

1 **Linking species thermal tolerance to elevational range shifts in upland dung beetles**

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11

12 **Abstract**

13 Climate warming has been proposed as the main cause of the recent range shifts seen in many
14 species. Although species' thermal tolerances are thought to play a key role in determining
15 responses to climate change, especially in ectotherms, empirical evidence is still limited. We
16 investigate the connection between species' thermal tolerances, elevational range and shifts in
17 the lower elevational limit of dung beetle species (Coleoptera, Aphodiidea) in an upland
18 region in the northwest of England. We measured thermal tolerances in the laboratory, and
19 used current and historical distribution data to test specific hypotheses about the area's three
20 dominant species, particularly the species most likely to suffer from warming: *Agollinus*
21 *lapponum*. We found marked differences between species in their minimum and maximum
22 thermal tolerance and in their elevational range and patterns of abundance. Overall,
23 differences in thermal limits among species matched the abundance patterns along the
24 elevation gradient expected if distributions were constrained by climate. *A. lapponum*
25 abundance increased with elevation and this species showed lower maximum and minimum
26 thermal limits than *Acrossus depressus*, for which abundance declined with elevation.
27 Consistent with lower tolerance to high temperature, we recorded an uphill retreat of the low
28 elevation limit of *A. lapponum* (177 m over 57 years) in line with the increase in summer
29 temperature observed in the region over the same period. Moreover, this species has been
30 replaced at low and mid-elevations by the other two warm-tolerant species (*A. depressus* and
31 *Agrilinus ater*). Our results provide empirical evidence that species' thermal tolerance
32 constrains elevational ranges and contributes to explain the observed responses to climate
33 warming. A mechanistic understanding of how climate change directly affects species, such
34 as the one presented here, will provide a robust base to inform predictions of how individual
35 species and whole assemblages may change in the future.

36

37 **Introduction**

38 Species' ability to tolerate low and high temperatures can be crucial in determining their
39 latitudinal and elevational distribution (Gaston and Chown 1999, Addo-Bediako et al. 2000,
40 Deutsch et al. 2008, Sunday et al. 2012, Buckley et al. 2014, Sunday et al. 2014). This is
41 particularly true for ectotherms as their body temperature regulation is entirely reliant on
42 external temperatures (Deutsch et al. 2008, Sunday et al. 2011, Wilson and Maclean 2011,
43 Buckley et al. 2013, Khaliq et al. 2014) and temperature can affect egg and larval survival
44 and adult fecundity as well as metabolic processes (Klok and Chown 2001, Bowler and
45 Terblanche 2008, Somero 2010, Radchuk et al. 2013). Thus, species with low minimum and
46 maximum thermal tolerance have been found to be prevalent in cold environments at high
47 elevations and latitudes in various regions (Addo-Bediako et al. 2000, Sørensen et al. 2005,
48 Buckley et al. 2013, Warren and Chick 2013).

49 This relationship between geographic range and physiological tolerance has led to the
50 suggestion that species or populations most likely to persist under, or even benefit from,
51 climate change are those with high maximum thermal tolerance limits (Deutsch et al. 2008,
52 Khaliq et al. 2014). Indeed, numerous studies have reported expansions towards higher
53 latitudes and elevations of species from warmer regions, as climate warming is increasing the
54 availability of suitable habitat in areas that were previously too cold for the species to survive
55 (e.g. Parmesan et al. 1999, Lobo and Halfpeter 2000, Konvicka et al. 2003, Parmesan and
56 Yohe 2003, Wilson et al. 2005, Franco et al. 2006, Chen et al. 2011, Hill et al. 2011).

57 On the other hand, species adapted to cold climates are predicted to suffer from warming if
58 rising temperatures exceed species maximum thermal limits (Thomas et al. 2004, Menéndez
59 2007, Colwell et al. 2008, García-Robledo et al. 2016). This in turn will result in local

60 extinction of populations at lower latitudes and elevations as has been reported for several
61 groups of ectotherms (e.g. Wilson et al. 2005, Franco et al. 2006, Hickling et al. 2006,
62 Dingemanse and Kalkman 2008, Merrill et al. 2008, Chen et al. 2011, Wilson and Maclean
63 2011, Sunday et al. 2012, Menéndez et al. 2014). The connection between maximum thermal
64 tolerance and climate driven extinctions appears to be intuitive but, as highlighted by many
65 authors, there is still little empirical evidence supporting this connection (e.g. Cahill et al.
66 2013, Kaspari et al. 2015, García-Robledo et al. 2016). A recent review by Cahill et al.
67 (2014) found that only a few studies of terrestrial ectotherms successfully identified thermal
68 tolerance limits as a proximate cause of the climate related shift reported at the lower/warm
69 range boundary. Thus this is a critical gap in our current understanding of the implications of
70 future climate change (Thomas et al. 2004, Thomas et al. 2006).

71 In this paper we investigate the connection between species thermal tolerances, elevational
72 range and shifts in the lower elevational limit of dung beetle species in an upland region in
73 northern England. We focus particularly on the elevational distribution of the northern dung
74 beetle *Agoliinus lapponum* (Gyllenhal, 1808) which has historically been the dominant
75 species in the study region (White 1960, Pearson and White 1963) but which has declined
76 recently; and compare it with two other dung beetle species, *Agrilinus ater* (De Geer, 1774)
77 and *Acrossus depressus* (Kugelann, 1792), that have become more abundant in the region. *A.*
78 *lapponum* is restricted to northern latitudes in Europe and reaches its southern geographic
79 limit in the British uplands (White 1960, Key 1982), hence populations in Britain are likely to
80 be particularly susceptible to local extinction caused by climate change (Franco et al. 2006).
81 *A. ater* and *A. depressus* are more widespread species both reaching their southern limits
82 much further south in Europe. We tested the specific hypotheses that the species with the
83 lowest minimum and maximum thermal tolerance limits: (1) will be more abundant at high

84 elevations, unlike the species that are able to tolerate the highest temperatures; (2) has shifted
85 its lower elevational limit uphill in line with changes in temperature observed in the study
86 region during the last 50 years; and (3) has declined in abundance at low and middle
87 elevations, where the other two species have become more dominant in the community as
88 they are better able to cope with the increase in temperature.

89

90 **Methods**

91 *Study area*

92 The study was carried out at the Moor House World Biosphere Reserve in the North
93 Pennines, northern England (Fig. 1). This is an internationally important upland research site
94 (Holden and Rose 2011) of *c.* 900 km² and with an elevation range from *c.* 200 m to 893 m
95 above sea level (a.s.l.) at the top of the Cross Fell (54°42'10"N, 2°29'14"W). The dominant
96 vegetation types are acid grassland, *Calluna vulgaris* heathland and mire (JNCC 1990), and
97 the area is regularly grazed by sheep from April to October. The dung beetle community is
98 dominated by members of Aphodiidae family (Supplementary material Appendix 1, Table
99 A1), which feed and reproduce inside the dung (Hanski 1991).

100 *Species abundance along the elevational gradient*

101 We assess the relationship between species abundance and elevation across the study area for
102 the three dominant dung beetle species: *Agoliinus lapponum* (Gyllenhal, 1808), *Agrilinus ater*
103 (De Geer, 1774) and *Acrossus depressus* (Kugelann, 1792) (Supplementary material
104 Appendix 1, Table A1). To replicate methods used historically at the site (White 1960,
105 Pearson and White 1963) from which data were incorporated into this study (see below),
106 beetles were collected by hand sorting from naturally occurring sheep dung (sampling effort

107 standardised to 30 minutes per visit per site) during May, June and July in 2008 and 2013 in a
108 total of 20 sites along three elevation transects (Fig.1), following the same routes as used in
109 the historic survey. The abundance of each species at a particular site was calculated by
110 pooling together the data from both survey years (2008 and 2013). The relationship between
111 the abundance of a species and elevation was tested using Generalised linear models with
112 negative binomial error structure to account for overdispersion (Zuur et al. 2009, Thomas et
113 al. 2013), considering elevation as a quadratic or a linear term during model selection. For
114 *A.lapponum*, due to the observed zero values at low elevations, we fitted an exponential
115 function with log (x+1) transformation. These analyses were carried out with MASS package
116 (Venables and Ripley 2002) in R (R Core Team 2016).

117 *Change in the elevational range of Agoliinus lapponum*

118 Historic elevational distribution data in the study area were only available for *A. lapponum*
119 from a survey carried out in 1956 (White 1960, Pearson and White 1963). During the 1956
120 survey (“historic survey” hereafter) beetles were collected in July by hand sorting from
121 naturally occurring sheep dung at 21 sites from 275 m to 610 m a.s.l. (Pearson and White
122 1963). The information from this survey included the name and elevation of the sampling
123 localities (no specific coordinates), the abundance of *A. lapponum* at each sampling site and a
124 measure of sampling effort (total number of dung beetles collected at each site). We
125 compared the historic data with those collected during June and July in 2008 and 2013
126 (hereafter referred to as “current surveys”) at 20 sites from 292 m to 810 m a.s.l. as described
127 above (Fig. 1). We recorded the abundance of all species, and in each surveyed year (2008
128 and 2013) data from the two sampling months were pooled per site for analysis.
129 We calculated changes in the lower elevational limit of *A. lapponum* between the historic and
130 the current surveys as the difference between the minimum elevations occupied by the

131 species in each year (1956 versus 2008 and 1956 versus 2013). Changes in the upper
132 elevational limit were not measured as the historic survey did not sample the whole elevation
133 gradient present in the region. To assess the accuracy of each survey in detecting the actual
134 limit of the species in each year we used the likelihood ratio (LR) method described by
135 (Rowe et al. 2010). This determines the likelihood of failing to find the species beyond its
136 observed range with a known number of sampling opportunities (the total number of
137 individuals collected outside the observed range of the species). Thus, the LR for a particular
138 elevation (i) where the species has not been found is defined as:

$$139 \text{LR}_i = (1 - [(F-2)/(S-2)])^{-G_i}$$

140 where F is the number of individuals of the target species captured over its observed range, G_i
141 is the total number of individuals of all species captured at elevation i where the target
142 species was not captured and S is the sum of the total number of individuals of all species
143 captured along the observed range of the target species and in elevation i . A cut off of $\text{LR} > 8$
144 was used as strong evidence that the target species do not occur beyond the observed range
145 and as an indication that the real limit of the species has been successfully identified in each
146 surveyed year (Royall 1997).

147 To assess changes in *A. lapponum* abundance between survey periods, we analysed the effect
148 of elevation on the proportional abundance of *A. lapponum* at each sampling locality during
149 the historic survey and the current surveys (data from 2008 and 2013 pooled together) using
150 Generalised Linear Models (using *lme4* package (Bates et al. 2015) in R) with a binomial
151 error distribution (binomial proportion).

152 *Regional changes in climate*

153 We obtained climate data for the period 1956 to 2013 from the Moor House meteorological
154 station (560 m a.s.l.) in the centre of the study area (Fig. 1) and provided by the UK
155 Environmental Change Network (<<http://www.ecn.ac.uk>>). For each year, we obtained data
156 for three temperature variables: the mean annual temperature, the mean temperature of the
157 coldest months (January-February) and the mean temperature of the warmest months (July-
158 August) as well as the annual rainfall. Changes in climate between survey periods were
159 calculated from the predicted values generated by the significant linear regression between
160 the variables (temperature or rainfall) and time (year). Changes in the isotherm between
161 survey periods was calculated using the adiabatic lapse rate at Moor House (decrease in air
162 temperature with increase in elevation) provided by Holden and Rose (2011).

163 *Thermal tolerance*

164 We assessed thermal tolerance for the three species (*A. lapponum*, *A. ater* and *A. depressus*),
165 which are the dominant species in the study area (see Supplementary material Appendix 1,
166 Table A1). We measured the critical thermal minimum (CT_{min}) and maximum (CT_{max}) for
167 each species as the temperature at which adult beetles suffered total paralysis (loss of ability
168 to move any legs, not including convulsions). Beetles of each species were collected from the
169 field along the southerly elevation transect (Fig. 1). Ten individuals (five for CT_{max} and five
170 for CT_{min} assays) of each species were taken from each of the elevations at which that species
171 was abundant (Supplementary material Appendix 1, Table A2). We placed five beetles of the
172 same species in individual 35 mm diameter petri dishes in two rows in the middle shelf of a
173 Sanjo MIR553 cooled incubator. We set the incubator at a constant starting temperature of 30
174 °C for CT_{max} , and 15 °C for CT_{min} assays, and as initial ambient temperature is known to
175 affect thermal tolerance estimates (Terblanche et al. 2007), the beetles were left in these
176 conditions for five minutes to acclimatise before beginning the tests. These temperatures are

177 tolerated by many ectotherms (Sunday et al. 2011) and were used here to reduce the duration
178 of the experiments and thus prevent beetle exhaustion. If a beetle was not moving during the
179 acclimatisation period it was replaced so that all beetles were active at the start of the assay.
180 We then set the incubator to increase (for CT_{max}) or decrease (for CT_{min}) the temperature
181 automatically at a rate of 0.5 °C/minute, ensuring that all beetles were subject to the same
182 rate of temperature change (Terblanche et al. 2007). Temperature inside the incubator was
183 recorded every 30 seconds using a data logger (DS1923 temperature/humidity logger
184 iButton®) located in a 35 mm petri dishes positioned alongside the dishes containing beetles.
185 Beetles were observed constantly and we noted the time at which each beetle reached total
186 paralysis. To calculate the thermal tolerance limit of each individual beetle, we extracted
187 three temperature values from the data logger: the temperature at the exact time of total
188 paralysis and the temperature 30 seconds before and after the total paralysis, from which a
189 mean was calculated. Once all beetles in the incubator had reached paralysis, they were
190 removed from the incubator and stored in ethanol 70% for further measurements. We
191 recorded the length of elytra and sex of each individual. Length of elytra was used as a proxy
192 for body size, as they were positively correlated ($R^2 = 0.876$, $F_{1;125} = 884.6$, $p < 0.001$) and
193 elytra length is not affected by expansion of joints and abdomen of beetles preserved in
194 ethanol, making the measurement more reliable.

195 Thermal tolerance data were checked graphically for outliers followed by Dixon Q test using
196 the package *outliers* in R (Komsta 2011) and highly significant outliers ($Q > 0.58$, $p < 0.001$)
197 were excluded from the analysis ($n = 2$). We used Linear Models to test for the effect of sex,
198 elytra length and elevation on CT_{max} and CT_{min} of each species separately. We tested
199 differences among the three dung beetle species on CT_{max} and CT_{min} using a Linear Mixed
200 Model using *lme4* package (Bates et al. 2015) in R, including elevation from which the beetle

201 was collected as a random effect. This was done to control for the effect of elevation on
202 thermal limits as beetles from different species were taken from different elevations (see
203 Supplementary Material Appendix 1, Table A2) due to the natural distribution of species. We
204 used Tukey multiple comparison tests to assess pair-wise differences among species at $p <$
205 0.05 using *multcomp* package (Hothorn et al. 2008) in R.

206

207 **Results**

208 *Species abundance along the elevational gradient*

209 Abundance significantly increased with elevation for *A. lapponum* ($R^2 = 0.82$, $F = 76.81$, df
210 $= 1, 16$, $p < 0.001$, $\log(\text{abundance} + 1) = 0.013 * \text{elev} - 4.48$) and decreased for *A. depressus*
211 (pseudo- R^2 (explained deviance) = 0.45, $df = 16$, $p < 0.001$, $\text{abundance} = -0.005 * \text{elev} +$
212 5.90); while, *A. ater* was more abundant at middle elevations (pseudo- R^2 (explained
213 deviance) = 0.42, $df = 15$, $p < 0.011$, $\text{abundance} = 0.01 * \text{elev} - 1.46 * 10^{-5} * \text{elev}^2 + 1.71$) (Fig. 2).

214 *Change in the elevational range of Agoliinus lapponum*

215 The three surveys accurately detected the lower elevational limit of *A. lapponum* with $LR > 8$
216 for all recorded absences outside the species elevational range: 14.3 % of surveyed sites in
217 1956, 33.3 % in 2008 and 44.4 % in 2013 (Table S3). The lower elevational limit was located
218 at 335 m a.s.l. during the historic survey, at 467 m a.s.l. in 2008 and at 512 m a.s.l. in 2013
219 (Fig. 3). This represents an uphill contraction of the lower elevational limit of 132 m in 52
220 years (1956-2008) and 177 m in 57 years (1956-2013).

221 The proportional abundance of *A. lapponum* significantly increased with elevation in the
222 historic survey (elevation: *Coeff.* = 0.0705; *SE* = 0.0122; *z*-value = 5.79; $p < 0.001$ and
223 elevation²: *Coeff.* = -0.00005; *SE* = 0.00001; *z*-value = -4.05, $p < 0.001$), being the most

224 abundant species over 400 m a.s.l (Fig. 4). The proportional abundance of *A. lapponum* also
225 significantly increased with elevation during the current surveys (elev: *Coeff.* = 0.056; SE =
226 0.007; z-value = 8.27; $p < 0.001$ and elev²: *Coeff.* = - 3 10⁻⁵; SE = 6 10⁻⁶; z-value = -6.07, $p <$
227 0.001) but the species was no longer the dominant species at middle elevations, becoming
228 dominant only over 600 m a.s.l (Fig. 4).

229 *Changes in regional climate*

230 The mean annual temperature at the Moor House meteorological station increased
231 significantly during the period 1956-2013 ($R^2 = 0.17$, $F_{(1,56)} = 12.40$, $p < 0.001$, $b = 0.0188$,
232 *intercept* = - 31.94; Supplementary material Appendix A, Fig. A1b). This represents an
233 increase in mean annual temperature of 0.98 °C (1956-2008) and of 1.07 °C (1956-2013).
234 Both the mean temperature of the coldest months ($R^2 = 0.12$, $F_{(1,56)} = 8.58$, $p = 0.005$, $b =$
235 0.034, *intercept* = - 67.67; Supplementary material Appendix A, Fig. A1c) and of the
236 warmest months ($R^2 = 0.13$, $F_{(1,56)} = 9.48$, $p = 0.003$, $b = 0.026$, *intercept* = - 40.09;
237 Supplementary material Appendix A, Fig. A1d) also significantly increased between study
238 periods. This represents an increase in temperature of 1.78 °C (1956 to 2008) and of 1.95 °C
239 (1956-2013) for the coldest months (January-February) and an increase in temperature of
240 1.35 °C (1956 to 2008) and of 1.48 °C (1956-2013) for the warmest months (July-August).
241 Using the mean adiabatic lapse rate for the study area (annual: 0.77-0.78 °C, Jan-Feb: 0.71-
242 0.75 °C ; Jul-Aug: 0.80-0.83 °C per 100 m decrease in elevation) reported by Holden and
243 Rose (2011) the temperature increase between study years (1956-2013) equates to 137.4-
244 139.2 m rise in the isotherm for mean annual temperature, 259.9-274.6 m for mean
245 temperature of coldest months and 177.9-184.5 m for the mean temperature of the warmest
246 months. There was no significant change in annual rainfall over time ($R^2 = -0.02$, $F_{(1,44)} =$
247 0.15, $p = 0.701$, Supplementary material Appendix A, Fig. A1a).

248 *Thermal tolerance*

249 The elevation from which the beetle was collected had a significant positive effect on CT_{max}
250 of *A. depressus* and a negative effect on CT_{min} of *A. ater* (Table 1, Supplementary material
251 Appendix A, Fig. A2). There was also a negative relationship between CT_{max} and elevation
252 for *A. lapponum*, though this was only marginally approaching significance ($p = 0.059$, Table
253 1, Supplementary material Appendix A, Fig. A2). Elytra length and sex did not significantly
254 affect either CT_{max} or CT_{min} of any species (Table 1). Dung beetle species significantly differ
255 in their thermal tolerance, both CT_{max} ($\chi^2 = 81.87$, $p < 0.001$) and CT_{min} ($\chi^2 = 46.61$, $p <$
256 0.001). CT_{max} was lowest for *A. lapponum* (median = 41.2 °C; interquartile range = 1.4),
257 intermediate for *A. depressus* (median = 42.3 °C; interquartile range = 1.2) and highest for *A.*
258 *ater* (median = 44.4 °C; interquartile range = 0.7), while CT_{min} was lowest for *A. lapponum*
259 (median = -0.1 °C; interquartile range = 1.0) and *A. ater* (median = -0.4 °C; interquartile
260 range = 0.8) and significantly higher for *A. depressus* (median = 1.2 °C; interquartile range =
261 1) (Fig. 5).

262

263 **Discussion**

264 Our results show that the lower elevational limit of the dung beetle *A. lapponum* has retreated
265 uphill over the last 57 years consistent with the level of warming experienced in the region
266 during the same period. It is now locally absent below 500 m and has been replaced as the
267 dominant species at middle elevation sites by two other dung beetle species (*A. depressus* and
268 *A. ater*). The three dung beetle species differed significantly in their thermal tolerance, with
269 *A. lapponum* showing the lowest CT_{max} of the three study species and lower CT_{min} compared
270 to *A. depressus*. These results support the hypothesis that physiological tolerance to high
271 temperature could be a proximate cause of the decline of *A. lapponum* in the study region.

272 *Thermal tolerance and elevational range*

273 We found clear differences in thermal limits among the three dung beetle species for both
274 CT_{max} and CT_{min} . Species also differed in their elevational range, showing contrasting
275 patterns of abundance along the elevational gradient. Overall, differences in thermal limits
276 among species matched the elevational range occupied by each species if distributions were
277 constrained by the ability of species to tolerate the environmental conditions (temperature) at
278 each elevation. Recent reviews highlight that species from different latitudes and elevations
279 differ widely in CT_{min} and CT_{max} , with species from high elevations able to tolerate colder
280 temperatures but showing lower tolerance to high temperatures than lowland species (Sunday
281 et al. 2011, García-Robledo et al. 2016).

282 In our study system, *A. lapponum* which is now absent from low elevations and more
283 abundant towards high elevation, showed the lowest tolerance to high temperatures of the
284 three species but also a low CT_{min} . In contrast, *A. depressus*, which is more abundant towards
285 low elevations, has significantly higher CT_{max} and CT_{min} than *A. lapponum*. These results
286 support the hypothesis that, at least for these two species, thermal tolerances are likely
287 constraining their distribution along the environmental gradient. Adults of *A. depressus* were
288 unable to move at temperatures below 1 °C during our experimental assays, while most *A.*
289 *lapponum* adults were still moving even at lower temperatures. In our study area,
290 temperatures at 700 m or above can easily drop below 1 °C even in the summer (personal
291 observation). In addition, differences in CT_{max} between the two species, significantly higher
292 for *A. depressus* than *A. lapponum*, were consistent with their occupancy and abundance at
293 low elevations. Warren and Chick (2013) found similar patterns of distribution and thermal
294 tolerance differences between two ant species in the Appalachian Mountains; with a clear

295 dominance of the cold-habitat species at high elevations and a replacement by the warm-
296 habitat species at low elevations.

297 We found contrasting results for the third species, *A. ater*. Despite tolerating temperatures as
298 low as those tolerated by *A. lapponum* and even higher temperatures than tolerated by
299 *A. depressus*, its abundance declined both at high and low elevations and peaked at mid-
300 elevations, where neither *A. lapponum* nor *A. depressus* were highly abundant (Fig. 2). Both
301 abiotic (climate) and biotic (species interactions) factors have been reported to shape species
302 geographic ranges (Gaston and Chown 1999, Parmesan 2006, Sunday et al. 2012, Sunday et
303 al. 2014) and competition between species at lower elevations has been suggested as the
304 constraining factor for the elevational distribution of some Alpine plants (Hautier et al. 2009,
305 Lenoir et al. 2010). Thus, local abundance of *A. ater* in the study region could potentially be
306 constrained by the abundance of potential competitor species. Adults of *Aphodius* species
307 have been shown to aggregate more intra- than inter-specifically at the dung pat level as a
308 mechanism to reduce species competition and promote coexistence (Hutton and Giller 2004).
309 Females of *A. ater* have been reported to actively avoid laying eggs in dung pats containing
310 large numbers of other coprophagous insects like fly larvae (Hirschberger and Degro 1996),
311 likely to avoid competition. We found this species, often in large numbers, in dung deposits
312 that contained no other dung beetles, supporting the idea that beetles avoid dung pats
313 colonised by other insects. However, high intraspecific density during larval development
314 results in smaller beetles that have lower fecundity (Hirschberger 1999), thus avoiding
315 interspecific competition could result in higher levels of intraspecific competition thus
316 constraining population growth.

317 *Range shifts, regional climate warming and thermal tolerances*

318 Our results show that the lower elevation limit of the dung beetle *A. lapponum* has retreated
319 uphill by around 177 m, which is consistent with the expected retreat resulting from the
320 increase in temperature during the warmest months (177.9 to 184.5 m shift in the isotherm
321 based on mean temperature of July and August). The results agreed with those reported for
322 other cold-adapted species, including dung beetles (Menéndez et al. 2014), in other mountain
323 regions (Wilson, et al. 2005, Moritz, et al. 2008). At the microclimate scale, maximum
324 temperatures at sward level have been reported to be considerable higher than air
325 temperatures (up to 14 °C higher reported by Bennie et al. 2008) and to increase in southerly-
326 facing slopes and in short vegetation (Suggitt et al. 2011). Indeed Sunday et al. (2014) found
327 that the temperatures experienced by ectotherms (operative body temperature) in open
328 habitats often exceed maximum ambient temperatures by over 20 °C in many regions. The
329 average maximum daily air temperature recorded at 556 m (data from the meteorological
330 station) during July 2013 was 19.3 °C, which could represent a potential exposure to
331 temperatures closer to the maximum tolerated by *A. lapponum* adults (average $CT_{max} = 41.3$
332 °C), particularly as maximum temperature within dung has been recorded to be considerably
333 higher than air temperature (Matthiessen and Palmer 1988). Thus, the 1-3 °C lower CT_{max} of
334 *A. lapponum* in comparison to the other two species could result in them falling outside
335 thermal limits during the warmest months of the year more often today than in the past,
336 constraining their ability to survive at low elevations. Additionally, larval stages of several
337 *Aphodius* species have been shown to have lower tolerance to high temperatures than adults
338 (Landin 1961), suggesting that other critical life stages could be even more severely affected
339 by warming, driving rapid population decline (Bowler and Terblanche 2008, Radchuk, et al.
340 2013). The similarity between the extent of the retreat and the uphill shift in the July-August
341 isotherm (the hottest months of the year) over the same period provides support that climate

342 warming, through physiological constraints, is driving the observed changes in range (Cahill,
343 et al. 2013, Cahill, et al. 2014). Moreover, temperatures during the coldest months of the year
344 (January-February) have also increased, likely benefiting cold sensitive species, such as *A.*
345 *depressus*, and resulting in the observed uphill expansion recorded for this species.

346 Attributing lowland contractions to direct physiological effects of climate change on
347 organisms is always difficult due to the many confounding factors that can lead to population
348 extinction at low elevations, including loss of suitable habitat (Forister, et al. 2010) or
349 resources (Memmott, et al. 2007) and changes in species interactions (le Roux, et al. 2012).

350 Changes in resource availability (sheep dung) are unlikely to have played a role in the
351 observed decline of *A. lapponum*, as in our study region dung availability decreases with
352 elevation (Househam 2008), while *A. lapponum* abundance showed the opposite trend.

353 Competition imposed by new arriving warm-tolerant species, expanding as a result of climate
354 change, has been suggested as an explanation for the decline of cold-adapted species (Wetthey
355 2002, Durance and Ormerod 2010). Our results show that *A. lapponum* has been replaced at
356 low and middle elevations by the other two dung beetle species, which are likely to be
357 benefiting from winter warming. Warren and Chick (2013) report a similar replacement for
358 ant species in the Appalachian Mountains, where the cold-tolerant species has been replaced
359 by a warm-tolerant species at low elevations, potentially as a result of competition or
360 hybridisation. Evidence of competition exclusion in temperate dung beetle communities are
361 limited (Finn and Gittings 2003), and though our data suggest the species most likely to have
362 been affected by competition in this case is *A. ater*, we cannot rule out that the increase in
363 abundance of *A. depressus* may have imposed higher levels of interspecific competition to *A.*
364 *lapponum* at low and middle elevations. Adults of *A. depressus* appear earlier in the season
365 than *A. lapponum* which could give this species a competitive advantage in egg laying and

366 larval development. However, further experimental work is required to fully understand the
367 contribution of direct (thermal tolerance) and indirect (through species interactions) effects of
368 climate change on the observed decline of this and other cold-adapted species. Whatever the
369 mechanisms, our results show that if climate continues to warm as predicted, the persistence
370 of this cold-adapted species in the study region may depend on its ability to adapt
371 physiologically in response to rising temperatures (Angilletta Jr, et al. 2002, Colwell, et al.
372 2008, García-Robledo, et al. 2016). The evolutionary adaptations of ectotherms to rising
373 temperatures appears to be slow (Hoffmann, et al. 2013), and we found only a weak
374 indication ($p = 0.059$) of *A. lapponum* populations from lower elevations being able to
375 tolerate higher temperatures than those at high elevations. As *A. lapponum* occurs further
376 north and is abundant in Scotland, regional extinction from the UK is unlikely for this species
377 even under the more extreme climate change scenario (+4 °C by the end of the century,
378 Murphy et al. 2009), but local extirpation from the study region is much more likely, if local
379 adaptation does not occur.

380 In conclusion, our results provide evidence for the connection between thermal tolerances
381 and elevational range but more importantly for the detrimental effects of climate warming on
382 upland species through upper thermal constraints. A mechanistic understanding of how
383 climate change directly affects species, such as the one presented here, will provide a robust
384 base to inform predictions of how individual species and whole assemblages may change in
385 the future.

386

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548

549 Table 1. Results from linear models (F-value provided) assessing the effect of beetle elytra
 550 length, sex and the elevation of the source population on (a) CT_{max} and (b) CT_{min} of the three
 551 dominant dung beetle species (*Agoliinus lapponum*, *Agrilinus ater* and *Acrossus depressus*.
 552 Significant effects are highlighted bold.

553 (a) CT_{max}

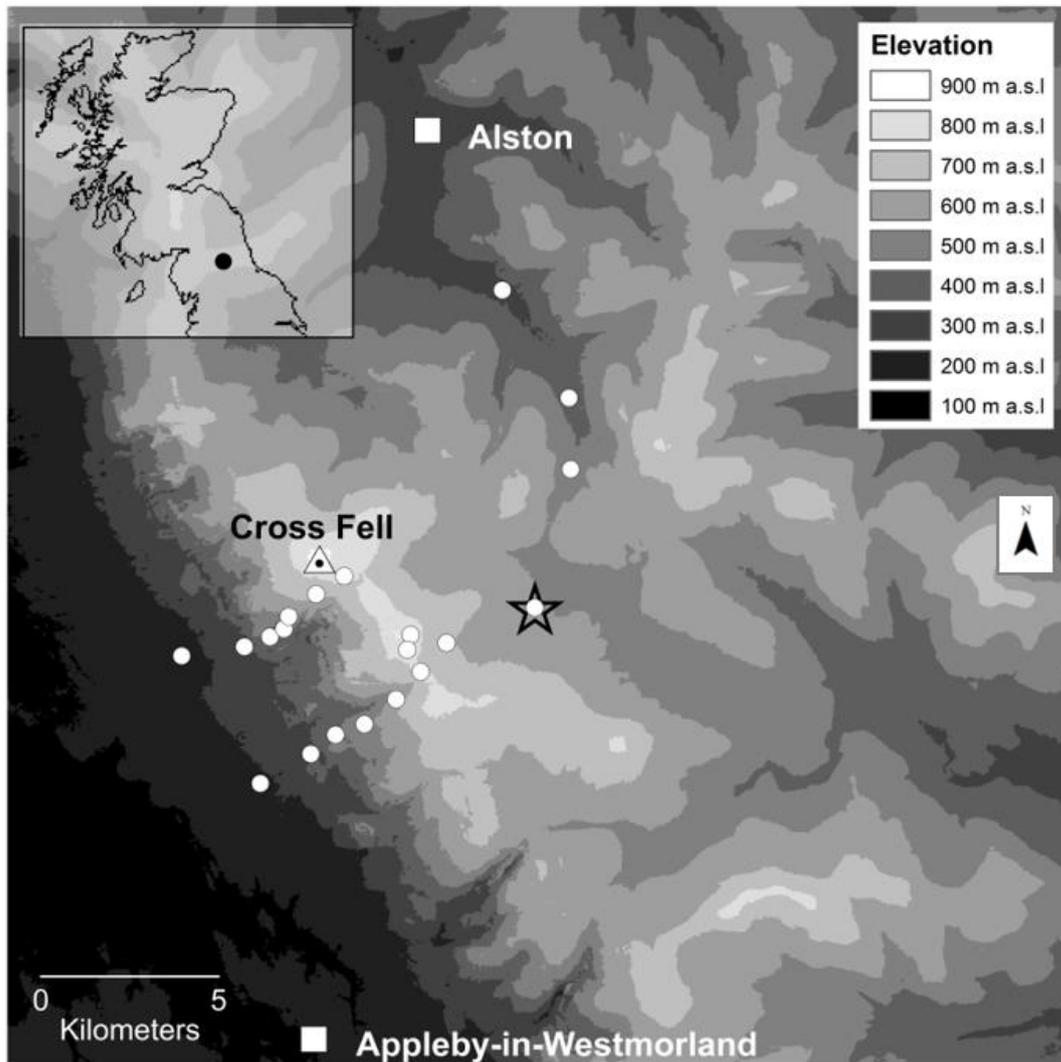
	<i>A. lapponum</i> (n = 20)			<i>A. depressus</i> (n = 19)			<i>A. ater</i> (n = 25)		
	F	d.f.	p value	F	d.f.	p value	F	d.f.	p value
Elytra length	0.047	1	0.831	0.357	1	0.558	0.798	1	0.381
Sex	0.876	1	0.362	1.52	1	0.234	0.203	1	0.657
Elevation	4.061	1	0.059	4.607	1	0.047	0.000	1	0.997

554

555 (b) CT_{min}

	<i>A. lapponum</i> (n = 19)			<i>A. depressus</i> (n = 19)			<i>A. ater</i> (n = 25)		
	F	d.f.	p value	F	d.f.	p value	F	d.f.	p value
Elytra length	0.007	1	0.936	0.944	1	0.335	0.082	1	0.777
Sex	0.881	1	0.361	0.313	1	0.583	0.156	1	0.696
Elevation	0.004	1	0.953	0.076	17	0.787	20.44	1	< 0.001

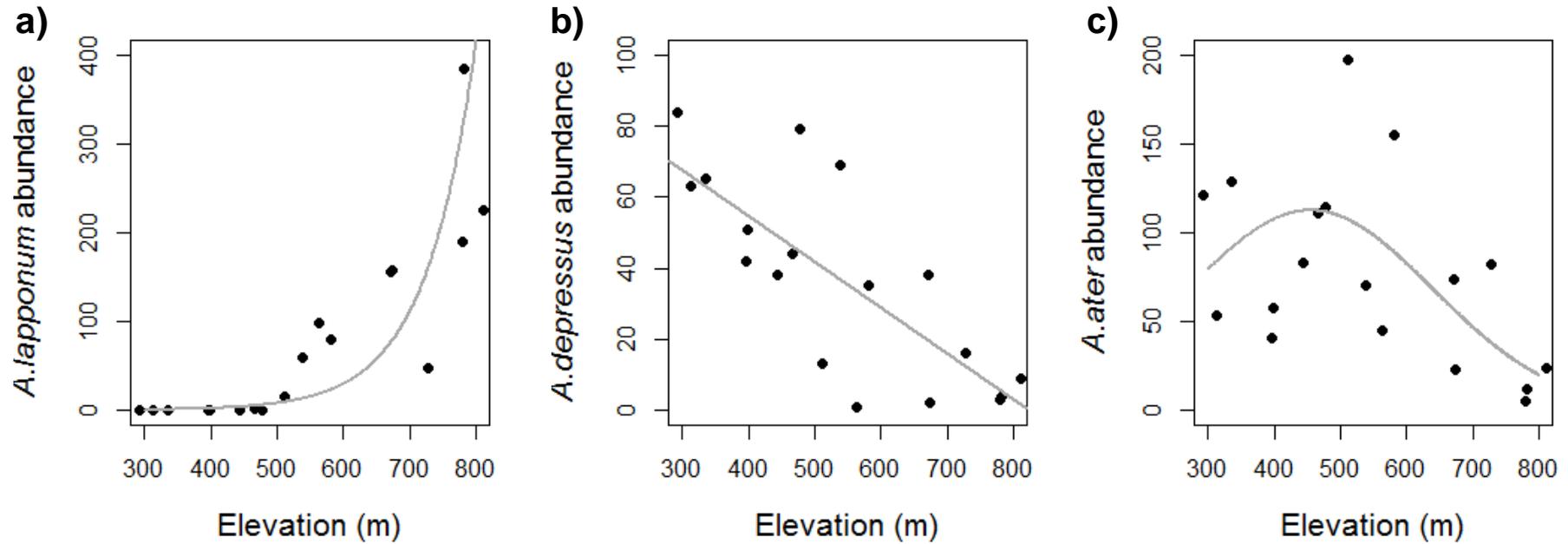
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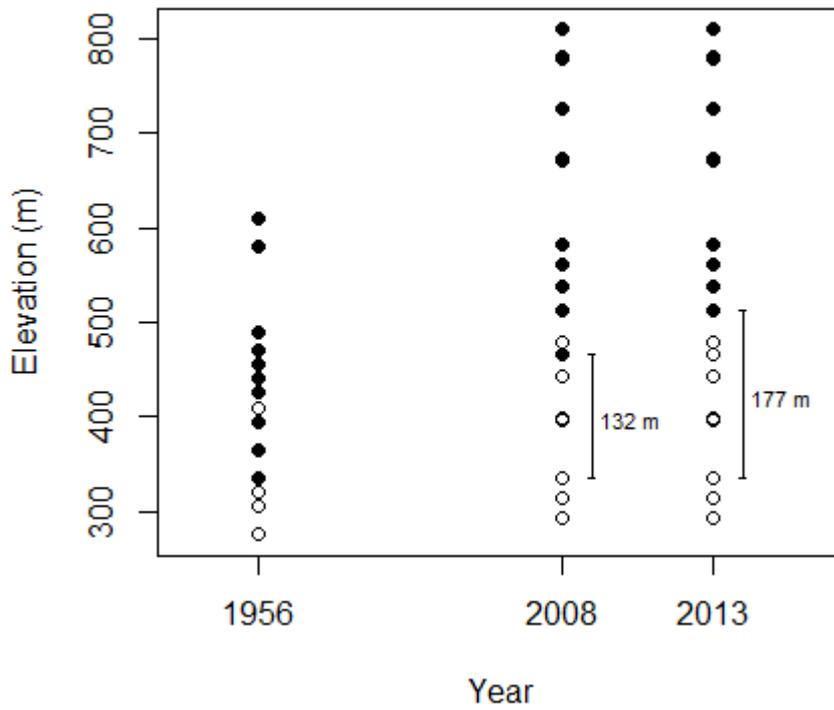
559

560 Figure 1. Location in the northern UK (inset) and digital elevation model of Moor House World
 561 Biosphere Reserve study area. The location of the meteorological station at 560 m (star) and
 562 the 2008 & 2013 dung beetle elevational distribution survey sampling sites (circles) are shown.



565 Figure 2. Current abundance along the elevational gradient for the three dung beetle species (a) *Agoliinus lapponum*; (b) *Acrossus depressus* and
 566 (c) *Agrilinus ater* in the study area (abundance per site for 2008 and 2013 surveys pooled together). Lines represent significant trends.

567



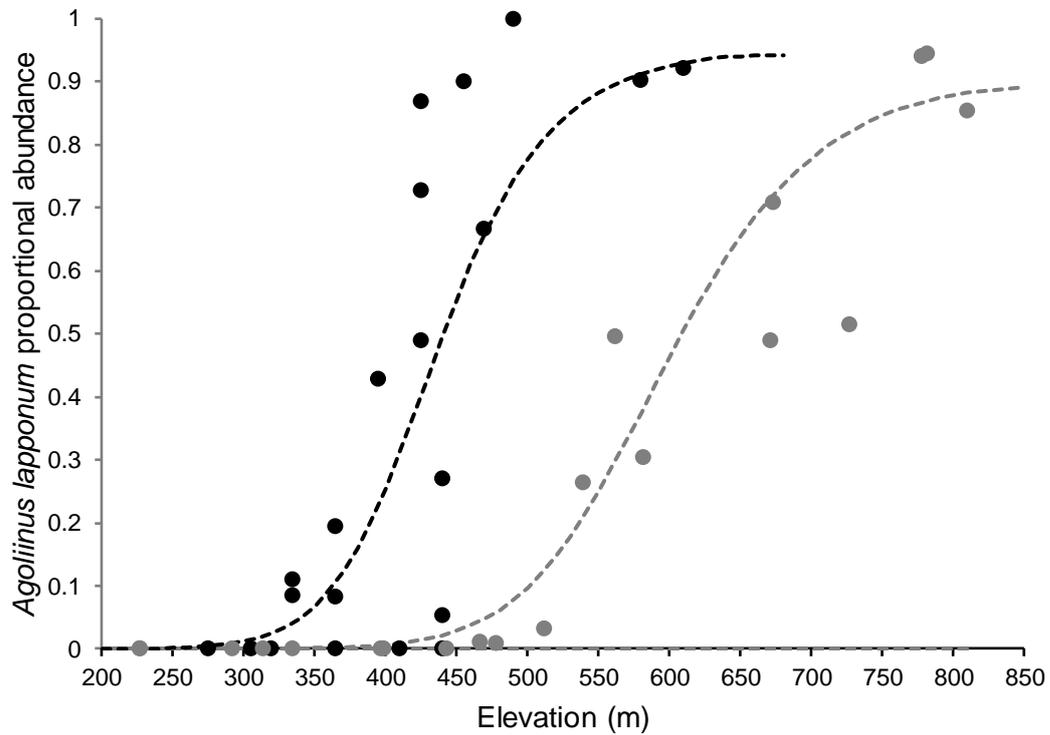
568

569 Figure 3. Elevational distribution of *Agoliinus lapponum* in the study area in each survey year.

570 Observed occupancy with elevation (white circles indicate absences and black circles

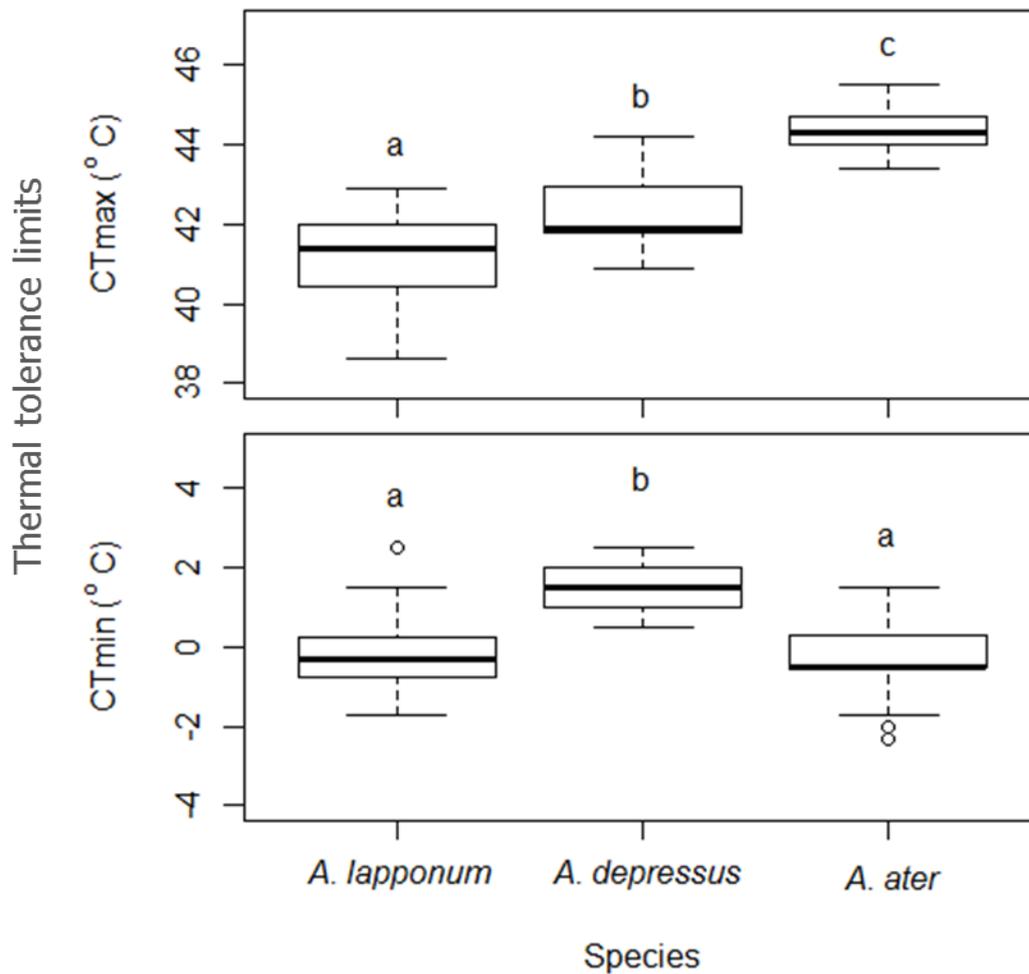
571 presences) and change in lower elevational limit (m) between the historic survey (1956) and

572 the two current surveys (2008 and 2013) are displayed.



574

575 Figure 4. Proportional abundance of *Agoliinus lapponum* by elevation in the historic survey
 576 (black circles, data from 1956) and in the current surveys (grey circles, data from 2008 and
 577 2013 pooled together). Dashed lines depict significant fitted GLM models for each survey
 578 period including both linear and quadratic effects of elevation.



580

581 Figure 5. Maximum (CTmax) and minimum (CTmin) thermal tolerance limits (°C) of the three
 582 dung beetle species (*Agoliinus lapponum*, *Acrossus depressus* and *Agrilinus ater*). Boxplots
 583 displaying the median, the first and third quartile and the maximum and minimum values.
 584 Different letters denote significant differences between species at $p < 0.05$.

Table A1. Dung beetle species and the number of individuals collected during the surveys in 2008 and 2013 in the Moor House study area. Shown in bold are the three focal species of the study (*Acrossus depressus*, *Agoliinus lapponum* and *Agrilinus ater*).

Species	Number of individuals
APHODIIDAE	
<i>Acrossus depressus</i> (Kugelann 1792)	701
<i>Acrossus rufipes</i> (Linnaeus 1758)	74
<i>Agoliinus lapponum</i> (Gyllenhal 1808)	1282
<i>Agrilinus rufus</i> (Moll 1782)	25
<i>Agrilinus ater</i> (De Geer 1774)	1524
<i>Agrilinus constans</i> (Duftschmid 1805)	56
<i>Aphodius fimetarius</i> (Linnaeus 1758)	75
<i>Esymus merdarius</i> (Fabricius 1775)	9
<i>Melinopterus prodromus</i> (Brahm 1790)	27
<i>Nimbus contaminatus</i> (Herbst 1783)	136
<i>Planolinus borealis</i> (Gyllenhal 1827)	10
<i>Planolinus uliginosus</i> (Hardy 1847)	21
<i>Teuchestes fossor</i> (Linnaeus 1758)	4
<i>Aphodiidae sp</i>	2
GEOTRUPIDAE	
<i>Anoplotrupes stercorosus</i> (Scriba 1791)	1
<i>Geotrupes stercorarius</i> (Linnaeus 1758)	2

Table A2. Number of individuals of the three species (*Agoliinus lapponum*, *Agrilinus ater* and *Acrossus depressus*) used in thermal tolerance assays, by the elevation from which they were collected along the southerly transect of the Moor House study site (Fig. 1). Data in brackets are the total number of individuals recorded of each species at the corresponding 100 m elevation band during the 2008 and 2013 surveys. Elytra length range also provided as proxy for body size.

Source elevation (m a.s.l.)	<i>A. lapponum</i>	<i>A. depressus</i>	<i>A. ater</i>
335	(0)	10 (212)	10 (303)
443	(0)	10 (131)	10 (182)
512	10 (17)	10 (136)	10 (422)
582	10 (237)	10 (105)	10 (270)
727	10 (361)	(56)	10 (179)
782	10 (799)	(16)	(41)
Elytra length	4.5 – 6.5 mm	5.8 – 7.6 mm	3.5 – 5.2 mm

Table A3. Abundance and likelihood ratio (LR) of *Agoliinus lapponum* along the elevational gradient in (a) 1956, (b) 2008 and (c) 2013 surveys. LR for elevations outside the recorded species range is calculated based on the abundance of the species across its observed range relative to the overall sampling effort at that elevation (total number of beetles collected). Values of LR above 8 indicate strong evidence that the target species do not occur at that elevation (Rowe, et al. 2010).

a) 1956 survey

Elevation (m a.s.l.)	Total captured individuals	<i>A. lapponum</i> abundance	LR
275	94	0	9.3×10^{19}
305	56	0	2.7×10^{12}
320	106	0	1.6×10^{22}
335	47	4	-
335	54	6	-
365	94	0	-
365	36	3	-
365	82	16	-
395	63	27	-
410	76	0	-
425	46	40	-
425	55	27	-
425	48	35	-
440	63	17	-
440	42	0	-
440	37	2	-
455	40	36	-
470	63	42	-
490	38	38	-
580	62	56	-
610	102	94	-
Total	1304	443	

b) 2008 survey

Elevation (m a.s.l.)	Total captured individuals	<i>A. lapponum</i> abundance	LR
292	83	0	4.9 x10 ¹⁷
314	53	0	3.9 x10 ¹¹
335	115	0	7.0 x10 ²³
397	61	0	1.8 x10 ¹³
399	44	0	5.1 x10 ⁰⁹
443	82	0	3.1 x10 ¹⁷
467	107	2	-
478	255	0	-
512	124	2	-
539	119	26	-
562	46	10	-
582	88	15	-
671	134	72	-
673	74	56	-
727	58	15	-
778	131	125	-
782	177	170	-
810	94	86	-
Total	1845	579	

c) 2013 survey

Elevation (m a.s.l.)	Total captured individuals	<i>A. lapponum</i> abundance	LR
292	149	0	4 x10 ⁵³
314	72	0	1.6 x10 ²⁸
335	107	0	1.9 x10 ⁴⁰
397	32	0	1.4 x10 ¹³
399	79	0	4.9 x10 ³⁰
443	85	0	6.2 x10 ³²
467	76	0	4.2 x10 ²⁹
478	85	0	6.2 x10 ³²
512	134	13	-

539	92	34	-
562	104	88	-
582	195	64	-
671	141	83	-
673	110	102	-
727	95	33	-
778	68	64	-
782	226	214	-
810	167	140	-
Total	2017	835	

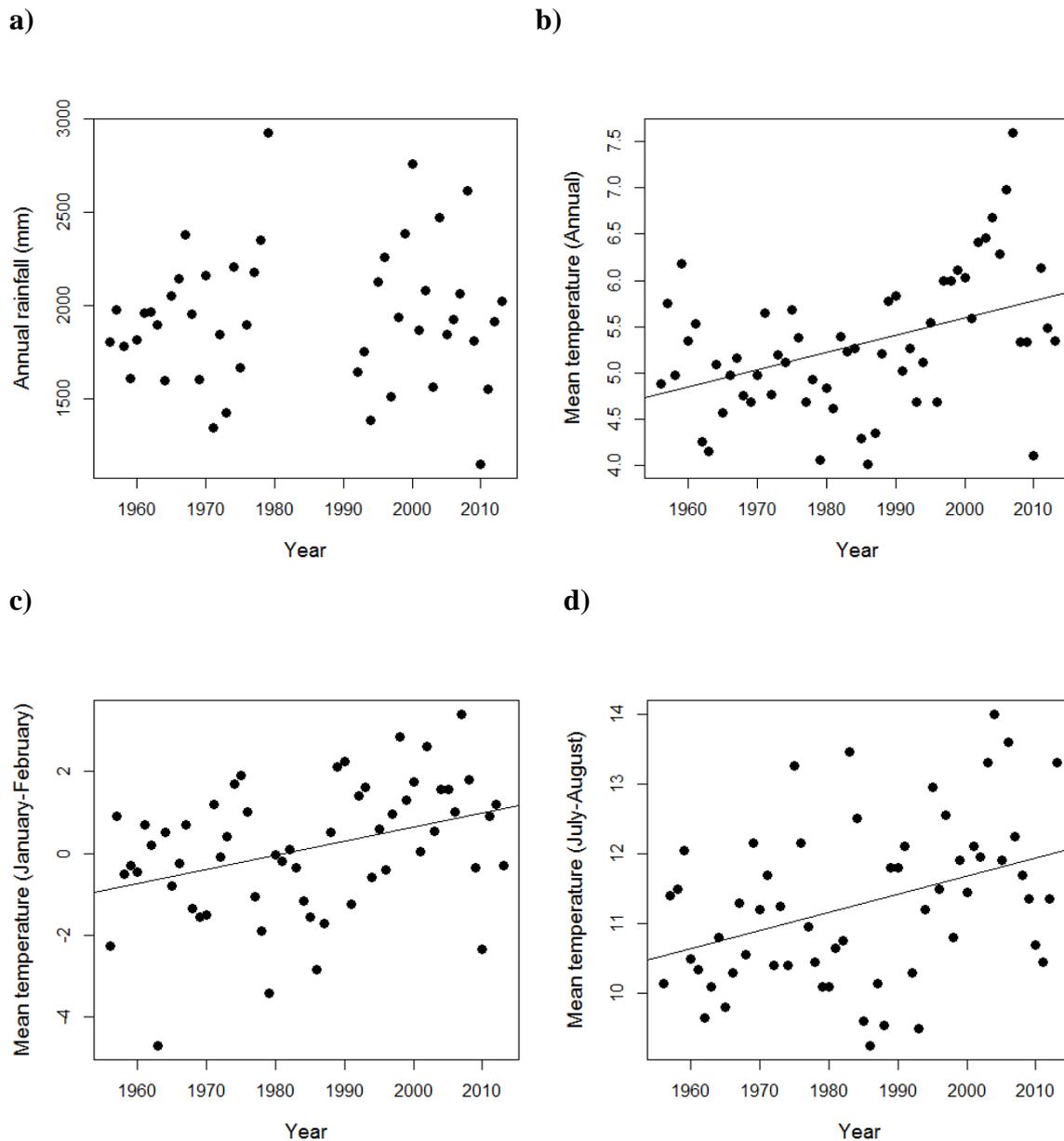


Figure A1. Climate trends from 1956 to 2013, showing (a) mean annual rainfall, (b) mean annual temperature in °C (c) mean temperature of the coldest months (January-February) in °C and (d) mean temperature of the warmest months (July-August) in °C. Lines depict significant linear trend over time. Data were recorded at the meteorological station situated in the study area at 560 m a.s.l. (Fig. 1), and provided by the UK Environmental Change Network (<<http://www.ecn.ac.uk>>). For the period of 1980-91 there is a gap in the climate records so not rainfall data are available for this period, temperature data for this period have been calculated from a nearby station 6.6 km away, situated at similar elevation and habitat type (see

Holden & Rose 2011 for more details on how the data were calibrated to make all data comparable for the period 1956-2013).

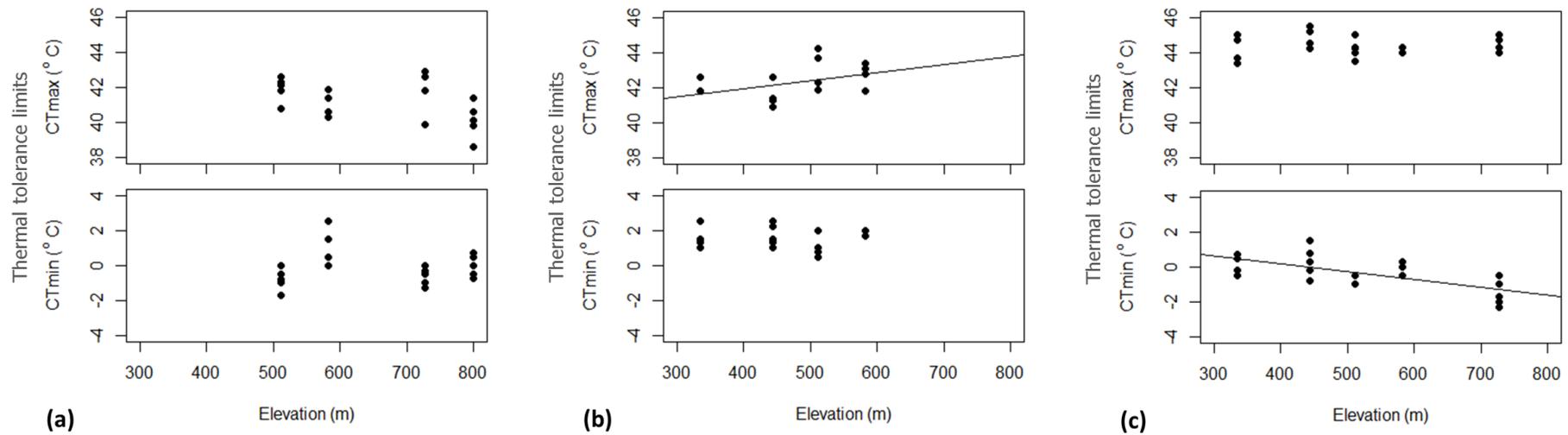


Figure A2. Relationship between the elevation from which the beetle was collected and the maximum (CTmax: top) and minimum (CTmin: bottom) thermal limits (°C) of each individual of (a) *Agoliinus lapponum*, (b) *Acrossus depressus* and (c) *Agrilinus ater* tested. Lines depict significant trend.