia bicornis* (Ostap-Chec et al. 2021) and the flat-rock spiders *Hemicloea major* (Pike et al. 2012).

By contrast, the importance of temperature on oviposition site selection becomes less clear when mothers can transport their eggs from one location to another, as it may allow them to secondarily adjust the temperature of their eggs throughout development. This transport, included in a broader phenomenon called egg brooding in insects (Machado and Trumbo 2018), is known to allow parents to limit the risk of egg predations or promote egg oxygenation in aquatic species. For instance in the golden egg bug *Phyllomorpha laciniata*,

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females lay their eggs on conspecifics whose mobility improves the avoidance of egg parasitoids (Carrasco and Kaitala 2009). Similarly in the water bug *Abedus herbeti*, females lay their eggs on the males' back, which then move (with the eggs) to ensure that they receive a proper level of oxygenation (Smith 1997). However, whether active egg transport (e.g. parents actively moving their eggs from one location to another by holding them between their mouth parts) could be an adaptive behaviour by which mothers adjust the thermal needs of the embryo during development remains unexplored. Yet, this process could operate in the European earwig *Forficula auricularia*. In this complex of cryptic species (Wirth et al. 1998, González-Miguéns et al. 2020), females usually lay their eggs just before winter (or during winter), and then remain with their eggs until hatching (Lamb 1976). During this period, females provide extensive forms of egg care including, for instance, grooming behaviours to remove pathogens and applying chemical compounds on eggshells (Liu et al. 1997, Boos et al. 2014) and fierce protection against predators (Thesing et al. 2015, Van Meyel et al. 2019). Moreover, females are frequently observed transporting their eggs from one location to another by holding them individually between their mouth parts (Diehl and Meunier 2018, Meunier et al. 2020).

Recent results and observations suggest that earwig eggs could benefit from temperature-dependent oviposition site selection and temperature-dependent maternal transport during development. First, the duration of egg development in winter varies from three weeks to several months depending on the population (Ratz et al. 2016, Tourneur 2018). This suggests that eggs could be exposed to extremely low (and damaging) temperatures for a very long time if females do not select the oviposition site accordingly and/or do not transport the eggs during development - particularly in natural populations in North America (Gingras and Tourneur 2001, Tourneur and Meunier 2020). Second, eggs exposure to cold can

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have both short and long-term effects on fitness-related traits in the European earwig: shorter exposure to cold speeds up egg hatching, accelerates the development of the resulting offspring to adulthood, and alters the immune system of the resulting adults (Körner et al. 2018). Finally, numerous laboratory breeding trials indicate that eggs of some populations need to be exposed to near-zero temperatures to trigger embryo development and then the temperature needs to be increased to continue this development, while others do not (e.g. Wirth et al. 1998; Meunier et al. 2012; Ratz et al. 2016; Tourneur and Meunier 2020).

In this study, we investigated whether European earwig females select an oviposition site according to environmental temperature, move their eggs depending on both environmental temperature and eggs age, and whether these behaviours are population specific. We set up 60 females from two Canadian populations in experimental devices allowing thermal gradients and then recorded 1) the range of temperatures explored by each female during the 15 days preceding oviposition, 2) the temperature at which females laid their eggs, 3) whether and how mothers transported their clutch along three thermal gradients throughout egg development and finally, 4) how these gradients affected juveniles production. Overall, our results reveal that environmental temperature shapes female exploration before oviposition, oviposition site selection, egg transport during development and the production of juveniles and that both oviposition site selection and the dynamic of egg transport during development are population specific.

MATERIAL AND METHODS

Earwig sampling and experimental process

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We field sampled earwig males and females in Harvey station (New Brunswick, Canada; HNB) and St John's (Newfoundland and Labrador, Canada; SJNL) in September 2020. We immediately brought back these adults to the laboratory and set them up in plastic containers lined with wet sand and containing two small shelters. We maintained these containers at room temperature under natural day: light until October 2020. At that time, we transferred 36 females (18 from HNB and 18 from SJNL) to the middle of individual aluminium rails (18 x 18 x 720 mm = height x width x length) lined with a layer of wet sand and closed with a plastic cover. We then deposited the aluminium rails on thermal bridges (AHP-1200CPV-Thermoelectric Cooloing America Corporation. 4049W Schubert Avenue. Chicago IL. USA) with temperatures linearly ranging from 0°C to 20°C (Figure S1) and insulated with thick foam to ensure complete darkness. To determine the temperature range explored by each female before egg-laying, we then measured the distance between these females and the coldest edge of the rail daily until they laid eggs. 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. Finally, to increase the number of females with eggs available for the second part of the experiment, we also isolated 24 additional females (12 from HNB and 12 from SJNL) in Petri dishes (diameter 10 cm) lined with wet sand and maintained at 10°C under complete darkness until egg production. From field sampling to oviposition, we fed the 36 and 24 females twice a week with fresh carrots placed on a soaked cotton pad, plus an artificial diet composed of 1/3 dry power of egg yolk, 1/3 bee collected pollen (Community Apiaries. 576 Plymouth road, Richmond Corner,New-Brunswick. Canada) and 1/3 cricket powder (Entomo Farms. 31 industrial drive Norwood, Ontario. Canada).

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Three days after oviposition, we transferred the 36 mothers and eggs previously maintained in aluminium rails and the 24 mothers and eggs previously maintained in Petri dishes to the middle of shorter aluminium rails (18 x 18 x 660 mm) also lined with a layer of wet sand and closed with a plastic cover to ensure complete darkness. We then deposited these aluminium rails into a climate cabinet (insulated with 2 thick foam to ensure complete darkness; 24h) providing either warm (0.87°C to 6.96°C; 10 HNB and 10 SJNL females), intermediate (-3.64°C to 2.73°C; 10 HNB and 10 SJNL females) or cold (-4.45°C to 1.61°C; 10 HNB and 10 SJNL females) non-linear thermal ranges (Figure S2), with eggs deposited at 5.22°C, 1.18°C and 0.11°C, respectively. These temperature ranges encompass the above-ground variation in temperatures measured in these populations during the natural period of egg care (Gingras and Tourneur 2001)(Figure 1). To test whether and how mothers transported their clutch throughout egg development, we then measured the distance between the centre of the clutch 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. Note that the rails used in the climatic cabinet were slightly shorter than those used in the thermal bridge because they had to fit into the climatic cabinet which was shallower.

Fifteen weeks after oviposition, we transferred the shorter aluminium rails of a random subset of 10 mothers and eggs per thermal range and population (i.e. a total of 60 mothers and eggs) to the thermal bridges used before oviposition (see above). We then checked every rail daily to record the date of egg hatching, the location of the clutch at hatching (based on the distance between the centre of the clutch and the coldest edge of the rail) and more generally, to determine whether at least one egg eventually hatched. We did not feed females

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from the day of oviposition to egg hatching, as earwig mothers typically stop foraging during this period (Kölliker 2007).

We measured the thermal gradients present in the aluminium rails using thermometers (4 channel K type Thermometers SD. Amazon. CA) connected to four probes located in the sand either at 2, 25.5, 49 and 72 cm (longer aluminium rails) or 1.5, 22.5, 43.5 and 64.5 cm (shorter aluminium rails) of the coldest edge of the rails. The recordings occurred every hour during the entire experiment and focused on four aluminium rails for measurements conducted 15 days before oviposition and after the 15 weeks following oviposition (thermal bridges), and six aluminium rails distributed among the three thermal constraints for measurements conducted during the 15 weeks following oviposition (climate cabinet). We then used these recordings to compute linear (thermal bridges) and non-linear (climate cabinet) equations linking distance to temperature (Figures S1 and S2), which we then used to obtain the temperature of the location of the females and their eggs.

Genetic analyses

To determine whether HNB and SJNL females belonged to the same subspecies of the European earwig (Wirth et al. 1998, González-Miguéns et al. 2020), we analysed the Cytochrome Oxidase I (COI) gene of 6 females per population. This number of females is enough to robustly assess the origin of an entire population, as previous work demonstrated that the two species do not co-exist in the same population (Wirth et al. 1998, Guillet et al. 2000b). Genomic DNA was extracted from whole individuals with the NucleoMag® Tissue kit (Macherey Nagel) following manufacturer instructions. The COI gene (658 bp) was amplified from each individual using routine barcoding primers LepF and LepR (Hajibabaei et al. 2006).

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PCR amplifications was performed with the DreamTaq® PCR Master Mix Kit (Thermo Scientific) using an ESCO Swift Maxi® thermocycler with an initial denaturation step at 95°C (2 min) followed by 35 cycles at 95°C (45 sec), 52°C (60 sec) and 72°C (90 sec) and finally an extension step at 72°C (10 min). PCR products were purified, and Sanger sequenced in both direction using an ABI 3730XL sequencing system (Thermo Fisher Scientific) at Eurofins Genomics Company. Sequences obtained were corrected using Geneious® 9.1.8. The subspecies status of each female was identified from NCBI databases using the BLAST tool (Altschul et al. 1990). The BLAST results were ranked by percent identity and the reference sequences with at least 100% identity to our sequences were used to assign the subspecies status. All sequences obtained in this study have been submitted to GenBank; their accession numbers are OL512959 to OL512964 for the 6 HNB females and OL512965 to OL512970 for the 6 SJNL females.

Statistical analyses

We used a series of eight Exact Mann-Whitney Rank Sum tests correcting for tied observations to test the effect of population on the warmest and coldest temperatures reached by females before oviposition, the amplitude of temperatures at which females were observed before oviposition, the location of females at oviposition, the date of oviposition, the number of eggs produced, the temperature of the area of egg hatching and the number of weeks until egg hatching. Potential changes in the location of the clutch after oviposition were analysed using Linear Mixed-effects models in which the week (1 to 15), population (SJNL and HNB), range of temperature (warm, intermediate and cold) and the interaction between these three factors were entered as explanatory factors, while the ID of each female was used as a random factor

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to correct for multiple measurements. To interpret the significant interaction between these three factors, we first run the same models for each range of temperature (thus without temperature range as an explanatory factor) and when the interactions between weeks and population were significant, we conducted post hoc pairwise comparisons between the initial location of the eggs and their location at each week using Exact Mann-Whitney Rank Sum tests. To correct for multiple testing, the P-values of these pairwise comparisons were adjusted using the False Discovery Rate (fdr) method (Benjamini and Hochberg 1995). The likelihood to produce at least one larva was tested using a Generalized linear model with binomial error distribution. In this model, the presence of at least one hatched juvenile was used as a binary response (1 or 0), while the population, the range of temperatures and their interaction were entered as explanatory factors. The analyses were conducted with the software R v4.1.1 (R Core Team, 2017) loaded with the packages *car* (Fox and Weisberg 2019), *exactRankTests* (Hothorn and Hornik 2021), *emmeans* (Lenth 2021) and *DHARMa* (Hartig 2020).

RESULTS

The maximum temperature range at which each female was observed during the 15 days preceding oviposition was greater in SJNL compared to HNB females (Fig 2A; $W = 53$, $P < 0.001$). Interestingly, the warmest location at which females have been observed was larger in SJNL compared to HNB females (Fig 2B; $W = 2$, $P < 0.001$), whereas the coldest location was independent of the population (Fig 2C; $W = 135.5$, $P = 0.404$).

The temperature of the area where females laid their eggs was higher in SJNL compared to HNB females (Fig 3A; $W = 17.5$, $P < 0.001$). Moreover, HNB females produced

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their eggs earlier in the season (Fig 3B; W = 38, P < 0.001) and laid overall more eggs (Fig 3C; W = 261, P < 0.001) compared to SJNL females.

During the 15 weeks following oviposition, the location of the eggs varied according to the weeks, population and temperature range (Figure 4; Triple interaction between weeks, population and range of temperature: χ^2_5= 33.13, P < 0.001). Egg location depended on an interaction between weeks and population in the warmest range of temperatures (χ^2= 12.43, P < 0.001), which reveals that SJNL females moved their eggs towards warmer locations about 7 weeks after oviposition, whereas HNB females did not specifically target warmer locations (Figure 4). Moreover, egg location also depended on an interaction between weeks and population in both the intermediate and coldest range of temperatures (χ^2= 16.53, P < 0.001 and χ^2= 6.27, P = 0.012, respectively), showing that both SJNL and HNB females moved their eggs towards warmer locations but that this move started earlier in SJNL compared to HNB populations (Figure 4). Interestingly, egg transport was always associated with the construction of a new nest in which the eggs were moved.

Finally, the likelihood to produce juveniles (i.e. that at least one egg hatched) was overall higher in HNB compared to SJNL females (Figure 5A; Likelihood ratio χ^2= 8.52, P = 0.004), overall higher in females previously maintained under the warmest range of temperature compared to the two others ranges (Figure 5B, Likelihood ratio χ^2= 8.09, P = 0.018), and independent on an interaction between these two factors (Likelihood ratio χ^2= 3.49, P = 0.175). When they hatched, the temperature of the place at which the first juveniles has been observed was not population-dependent (Figure 5C; W = 60, P = 0.396), while the eggs took overall more time to develop in HNB compared to SJNL females (Figure 5D; W = 80.5, P = 0.012).

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The COI analyses revealed that each population contained a different member of the complex of species composing the European earwig. All (6/6) HNB females belonged to the species "*Forficula auricularia* A" and all (6/6) SJNL females belonged to the species "*Forficula auricularia* B".

DISCUSSION

While temperature often drives oviposition site selection to ensure that the lack of egg mobility does not result in exposure to sub-optimal or extreme temperatures (Pike et al. 2012, Li et al. 2018, Ostap-Chec et al. 2021), the role of temperature on oviposition site selection is less clear when mothers have the capability to transport the eggs after oviposition. Here, we show in the European earwig that this capability does not prevent females from selecting oviposition sites according to temperature, that egg transport can indeed help mothers to adjust egg temperature after oviposition and, interestingly, that the selected temperatures by mothers are population-specific. In particular, we show that St John's (Newfoundland and Labrador, Canada) females explore a greater ranges of temperatures before oviposition, lay their eggs in warmer areas, move their eggs quicker toward warm locations when suddenly exposed to cold, but were overall less likely to produce juveniles under our experimental conditions compared to Harvey station (New Brunswick, Canada) females.

Our data first reveal that earwig females of the two populations chose oviposition sites with temperatures above 10°C, which is in stark contrast to the sub-zero temperatures measured above ground in these two locations during the oviposition period (Figure 1). This finding highlights the fact that some ectotherms, such as earwigs, cannot only develop physiological mechanisms to withstand their own freezing during wintering (Toxopeus and

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Sinclair 2018) but also need to find places exhibiting a large thermal difference from the ground surface to establish their nests and deposit their eggs (Leather et al. 1993). Multiple strategies have been reported in insects to achieve this goal, among which digging nests or burrows to get efficient isolation from above-ground temperatures (Davis et al. 2015, Huang et al. 2020), hiding under rocks and nesting into trunks to use the thermal inertia of the substrate as a shelter (Brower et al. 2009, Trájer et al. 2014), and nesting close to human constructions (e.g. underground pipeline, houses, building walls, etc) to benefit from their constant source of heat during winter (Labrie et al. 2008, Trájer et al. 2014). These three strategies are likely to be adopted by the European earwig, as earwig adults are frequently found in human habitations, underground burrows and under rock and trunks during winter (Goodacre 1997, Gingras and Tourneur 2001, Kölliker and Vancassel 2007, Binns et al. 2021). Moreover, a study suggests that the nest proximity to human constructions could be an effective overwintering strategy for Canadian populations of earwigs (Goodacre 1997).

After oviposition, we showed that earwig mothers of the two populations experimentally exposed to temperatures below 5°C transported their eggs to warmer locations and that the eggs subsequently kept above 5°C (i.e. a temperature reachable in the warmest temperature range) were most likely to hatch. These results overall support the hypothesis that egg transport is an adaptive post-oviposition behaviour by which earwig mothers adjust the thermal needs of their embryos. This discovery sheds light on a new strategy in female insects that overwinter with their eggs for coping with temperature changes (Lee and Dellinger 1991, Sinclair et al. 2003), and emphasizes that the potential costs associated with building a nest and finding a burrow in another location during winter do not prevent such egg transport (Danks 2002). More generally, the egg transport capability of earwig females combined with their oviposition site selection based on temperature could

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explain, at least in part, how such an insect with long-overwintering eggs has been able to invade extremely cold climates (Guillet et al. 2000a, Quarrell et al. 2018, Hill et al. 2019, Tourneur and Meunier 2020).

Interestingly, St John's females transported their eggs much earlier than Harvey station females. This suggests that the cold tolerance of eggs in the first few weeks after egg laying is less effective at St John's than at Harvey station due to either population-specific egg quality, as reported in numerous oviparous species (Jing and Kang 2003, Stålhandske et al. 2015), or population-specific egg development time, as eggs sensitivity to cold often increases when they are closer to hatching (e.g. Gray, 2009). In line with the last scenario, egg development time greatly varies between natural populations of the European earwig (Tourneur and Gingras 1992, Meunier et al. 2012, Ratz et al. 2016, Tourneur and Meunier 2020), and our data shows that eggs produced by St John's females indeed take less time to hatch compared to eggs produced by Harvey station females when reared in similar conditions. On a more general level, it is typically expected that a population-specific cold tolerance of eggs should lead to population-specific timing of oviposition, the less cold-tolerant eggs being laid later in winter than the more cold-tolerant eggs (Tourneur and Meunier 2020). This is again in line with our results: St John's females laid eggs about one month after Harvey station females. Overall, our results thus demonstrate that winter temperatures are an important factor in the pre-and post-oviposition strategies of earwig females.

Overall, our data reveal population-specific patterns regarding the thermal preferences of females before and at the time of oviposition, and their egg transport dynamics. The European earwig is a complex of cryptic species, for which genetic divergence and reproductive isolation are well established (Wirth et al. 1998, Guillet et al. 2000a, González-

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Miguéns et al. 2020) but the specificity of life-history traits remains largely unexplored. The only reported species-specific trait refers to their reproduction, with females of species “A” producing one clutch and females of species “B” producing two clutches (Wirth et al. 1998, Tourneur 2018). However, some studies revealed that the number of clutches produced by a female is not always fixed and can vary between populations of the same *F. auricularia* species (Tourneur and Gingras 1992, Ratz et al. 2016), as well as depend on numerous parameters acting during the early life of an earwig female (Meunier et al. 2012, Meunier and Kölliker 2012, Wong and Kölliker 2014). This could suggest that local adaptations, rather than membership of species 'A' or 'B', might be the prime driver of life-history traits in this complex of species (Quarrell et al. 2018, Tourneur and Meunier 2020). In this study, our genetic analyses demonstrate that females of Harvey station belong to species “A” and females of St John’s to species “B” (note that this is the first time that species “B” is found in Canada outside British Columbia) which could support species-specific thermal preferences and dynamic of egg transport, but both Harvey station and St John’s exhibit very close temperature regimes (Figure 1), which could also suggest that species-specific traits are a likely explanation. Further studies such as transplant experiments are thus needed to disentangle whether the reported patterns reflect local adaptation and/or novel species-specific traits within the European earwig complex (Wirth et al. 1998, Guillet et al. 2000a, González-Miguéns et al. 2020).

Whereas ectotherms typically have very limited to no physiological capacities to limit the costs of exposure to extreme temperatures via internal regulations (Stevenson 1985), some have evolved forms of behavioural thermoregulation to avoid locations with extreme temperatures (Lee Jr. 1991, Terrien 2011). In this study, we demonstrate that ectotherm females of the European earwig adopt a broad set of behavioural thermoregulations when tending their eggs overwinter. In particular, they select oviposition sites exhibiting specific

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temperatures and transport their eggs to warmer locations when experimentally exposed to cold. While this set of behavioural thermoregulations is present in the two studied populations, its modality of expression varied between Harvey station and St John's – either as a result of local adaptation or as species-specific traits among the complex of species composing the European earwig (Wirth et al. 1998, Guillet et al. 2000a, González-Miguéns et al. 2020). Overall, our findings emphasize that earwig females have evolved behavioural strategies to mitigate the risks inherent to tending eggs during several months and extreme winter cold. More generally, they highlight the diversity of behaviours that insects can adopt to cope with extreme temperatures, and which could favour their tolerance to the effects of moderate climate change.

DATA ACCESSIBILITY

The complete data set and R script are archived and freely available in the open data repository Zenodo (https://doi.org/10.5281/zenodo.5718267).

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Figure 1 – Locations of Harvey Station (HNB, Canada; Blue) and St Johns (SJNL, Canada; Orange), and their monthly variation of temperatures recorded from 1970 to 2000. Dots depict mean monthly temperatures, with whiskers extending from minimum to maximum mean temperatures. These data were extracted from the WorldClim database.

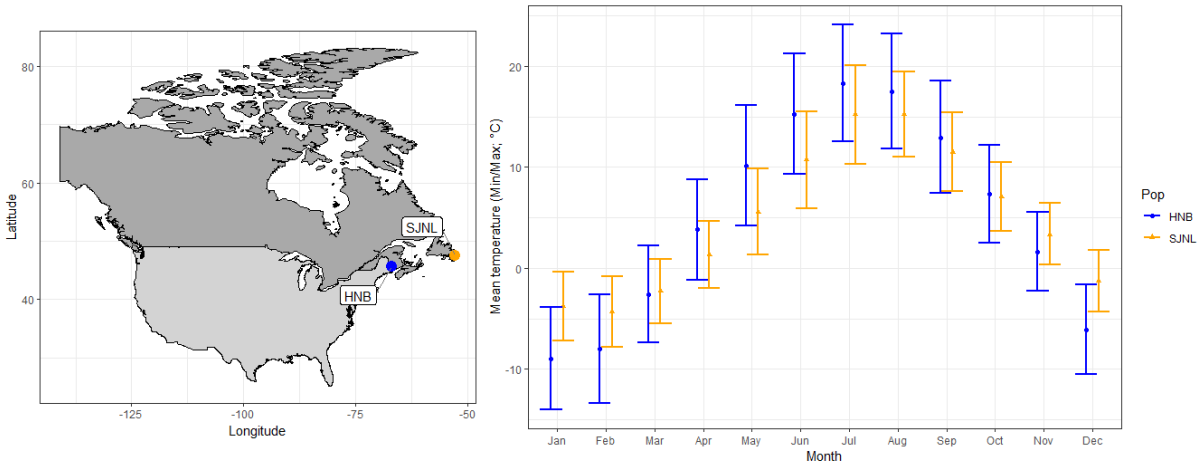

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Figure 2 – Effect of population on the (A) maximum temperature range, (B) warmest location and (C) coldest location at which females were observed during the 15 days preceding oviposition. Box plots depict median (middle bar) and interquartile range (light bar), with whiskers extending to 1.5 times the interquartile range and dots representing experimental values. ***P<0.001; ns P> 0.05. HNB: Harvey Station. SJNL: St Johns.

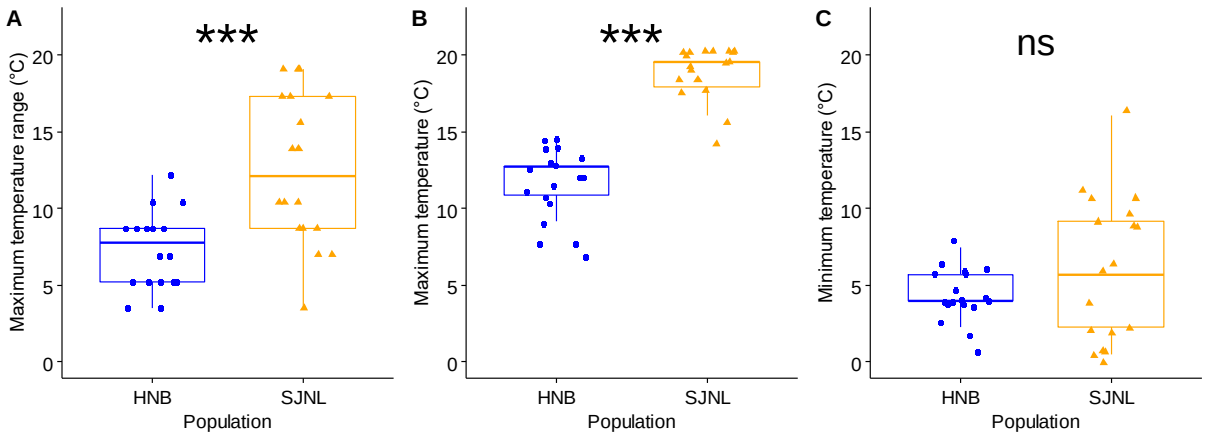

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Figure 3 – Effect of population on the (A) temperature of the location at which females laid eggs, (B) day at which females laid eggs, and the (C) number of eggs produced. Box plots depict median (middle bar) and interquartile range (light bar), with whiskers extending to 1.5 times the interquartile range and dots representing experimental values. The oviposition day corresponds to the number of day after the 16 November 2020. ***P<0.001. HNB: Harvey Station. SJNL: St Johns.

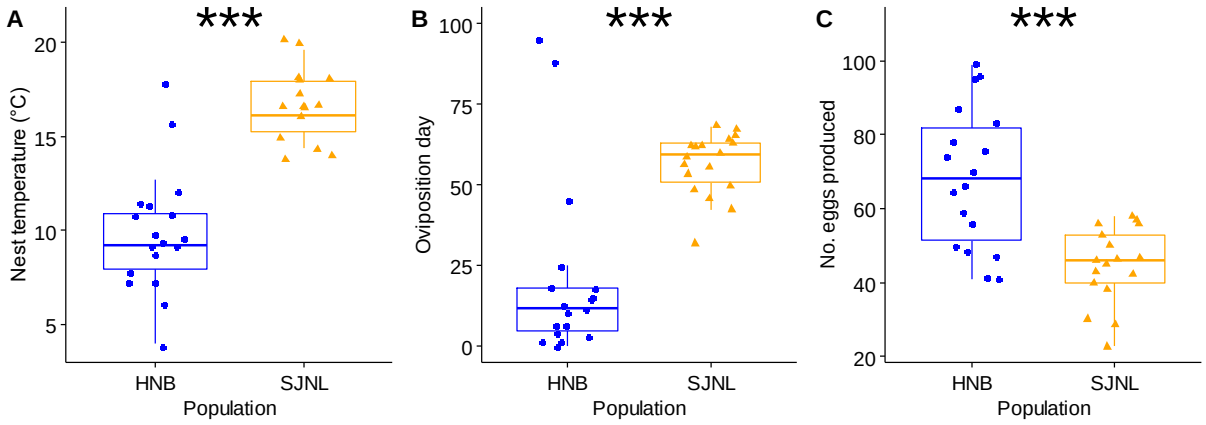

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Figure 4 – Effect of population and thermal ranges on egg location during the 15 weeks following oviposition. Brackets indicate thermal ranges. Dashed lines indicate the location of mothers and eggs when the experiment was set up. Grey dots are raw values for each clutch of eggs. Coloured dots are mean values ± SE per week. Exact Mann-Whitney tests to compare values of each week to the initial temperature: **P<0.01, *P<0.05. P-values corrected for multiple comparisons. HNB: Harvey Station. SJNL: St Johns.

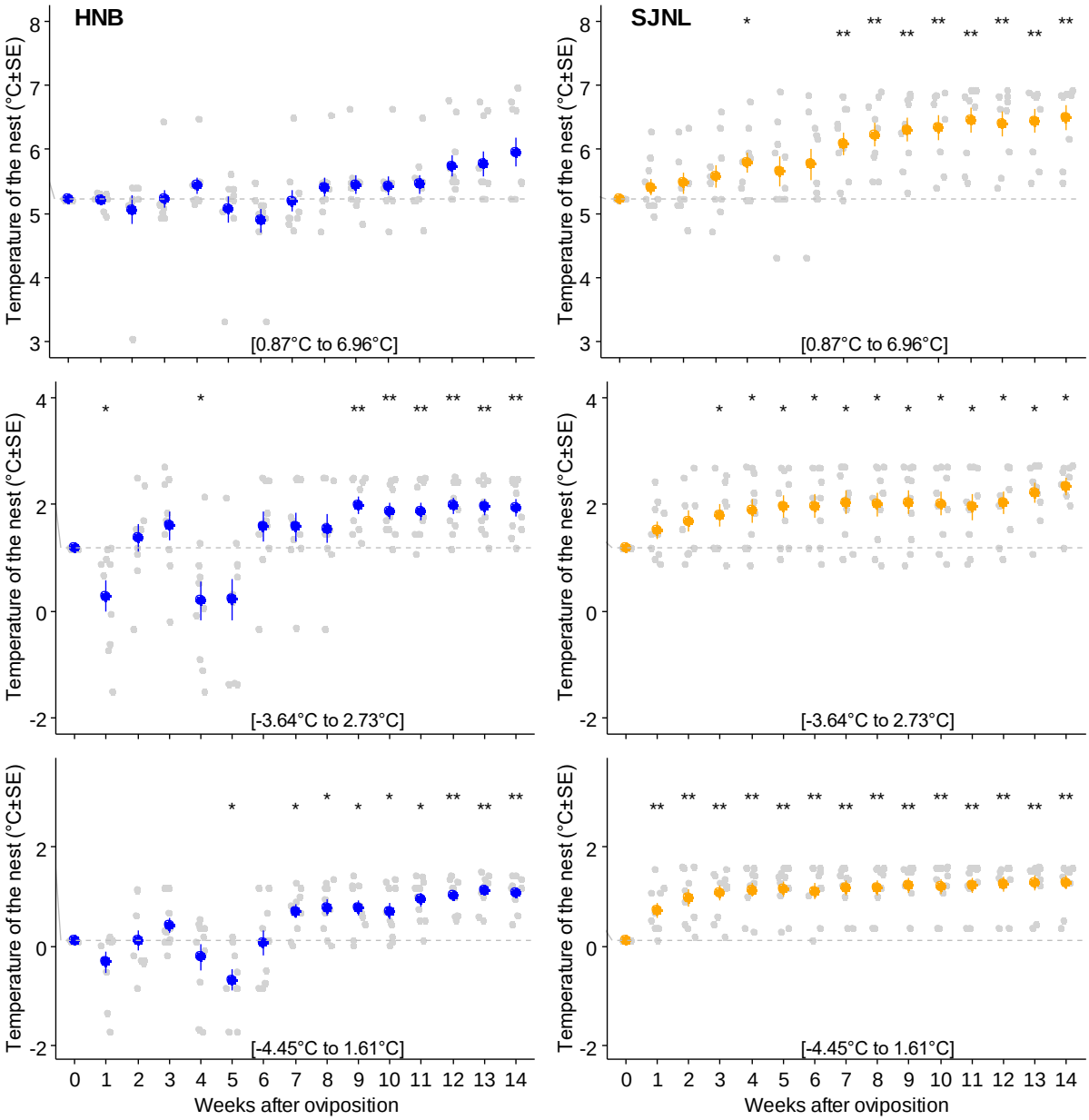

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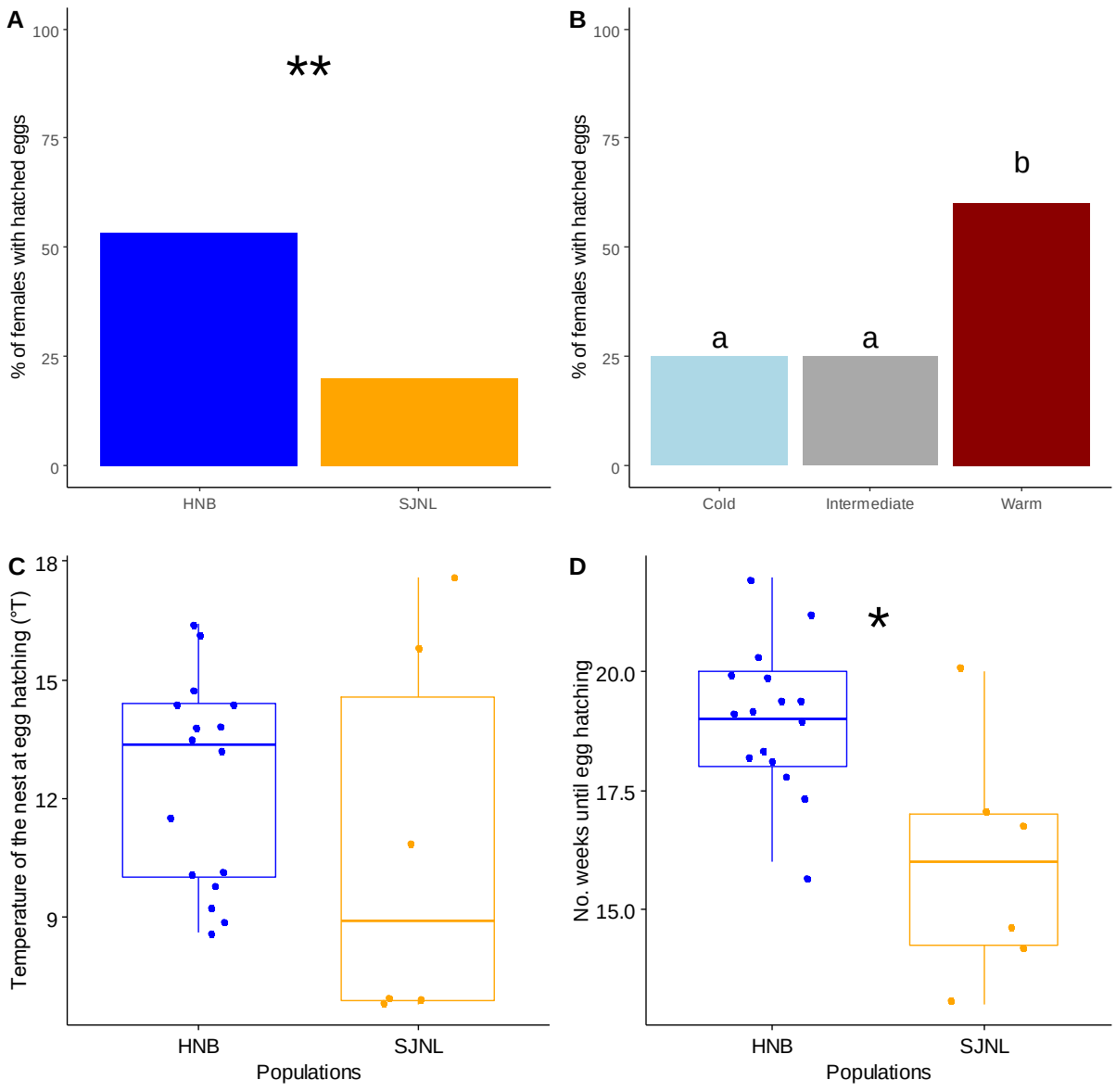
Figure 5 – Effect of (A, B) population and thermal constraints on the percentage of females with at least one hatched egg, (C) population on the location of eggs at egg hatching and of (D) population on the number of weeks between oviposition and egg hatching – when present. Figures C and D only involved females from the warmest range of temperature, as their number were too limited in the other range of temperatures. Box plots depict median (middle bar) and interquartile range (light bar), with whiskers extending to 1.5 times the interquartile range and dots representing experimental values. ns: P>0.05; *P<0.05; **P<0.01; · p = 0.07. Different letters indicate P<0.05. HNB: Harvey Station. SJNL: St Johns.



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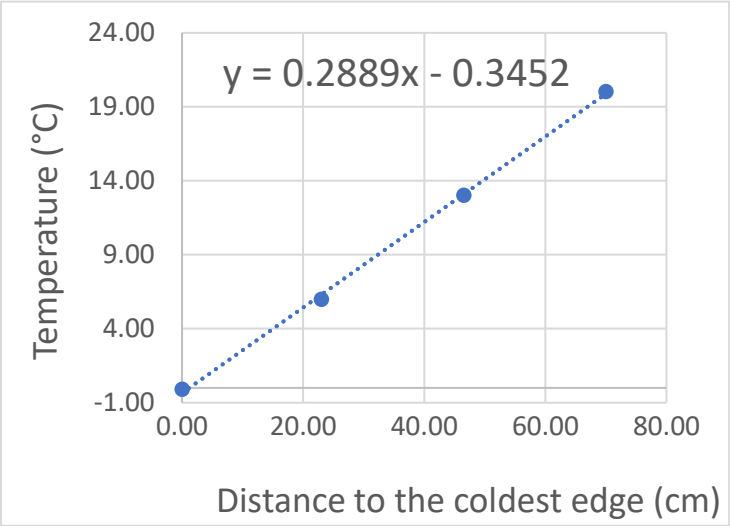
SUPPLEMENTARY MATERIAL

Figure S1 – Thermal gradient and equation of the thermal bridge – measurements before and at oviposition.



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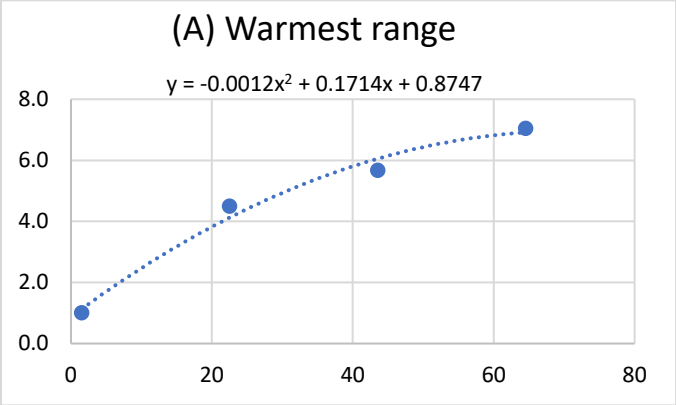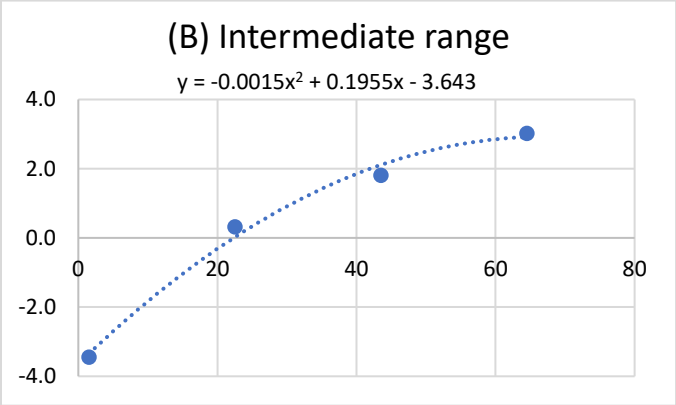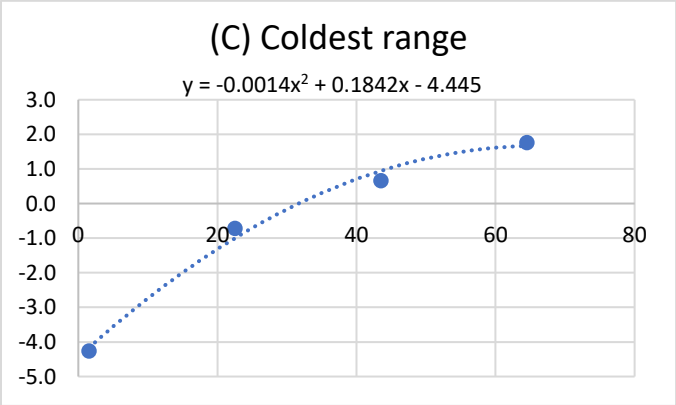
Figure S2 – Thermal gradients and equations of the (A) warmest, (B) intermediate and (C) coldest temperature ranges in the climate cabinet – measurements after oviposition.