

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

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ABSTRACT

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

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

INTRODUCTION

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

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

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

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

78 females lay their eggs on conspecifics whose mobility improves the avoidance of egg
79 parasitoids (Carrasco and Kaitala 2009). Similarly, in the water bug *Abedus herbeti*, females lay
80 their eggs on the males' back, which then move (with the eggs) to ensure that they receive a
81 proper level of oxygenation (Smith 1997). However, whether active egg transport (e.g. parents
82 actively moving their eggs from one location to another by holding them between their mouth
83 parts) could be an adaptive behaviour by which mothers adjust the thermal needs of the
84 embryo during development remains unexplored. Yet, this process could operate in the
85 European earwig *Forficula auricularia*. In this complex of cryptic species (Wirth et al. 1998,
86 González-Miguéns et al. 2020), females usually lay their eggs just before ~~winter~~ (or during
87 winter), and then remain with their eggs until hatching (Lamb 1976). During this period,
88 females provide extensive forms of egg care including, for instance, grooming behaviours to
89 remove pathogens and applying chemical compounds on eggshells (Liu et al. 1997, Boos et al.
90 2014) and fierce protection against predators (Thesing et al. 2015, Van Meyel et al. 2019).
91 Moreover, females are frequently observed transporting their eggs from one location to
92 another by holding them individually between their mouth parts (Diehl and Meunier 2018,
93 Meunier et al. 2020).

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

102 have both short and long-term effects on fitness-related traits in the European earwig: shorter
103 exposure to cold speeds up egg hatching, accelerates the development of the resulting
104 offspring to adulthood, and alters the immune system of the resulting adults (Körner et al.
105 2018). Finally, numerous laboratory breeding trials indicate that eggs of some populations
106 need to be exposed to near-zero temperatures to trigger embryo development and then the
107 temperature needs to be increased to continue this development, while others do not (e.g.
108 Wirth et al. 1998; Meunier et al. 2012; Ratz et al. 2016; Tourneur and Meunier 2020).

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


MATERIAL AND METHODS

121 Earwig sampling and experimental process 


122 We field sampled earwig males and females in Harvey station (New Brunswick, Canada; HNB)
123 and St John's (Newfoundland and Labrador, Canada; SJNL) in September 2020. We
124 immediately brought back these adults to the laboratory and set them up in plastic containers
125 lined with wet sand and containing two small shelters. We maintained these containers at
126 room temperature under natural day-light until October 2020. At that time, we transferred
127 36 females (18 from HNB and 18 from SJNL) to the middle of individual aluminium rails (18 x
128 18 x 720 mm = height x width x length) lined with a layer of wet sand and closed with a plastic
129 cover. We then deposited the aluminium rails on thermal bridges (AHP-1200CPV-
130 Thermoelectric Cooling America Corporation, 4049W Schubert Avenue, Chicago IL, USA) with
131 temperatures linearly ranging from 0°C to 20°C (Figure S1) and insulated with thick foam to
132 ensure complete darkness. To determine the temperature range explored by each female
133 before egg-laying, we then measured the distance between these females and the coldest
134 edge of the rail daily until they laid eggs. To limit stress on the females due to rail handling,
135 we divided each rail into 12 zones of 60 mm length and defined the distance between a female
136 and the coldest edge as the centre of the zone she was in. Finally, to increase the number of
137 females with eggs available for the second part of the experiment, we also isolated 24
138 additional females (12 from HNB and 12 from SJNL) in Petri dishes (diameter 10 cm) lined with
139 wet sand and maintained at 10°C under complete darkness until egg production. From field
140 sampling to oviposition, we fed the 36 and 24 females twice a week with fresh carrots placed
141 on a soaked cotton pad, plus an artificial diet composed of 1/3 dry power of egg yolk, 1/3 bee
142 collected pollen (Community Apiaries, 576 Plymouth road, Richmond Corner, New-Brunswick,
143 Canada) and 1/3 cricket powder (Entomo Farms, 31 industrial drive Norwood, Ontario,
144 Canada).



145 Three days after oviposition, we transferred the 36 mothers and eggs previously
146 maintained in aluminium rails and the 24 mothers and eggs previously maintained in Petri
147 dishes to the middle of shorter aluminium rails (18 x 18 x 660 mm) also lined with a layer of
148 wet sand and closed with a plastic cover to ensure complete darkness. We then deposited
149 these aluminium rails into a climate cabinet (insulated with 2 thick foam to ensure complete
150 darkness; 24h) providing either warm (0.87°C to 6.96°C; 10 HNB and 10 SJNL females),
151 intermediate (-3.64°C to 2.73°C; 10 HNB and 10 SJNL females) or cold (-4.45°C to 1.61°C; 10
152 HNB and 10 SJNL females) non-linear thermal ranges (Figure S2), with eggs deposited at
153 5.22°C, 1.18°C and 0.11°C, respectively. These temperature ranges encompass the above-
154 ground variation in temperatures measured in these populations during the natural period of
155 egg care (Gingras and Tourneur 2001)(Figure 1). To test whether and how mothers
156 transported their clutch throughout egg development, we then measured the distance
157 between the centre of the clutch of eggs and the coldest edge of the rail once a week during
158 the 15 following weeks. Because rail handling occurred only weekly in this part of the
159 experiment, we measured the distances between the (center of the pile of) eggs and cold edge
160 directly in cm. Note that the rails used in the climatic cabinet were slightly shorter than those
161 ~~used in the thermal bridge because they had to fit into the climatic cabinet which was~~
162 ~~shallower.~~

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

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

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


182 Genetic analyses

183 To determine whether HNB and SJNL females belonged to the same subspecies of the
184 European earwig (Wirth et al. 1998, González-Miguéns et al. 2020), we analysed the
185 Cytochrome Oxidase I (COI) gene of 6 females per population. This number of females is
186 enough to robustly assess the origin of an entire population, as previous work demonstrated
187 that the two species do not co-exist in the same population (Wirth et al. 1998, Guillet et al.
188 2000b). Genomic DNA was extracted from whole individuals with the NucleoMag® Tissue kit
189 (Macherey Nagel) following manufacturer instructions. The COI gene (658 bp) was amplified
190 from each individual using routine barcoding primers LepF and LepR (Hajibabaei et al. 2006).


191 PCR amplifications was performed with the DreamTaq® PCR Master Mix Kit (Thermo Scientific)
192 using an ESCO Swift Maxi® thermocycler with an initial denaturation step at 95°C (2 min)
193 followed by 35 cycles at 95°C (45 sec), 52°C (60 sec) and 72°C (90 sec) and finally an extension
194 step at 72°C (10 min). PCR products were purified, and Sanger sequenced in both direction
195 using an ABI 3730XL sequencing system (Thermo Fisher Scientific) at Eurofins Genomics
196 Company. Sequences obtained were corrected using Geneious® 9.1.8. The subspecies status
197 of each female was identified from NCBI databases using the BLAST tool (Altschul et al. 1990).
198 The BLAST results were ranked by percent identity and the reference sequences with at least
199 100% identity to our sequences were used to assign the subspecies status. All sequences
200 obtained in this study have been submitted to GenBank; their accession numbers are
201 OL512959 to OL512964 for the 6 HNB females and OL512965 to OL512970 for the 6 SJNL
202 females.

203 **Statistical analyses**

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

213 to correct for multiple measurements. To interpret the significant interaction between these
214 three factors, we first ~~run~~ the same models for each range of temperature (thus without
215 temperature range as an explanatory factor) and when the interactions between weeks and
216 population were significant, we conducted post hoc pairwise comparisons between the initial 
217 location of the eggs and their location at each week using Exact Mann-Whitney Rank Sum
218 tests. To correct for multiple testing, the P-values of these pairwise comparisons were
219 adjusted using the False Discovery Rate (fdr) method (Benjamini and Hochberg 1995). The
220 likelihood to produce at least one larva was tested using a Generalized linear model with
221 binomial error distribution. In this model, the presence of at least one hatched juvenile was
222 used as a binary response (1 or 0), while the population, the range of temperatures and their
223 interaction were entered as explanatory factors. The analyses were conducted with the
224 software R v4.1.1 (R Core Team, 2017) loaded with the packages *car* (Fox and Weisberg 2019),
225 *exactRankTests* (Hothorn and Hornik 2021), *emmeans* (Lenth 2021) and *DHARMA* (Hartig
226 2020).

RESULTS


227 The maximum temperature range at which each female was observed during the 15 days
228 preceding oviposition was greater in SJNL compared to HNB females (Fig 2A; $W = 53$, $P <$
229 0.001). Interestingly, the warmest ~~location~~ at which females ~~have been~~ observed was larger
230 in SJNL compared to HNB females (Fig 2B; $W = 2$, $P < 0.001$), whereas the coldest location was
231 ~~independent of the~~ population (Fig 2C; $W = 135.5$, $P = 0.404$). 

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

234 their eggs earlier in the season (Fig 3B; $W = 38$, $P < 0.001$) and laid overall more eggs (Fig 3C;
235 $W = 261$, $P < 0.001$) compared to SJNL females.

236 During the 15 weeks following oviposition, the location of the eggs varied according to
237 the weeks, population and temperature range (Figure 4; Triple interaction between weeks,
238 population and range of temperature: $\chi^2_5 = 33.13$, $P < 0.001$). Egg location depended on an
239 interaction between weeks and population in the warmest range of temperatures ($\chi^2 = 12.43$,
240 $P < 0.001$), which reveals that SJNL females moved their eggs towards warmer locations about
241 7 weeks after oviposition, whereas HNB females did not specifically target warmer locations
242 (Figure 4). Moreover, egg location also depended on an interaction between weeks and
243 population in both the intermediate and coldest range of temperatures ($\chi^2 = 16.53$, $P < 0.001$
244 and $\chi^2 = 6.27$, $P = 0.012$, respectively), showing that both SJNL and HNB females moved their
245 eggs towards warmer locations but that this move started earlier in SJNL compared to HNB
246 populations (Figure 4). Interestingly, egg transport was always associated with the
247 construction of a new nest in which the eggs were moved.

248 Finally, the likelihood to produce juveniles (i.e. that at least one egg hatched) was
249 overall higher in HNB compared to SJNL females (Figure 5A; Likelihood ratio $\chi^2 = 8.52$, $P =$
250 0.004), overall higher in females previously maintained under the warmest range of
251 temperature compared to the two others ranges (Figure 5B, Likelihood ratio $\chi^2 = 8.09$, $P =$
252 0.018), and independent on an interaction between these two factors (Likelihood ratio $\chi^2 =$
253 3.49 , $P = 0.175$). When they hatched, the temperature of the place at which the first juveniles
254 has been observed was not population-dependent (Figure 5C; $W = 60$, $P = 0.396$), while the
255 eggs took overall more time to develop in HNB compared to SJNL females (Figure 5D; $W =$
256 80.5 , $P = 0.012$).

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

DISCUSSION

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

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

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

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

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

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

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

325 Miguéns et al. 2020) but the specificity of life-history traits remains largely unexplored. The
326 only reported species-specific trait refers to their reproduction, with females of species “A”
327 producing one clutch and females of species “B” producing two clutches (Wirth et al. 1998,
328 Tourneur 2018). However, some studies revealed that the number of clutches produced by a
329 female is not always fixed and can vary between populations of the same *F. auricularia* species
330 (Tourneur and Gingras 1992, Ratz et al. 2016), ~~as well as depend~~ on numerous parameters
331 acting during the early life of an earwig female (Meunier et al. 2012, Meunier and Kölliker
332 2012, Wong and Kölliker 2014). This could suggest that local adaptations, rather than
333 membership of species 'A' or 'B', might be the prime driver of life-history traits in this complex
334 of species (Quarrell et al. 2018, Tourneur and Meunier 2020). In this study, our genetic
335 analyses demonstrate that females of Harvey station belong to species “A” and females of St
336 John’s to species “B” (note that this is the first time that species “B” is found in Canada outside
337 British Columbia) which could support species-specific thermal preferences and dynamic of
338 egg transport, ~~but both~~ Harvey station and St John’s exhibit very close temperature regimes
339 (Figure 1), ~~which could also suggest that species-specific traits are a likely explanation~~. Further
340 studies such as transplant experiments are thus needed to disentangle whether the reported
341 patterns reflect local adaptation and/or novel species-specific traits within the European
342 earwig complex (Wirth et al. 1998, Guillet et al. 2000a, González-Miguéns et al. 2020).

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

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

DATA ACCESSIBILITY

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

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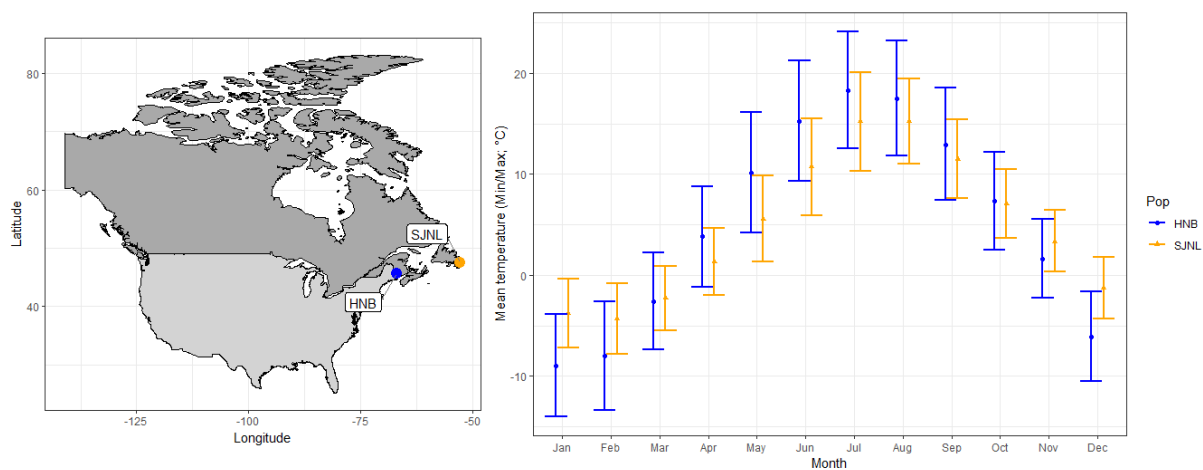
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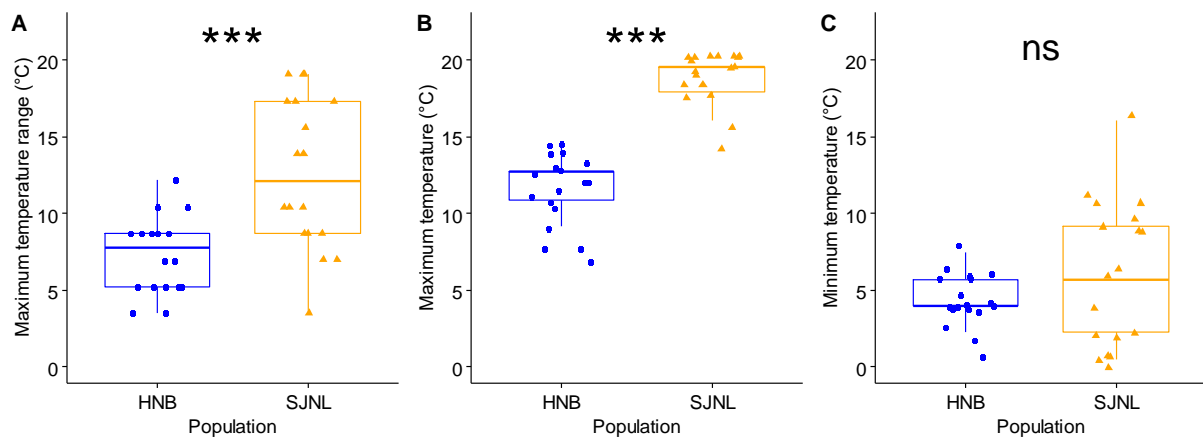
530 **Figure 1** – Locations of Harvey Station (HNB, Canada; Blue) and St Johns (SJNL, Canada;
531 Orange), and their monthly variation of temperatures recorded from 1970 to 2000. Dots
532 depict mean monthly temperatures, with whiskers extending from minimum to maximum
533 mean temperatures. These data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



534

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

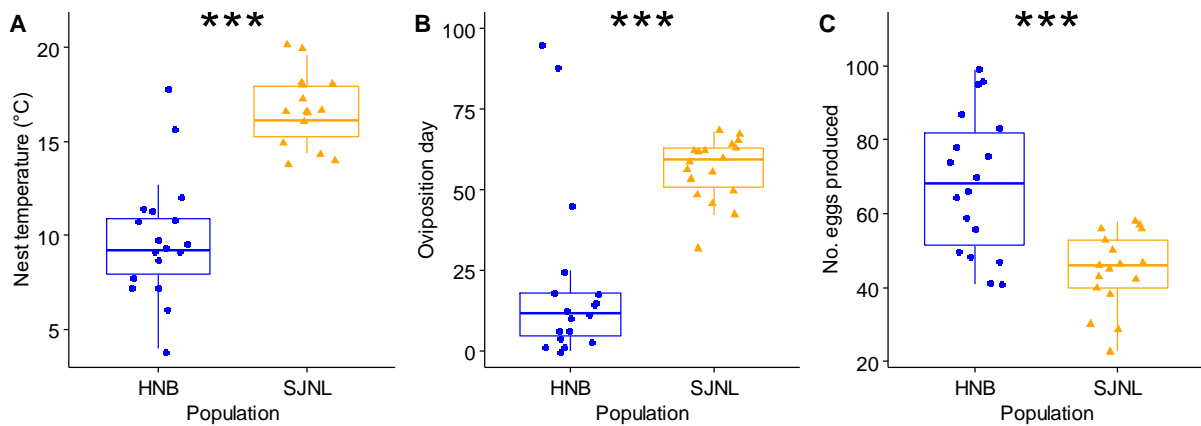
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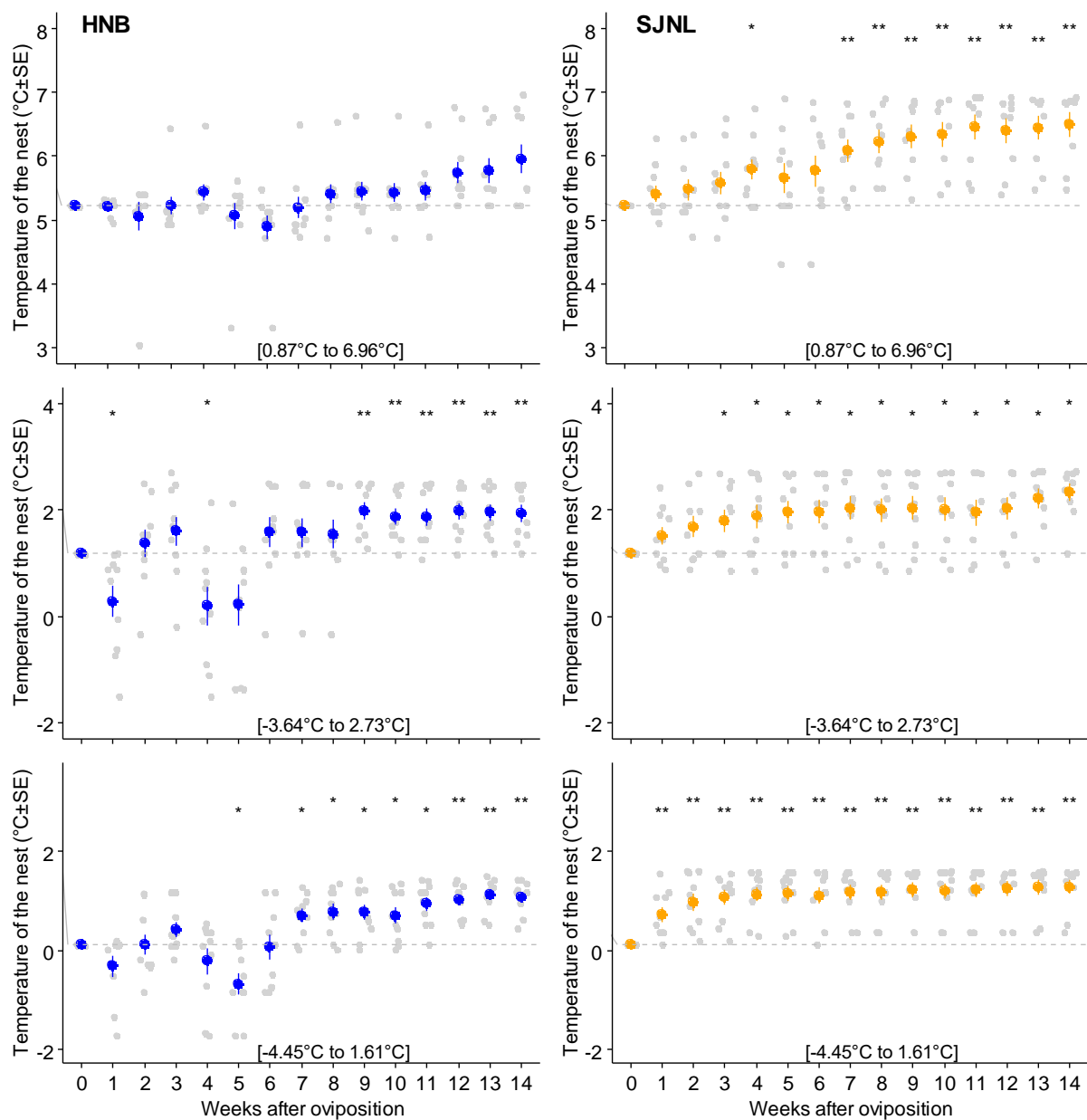
542 **Figure 3** – Effect of population on the (A) temperature of the location at which females laid
543 eggs, (B) day at which females laid eggs, and the (C) number of eggs produced. Box plots depict
544 median (middle bar) and interquartile range (light bar), with whiskers extending to 1.5 times
545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

548



549

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

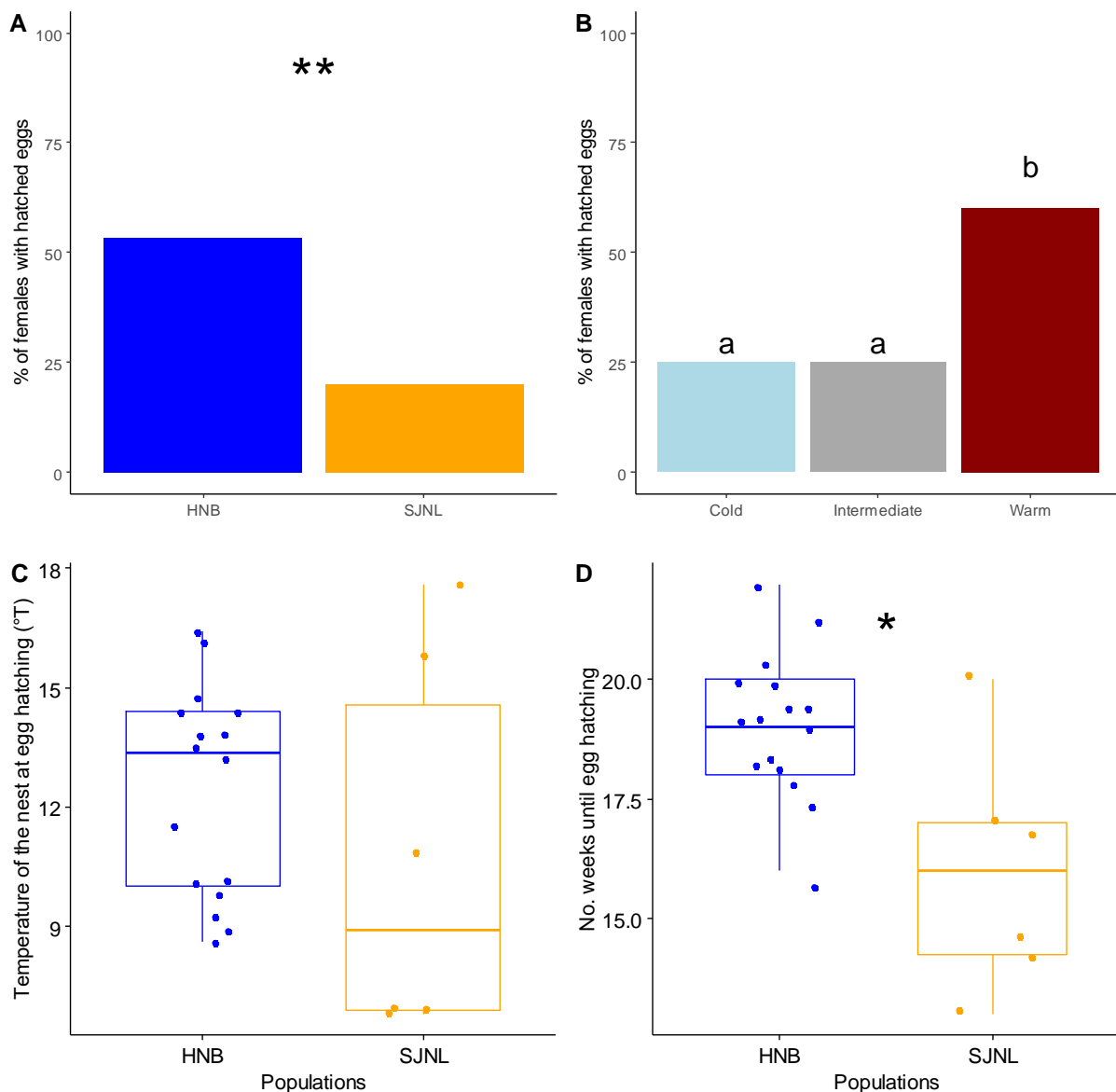


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558 **Figure 5** – Effect of (A, B) population and thermal constraints on the percentage of females
559 with at least one hatched egg, (C) population on the location of eggs at egg hatching and of
560 (D) population on the number of weeks between oviposition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



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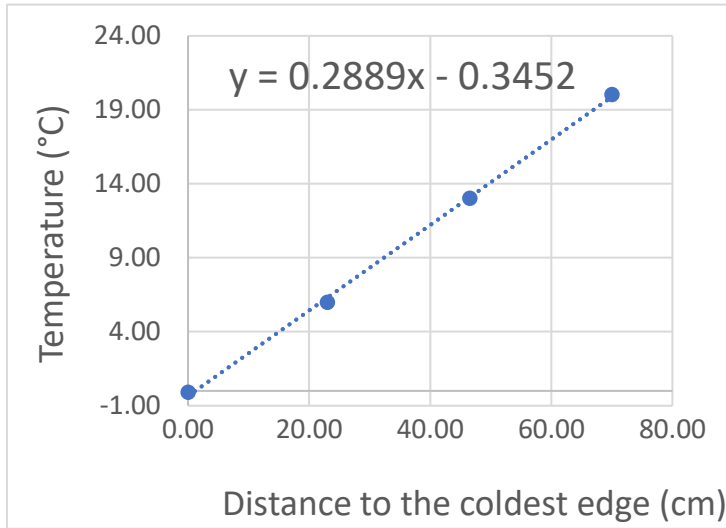
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SUPPLEMENTARY MATERIAL

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569 **Figure S1** – Thermal gradient and equation of the thermal bridge – measurements before and

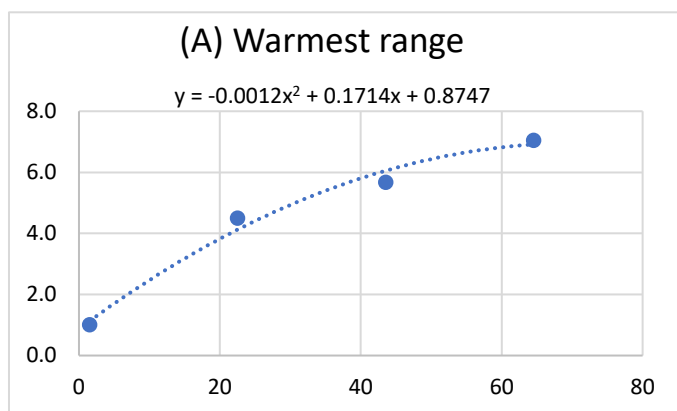
570 at oviposition.



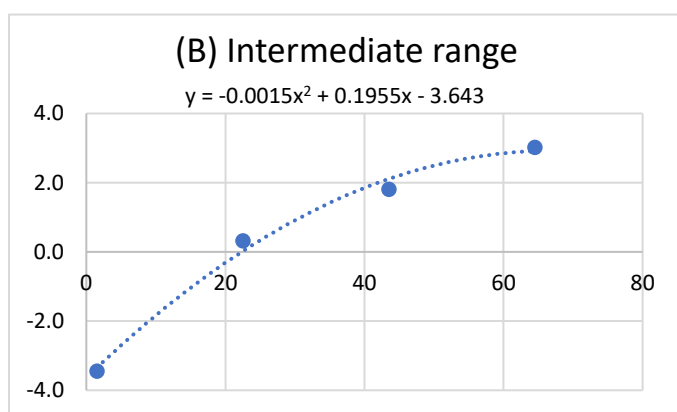
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572 **Figure S2** – Thermal gradients and equations of the (A) warmest, (B) intermediate and (C)
573 coldest temperature ranges in the climate cabinet – measurements after oviposition.

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