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**Experimental increases in temperature mean and variance alter reproductive behaviors in
the dung beetle *Phanaeus vindex***

William H. Kirkpatrick^{1,2} and Kimberly S. Sheldon^{1*}

¹Department of Ecology & Evolutionary Biology, University of Tennessee, Knoxville,
Knoxville, TN 37996-1610

²Department of Biological Sciences, University of Arkansas, Fayetteville, Arkansas, United
States of America

*Corresponding author: ksheldon@utk.edu

24 **Abstract**

25 Temperature profoundly impacts insect development, but plasticity of reproductive behaviors
26 may mediate the impacts of temperature change on earlier life stages. Few studies have
27 examined the potential for behavioral plasticity of adults to buffer developing offspring from
28 warmer, more variable temperatures associated with climate change. We used a field
29 manipulation to examine whether the dung beetle *Phanaeus vindex* alters breeding behaviors in
30 response to climate change and whether adult behavioral shifts protect offspring from
31 temperature increases. Dung beetles lay eggs inside brood balls made of dung that are buried
32 underground for the entirety of offspring development. Depth of the brood ball impacts the
33 temperatures offspring experience with consequences for beetle development. We placed
34 females in either control or greenhouse treatments that simultaneously increased temperature
35 mean and variance. We found that females produced smaller brood balls but buried them deeper
36 in the greenhouse treatment, suggesting burial depth may come at a cost to brood ball size, which
37 can impact offspring nutrition. Despite being buried deeper, brood balls from the greenhouse
38 treatment experienced warmer mean temperatures but similar amplitudes of temperature
39 fluctuation relative to the controls. Our findings suggest adult behaviors may buffer offspring
40 from increased temperature variation due to climate change.

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44 *Keywords:* behavioral plasticity, climate change ectotherms, global warming, nesting plasticity,

45 reproductive plasticity, Scarabaeidae

46

47 **1. Introduction**

48 Increases in temperature mean and variance associated with climate change can greatly
49 impact the physiology and ecology of ectotherms (Angilletta, 2009). However, phenotypic
50 plasticity, particularly behavioral plasticity, could play a key role in helping organisms cope with
51 stressful temperatures; behavior can respond rapidly to environmental changes, potentially
52 buffering organisms from warmer and more variable temperatures (Telemeco et al., 2009; Sih et
53 al., 2010; Huey et al., 2012; Snell-Rood, 2013; Zuk et al., 2014; Buckley et al., 2015; Muñoz &
54 Losos, 2018). Importantly, behavioral plasticity of adults during reproduction may protect
55 offspring from unfavorable conditions (Refsnider & Janzen, 2012; Snell-Rood et al., 2016;
56 Macagno et al., 2018). Understanding the capacity for reproductive plasticity is thus important
57 for predicting how organisms will respond to climate change (Telemeco et al., 2017).

58 In ectotherms, reproductive behaviors of adults greatly influence the thermal environment
59 in which offspring development takes place with consequences for phenotype and fitness
60 (Telemeco et al., 2017; Macagno et al., 2018; Holley & Andrew, 2019; Mamantov & Sheldon,
61 2021). The temperatures experienced during development have profound effects on ectotherm
62 metabolism, development rate, and body size of adults (Kingsolver et al., 2004; Ragland et al.,
63 2008; Woods, 2009; Klok & Harrison, 2013; Pettersen et al., 2019). In many ectotherms, early
64 life stages (e.g., eggs) are sessile and developing offspring cannot move to more favorable
65 microclimates (Huey et al., 2012). Adjustments by adults in nesting location can alter the
66 developmental environment of offspring with impacts on offspring survival and fitness (Snell-
67 Rood et al., 2016; Telemeco et al., 2017; Macagno et al., 2018; Mamantov & Sheldon, 2021).
68 However, a critical question is whether plasticity of reproductive behaviors can modify the

69 offspring environment enough to compensate for climate change (Telemeco et al., 2009;
70 Refsnider et al., 2013).

71 We studied the breeding behavior of the dung beetle *Phanaeus vindex* Macleay, 1819 to
72 understand 1) how simultaneous increases in temperature mean and variance associated with
73 climate change affect breeding behaviors of adults, and 2) whether behavioral plasticity of adults
74 could compensate for climate change by buffering offspring from warmer, more variable
75 developmental temperatures. *Phanaeus vindex* (Coleoptera: Scarabaeinae) is a medium-sized,
76 diurnal dung beetle that ranges in open habitats and woodlands from the upper east coast to the
77 southern USA and west to the Rocky Mountains (Edmonds, 1994). To breed, the beetles find and
78 mate at a dung source. Females construct a tunnel below the dung source and transport dung
79 from the soil surface to the bottom of the tunnel. The female shapes the buried dung into a brood
80 ball and lays a single fertilized egg in it. Once the egg hatches, the developing larva eats the dung
81 provided by the female, going through complete metamorphosis within the brood ball (Halffter
82 & Matthews, 1966).

83 Maternal behavior during reproduction, including brood ball size and burial depth, shapes
84 the environmental conditions of the offspring with consequences for survival and fitness (Hunt &
85 Simmons, 2000, 2002, 2004; Moczek & Emlen, 2000; Snell-Rood et al., 2016; Macagno et al.,
86 2018; Mamantov & Sheldon, 2021). Because dung from the brood ball provides the only
87 nourishment available to developing larvae, the size of the brood ball can affect adult body size
88 upon emergence (Lee & Peng, 1981; Moczek & Emlen, 1999; Shafiei et al., 2001; Kishi &
89 Nishida, 2006). Importantly, smaller individuals tend to have reduced fecundity and competitive
90 ability relative to larger individuals (Hunt & Simmons, 2000). Burial depth impacts the
91 temperature experienced by offspring in the brood ball; offspring in brood balls near the soil

92 surface experience warmer, more variable temperatures than those at greater soil depths (Snell-
93 Rood et al., 2016). In ectotherms, warmer temperatures are associated with faster development
94 rates, smaller body sizes, and reduced survival (Chown & Terblanche, 2006; Angilletta, 2009;
95 Colinet et al., 2015; Mamantov & Sheldon, 2021). However, few studies have examined the
96 capacity for behavioral plasticity to mediate the types of temperature changes occurring with
97 climate change (Refsnider et al., 2013; Telemeco et al., 2017). Specifically, climate change is
98 leading to warmer temperatures, but also more variable temperatures, which will likely be more
99 challenging than shifts in mean temperature alone (Folguera et al., 2011; Paaijmans et al., 2013;
100 Thompson et al., 2013; Vasseur et al., 2014; Sheldon & Dillon, 2016).

101 We conducted a field experiment to examine the plasticity of nesting behaviors of *P.*
102 *vindex* in response to climate change by simultaneously increasing temperature mean and
103 variance using mini-greenhouses. Our goals were to examine 1) if and how females altered
104 reproductive behaviors, including the size, burial depth, and number of brood balls, in response
105 to warmer and more variable temperatures, and 2) whether behavioral shifts of females buffered
106 offspring from warmer and more variable temperatures.

107

108 **2. Methods**

109 **(a) Beetle collection**

110 During two different trapping sessions in May and June 2018, we collected adult *P. vindex* near
111 Knoxville, Tennessee, USA (36°03'25.8"N, 84°04'19.8"W) using pitfall traps baited with cow
112 dung. We brought males (n = ~10-15) and females (n = ~20) from each trapping session back to
113 the lab and held them in a large plastic container (18 gallon; 59 L x 46 W x 38 H cm) filled with
114 a 4:1 mixture of topsoil:sand and covered with flexible fiberglass screen. We placed the

115 container near a window to provide natural light, kept the room temperature at $\sim 23^{\circ}$ C, and fed
116 beetles ad libitum autoclaved cow dung twice weekly. We held beetles in this colony for 3-4
117 weeks to allow them to mature and mate (Fincher, 1973; Blume & Alga, 1976). We checked the
118 soil in the colonies weekly to see if females had produced brood balls. Once the colony began to
119 form brood balls, the females were considered fertilized and ready for experimental trials.

120

121 **(b) Experimental trials**

122 We conducted the warming experiment in an open field near Knoxville, Tennessee, USA ($36^{\circ}1'$
123 N, $84^{\circ}3'$ W) using four, 10-day trials with start dates ranging from late June to early August
124 2018. We arranged 12, 7-gallon buckets (50 cm high, 28.5 cm inner diameter) in two rows with 2
125 m in between buckets. We drilled 5 holes into the bottom of the buckets for water drainage. We
126 buried buckets to the brim, backfilled them with soil from the field site, and compacted the soil
127 as much as possible. The soil in each bucket was 44 cm deep, leaving 6 cm of space between the
128 top of the soil and top of the bucket. We placed four HOBO pendant data loggers (Model: UA-
129 001-64, Onset, Bourne, MA) in the buckets spaced 14 cm apart (i.e., 1, 15, 29, and 43 cm below
130 the soil surface) and recorded temperatures every 1 hour.

131 For each trial (four total), we randomly assigned six buckets to a heated (hereafter
132 “greenhouse”) treatment and six to a control group. For the greenhouse treatment, we designed
133 mini-greenhouses capable of passively and simultaneously increasing temperature mean and
134 variance to simulate climate change. The greenhouses were made of 1/16” clear polycarbonate
135 and shaped like a cone with a 61 cm opening at the bottom and a 7 cm opening at the top. The
136 cone shape allowed for even heating around each bucket. Based on temperature data, the
137 greenhouse cones simulated the types of changes expected under climate change, with an

138 average increase of 2° C at the soil surface relative to the control buckets, though surface
139 temperature differences could reach 4° C (Fig. 1). Temperatures in both bucket types showed
140 natural, diurnal fluctuations and, with increasing soil depth, a decline in mean temperatures and a
141 dampening of the amplitude of temperature fluctuation. However, temperatures at the soil depths
142 where the data loggers were placed were warmer and more variable in the greenhouse buckets
143 than similarly placed loggers in the control buckets (Fig. 1), thus effectively simulating
144 conditions expected under climate change (IPCC 2013).

145 For trials, we removed females from the colony, recorded mass, and randomly assigned
146 them to a bucket. At the start of a trial, we added one female to each bucket, allowing us to
147 eliminate the confounding effects of male behavior. Fertilized females store seminal fluid and
148 will construct tunnels and create brood balls in the absence of males. After placing a female in a
149 bucket, we put flexible fiberglass screen over the top secured with a bungee cord and followed
150 by a large piece of ½ inch welded galvanized hardware cloth secured with stakes in the ground.
151 The screen and wire prevented beetles from escaping and small animals from disturbing the trials
152 while also allowing for more natural conditions. For warming treatments, we then placed
153 greenhouses over the buckets. We provided beetles with ~ 25g of autoclaved cow dung at the
154 start of the trial and then every two days thereafter to ensure they had enough for consumption
155 and continuous brood ball construction. During these feedings, we removed all dung left on the
156 soil surface from the previous feeding before placing new dung.

157 After ten days, we carefully went through the soil to uncover brood balls. We recorded
158 the mass, burial depth, and number of brood balls from each bucket. We also removed the four
159 temperature loggers and downloaded data. If a female did not produce brood balls, we did not
160 use her in subsequent trials and she was excluded from statistical analyses. If a female produced

161 brood balls, we used her in subsequent trials, typically, but not always, switching her from either
162 a greenhouse treatment to a control or vice versa. Fifty-two percent of females produced in both
163 bucket types. One bucket did not drain well during multiple trials, and we removed it from
164 further analyses.

165

166 **(c) Statistical analysis**

167 To test for behavioral shifts, we fit linear mixed effects (LME) models in R (package lme4, R
168 version 3.6.0, R Development Core Team 2019) with the response variables of brood ball mass,
169 burial depth, or number and the fixed effect of treatment type (greenhouse or control bucket). We
170 included female mass as a covariate since body size may affect how much dung she can
171 sequester (Lee & Peng, 1981). We included the random effects of trial and beetle identification
172 to account for the non-independence of brood balls from the same trial and female, respectively
173 (Crawley, 2007), and used model selection to decide on the random effects structure following
174 Zuur et al. (2009). In the models for brood ball number, we removed buckets where a female did
175 not produce a brood ball since she was not likely fertilized or where a female produced brood
176 balls but died during the trial. Null models included an intercept and the random effects of trial
177 and beetle identification. We selected the best-fit model considering AIC_c (Akaike information
178 criterion for small sample sizes) values and the normality of residuals (Burnham & Anderson,
179 2002; Zuur et al., 2009; Symonds & Moussalli, 2011). Specifically, we calculated the Akaike
180 weight of each model ($wAIC_c$), which estimates the probability that the model is the best model
181 among the candidate models considered (Burnham & Anderson, 2002). Because our model for
182 burial depth that included the fixed effect of treatment type (i.e., control or greenhouse) was only
183 marginally better than the null model (see Table S1), we also fit a model using the fixed effect of

184 surface temperature recorded in each bucket rather than treatment type and all other variables
185 described above. This allowed us to account for differences in temperatures among different
186 buckets of the same treatment.

187 To examine whether behavioral shifts of adults affected the development temperatures of
188 offspring, we used temperatures from the data loggers in each bucket to calculate temperatures
189 experienced by each brood ball. Specifically, we fit a linear model to temperatures between
190 neighboring pairs of data loggers to predict the mean and standard deviation of temperature for
191 every brood ball based on its burial depth. To test whether offspring in the different treatments
192 experienced different temperatures, we fit LME models with the response variables of either
193 temperature mean or standard deviation at the location where the brood ball was placed and the
194 fixed and random effects as described above.

195 For the final models, we computed the proportion of variance explained by fixed effects
196 (R^2_m ; Nakagawa & Schielzeth, 2013) using the `r.squaredGLMM` function (package `MuMIn`).

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199 **3. Results**

200 Our goals were to examine whether females altered reproductive behaviors in warmer, more
201 variable temperatures and whether behavioral shifts of females buffered offspring from
202 temperature changes. Over the four 10-day trials, 21 females produced 84 brood balls. Beetles
203 produced smaller brood balls in the greenhouse treatment (Fig. 2A); the best-fit model for brood
204 ball size included the type of treatment as the only covariate ($\beta_{\text{greenhouse}} = -13.24$; $wAIC_c = 0.61$;
205 $R^2_m = 0.21$; Table S1). In the second-best model that accounts for the effect of female mass, the
206 effect of the greenhouse treatment was consistent with that of the best-fit model ($\beta_{\text{greenhouse}} = -$
207 13.50 ; $wAIC_c = 0.39$). Beetles also buried brood balls deeper in the greenhouse treatment (Fig.

208 2B), although this model ($\beta_{\text{greenhouse}} = 3.80$, $w\text{AIC}_c = 0.31$; $R^2_m = 0.05$) was only marginally
209 better than the null ($w\text{AIC}_c = 0.28$; Table S2). However, when we examined burial depth in
210 response to the mean surface temperature of the buckets, the best-fit model that included mean
211 surface temperature ($\beta_{\text{surface temperature}} = 2.69$, $w\text{AIC}_c = 0.47$; $R^2_m = 0.19$) was much better than the
212 null ($w\text{AIC}_c = 0.13$; Table S3; Fig. S1). During the 10-day trials, females produced an average of
213 2.5 brood balls, and we found no difference in the number of brood balls produced in the
214 greenhouse and control buckets (Table S4; Fig. S2).

215 In greenhouse buckets, the brood balls were located in areas with higher mean
216 temperatures compared with control buckets (Fig. S3), but brood balls were located in areas with
217 similar temperature variation regardless of treatment (Fig. S3). The best-fit model for mean
218 temperature where brood balls were placed included the type of treatment and the effect of
219 female mass ($\beta_{\text{greenhouse}} = 0.72$, $w\text{AIC}_c = 0.54$; $R^2_m = 0.17$) (Table S5). In the second-best model
220 that included just the type of treatment, the effect of the greenhouse treatment was consistent
221 with that of the best-fit model ($\beta_{\text{greenhouse}} = 0.69$; $w\text{AIC}_c = 0.46$). We found no effect of treatment
222 on temperature variation where brood balls were placed (Table S6).

223

224 4. Discussion

225 We demonstrate that female *Phanaeus vindex* altered their breeding behaviors in
226 response to simulated climate change by producing smaller brood balls that were buried deeper
227 in the soil. This plasticity in burial depth did not fully compensate for warming temperatures.
228 Despite being buried deeper in the soil, brood balls from the greenhouse treatment were placed in
229 warmer mean temperatures. Warmer temperatures during offspring development can result in
230 faster development rates, smaller adult body sizes, and depending on the temperature, lower

231 survival (Macagno et al., 2018; Pettersen et al., 2019; Mamantov & Sheldon, 2021). However,
232 the mean temperatures experienced by *P. vindex* brood balls in the greenhouse treatment (26.6
233 °C) and control (26 °C) buckets are well below the temperatures that lead to high rates of
234 mortality in other dung beetle offspring (Macagno et al., 2018; Mamantov & Sheldon, 2021).

235 In contrast to mean temperatures, *P. vindex* offspring in brood balls experienced similar
236 amplitudes of temperature fluctuation in the greenhouse treatment and control buckets. Increased
237 temperature variation associated with climate change may be more stressful than shifts in mean
238 temperature alone (Vasseur et al., 2014) and could impact fitness by leading to smaller body
239 sizes in dung beetles (Carter & Sheldon, 2020). As an example, offspring of *O. taurus* were three
240 times smaller as adults when exposed to greater temperature fluctuations (Fleming et al., in
241 press). Thus, plasticity of nest depth in *P. vindex* may buffer offspring from increased
242 temperature variation that could otherwise negatively impact fitness (Snell-Rood et al., 2016).

243 Increased nest depth may protect dung beetle offspring from stressful temperatures, but
244 time spent on tunnel construction could impact other reproductive traits (Macagno et al., 2018).
245 *P. vindex* beetles in the greenhouse treatment produced the same number of brood balls as those
246 in the control buckets, but the brood balls were smaller in the greenhouse treatment. One
247 possibility for these observations is that increased nesting depth of *P. vindex* may come at a cost
248 to brood ball size, a fitness-linked trait (Lee & Peng, 1981; Moczek & Emlen, 1999; Shafiei et
249 al., 2001; Kishi & Nishida, 2006). Negative relationships between reproductive behaviors in
250 response to temperature changes have been observed in other dung beetle species. *O. taurus*
251 beetles exposed to warmer mean temperatures (Mamantov & Sheldon, 2021) and increased
252 diurnal temperature variation (Holley & Andrew, 2020) did not alter brood ball size, but they
253 produced fewer brood balls. Interestingly, *O. hecate* appeared to show no tradeoff in

254 reproductive behaviors in response to warming; individuals produced more brood balls of the
255 same size and buried them deeper in response to warmer mean temperatures (Mamantov &
256 Sheldon, 2021). However, fitness could be impacted in other ways, such as via reduced egg
257 production and quality. As an example, in the ball-rolling dung beetle *Sisyphus rubrus*, warmer
258 treatments did not affect the size or number of brood balls produced, but beetles buried fewer
259 brood balls, which are more likely to contain eggs than unburied brood balls (Holley & Andrew,
260 2019). Thus, shifts in one behavior in response to temperature changes may come at a cost to
261 other fitness-linked traits.

262 Is the behavioral plasticity we observed in *P. vindex* adaptive? Though we did not
263 examine fitness, we did find potential tradeoffs that suggest an adaptive response. Specifically,
264 brood balls were smaller in the greenhouse treatment, which can reduce fitness via impacts on
265 adult body size (Lee & Peng, 1981; Moczek & Emlen, 1999; Shafiei et al., 2001; Kishi &
266 Nishida, 2006). However, by digging deeper in the soil, beetles were able to buffer their
267 offspring from potentially stressful temperatures. This suggests that behavioral plasticity to
268 temperature change may be adaptive in *P. vindex* by increasing offspring survival even if the
269 reduced brood ball sizes result in smaller individuals. Though behavioral plasticity may be
270 adaptive in *P. vindex*, this is not the case for all ectotherms. For example, *Sceloporus tristichus*
271 lizards reduced nesting depth in response to warmer temperatures, thus increasing heat stress on
272 embryos (Telemeco et al., 2017). Understanding the behavioral adjustments made by ectotherms
273 in response to temperature change is a key step to predicting the potential impacts of climate
274 change (Huey & Tewksbury, 2009).

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410

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420

421 **Data accessibility**

422 Data are accessible as electronic supplementary material.

423

424 **Authors' contributions**

425 KSS conceived of the study and designed the experiment. WHK performed the experiment. KSS
426 analyzed data with assistance from WHK. Both authors wrote the manuscript, gave final
427 approval for publication, and agree to be held accountable for the work performed therein.

428

429 **Competing interests**

430 We declare we have no competing interests.

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440 **Figure 1.** Representative temperature profiles from control (left) and greenhouse (right) buckets.

441 Temperatures are shown from four data loggers placed at different soil depths in control and

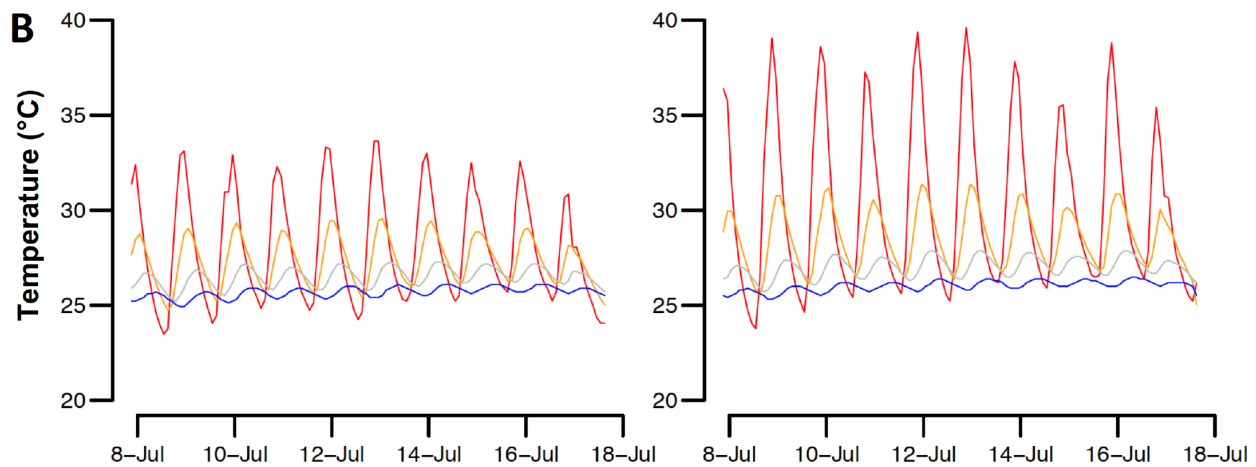
442 greenhouse buckets over nine days in July 2018 at the study site in Tennessee. Colors represent

443 temperatures at different depths below the soil surface, including 1 cm (red), 15 cm (orange), 29

444 cm (gray), and 43 cm (blue).

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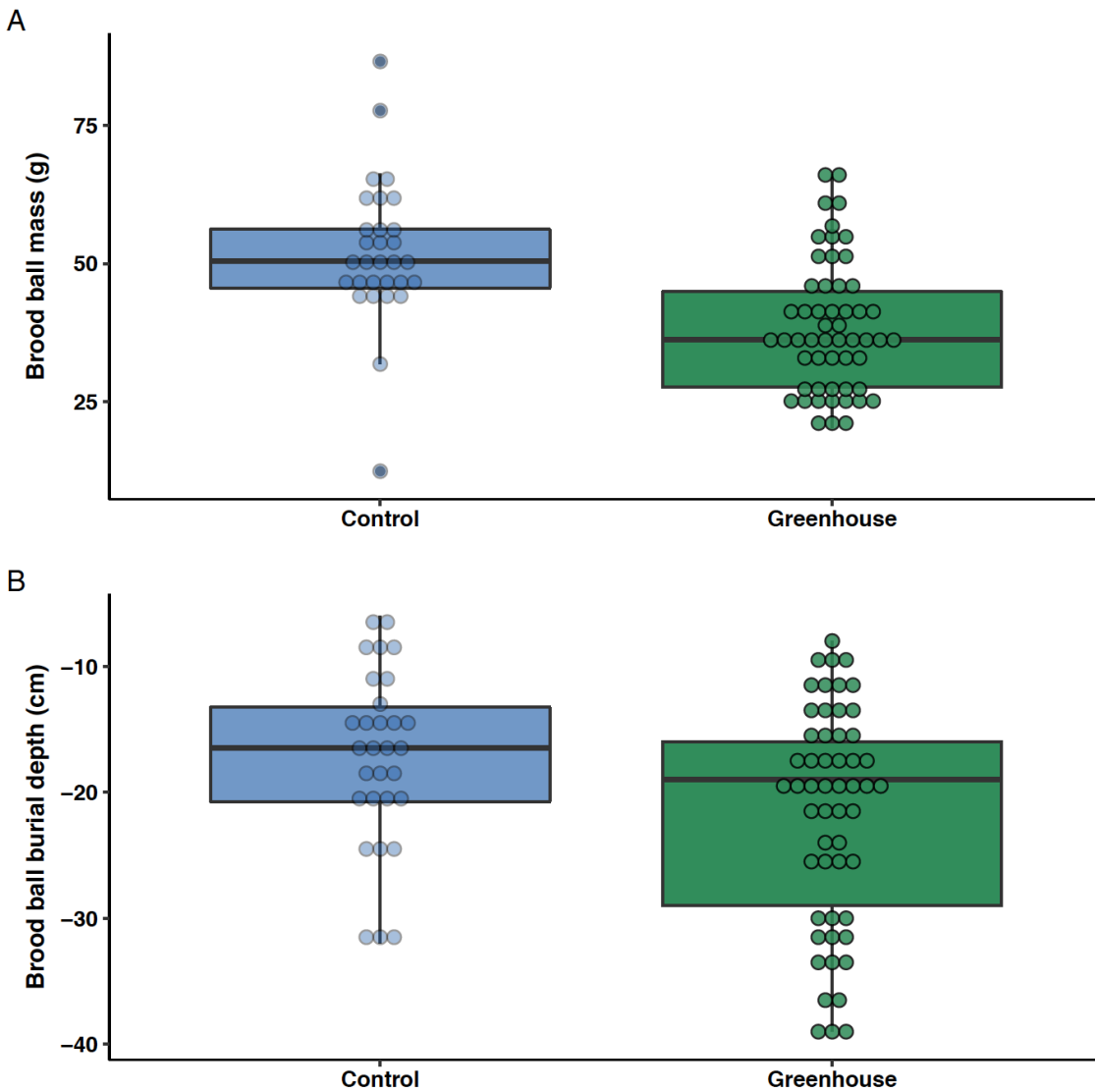
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458 **Figure 2.** Plasticity of reproductive behaviors of female *Phanaeus vindex* dung beetles. Females
459 in the warming treatment produced smaller brood balls (A) that were buried deeper in the soil
460 (B) compared with beetles in the control treatment. Points show individual brood balls. Boxes
461 show the median and first and third quartiles and whiskers show the minimum and maximum
462 values of either brood ball size (g) or brood ball burial depth (cm).

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