Individual plasticity drives boldness senescence in a territorial butterfly

Journal:	*Ethology*
Manuscript ID	ETH-20-0059.R1
Manuscript Type:	Research Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Kaiser, Aurélien; Universite catholique de Louvain Earth and Life Institute, Earth and Life Institute Eymard, Manon; Universite catholique de Louvain Earth and Life Institute Merckx, Thomas; Universite catholique de Louvain Earth and Life Institute; University of Oulu, Department of Ecology and Genetics Van Dyck, Hans; Universite catholique de Louvain Earth and Life Institute
keywords:	Animal personality, Behavioural syndrome, Movement ecology, Pararge aegeria, Routine movements, Thermal ecology

Individual plasticity drives boldness senescence in a territorial butterfly

Running title: Boldness senescence in a butterfly

Aurélien Kaiser[1], Manon Eymard[1], Thomas Merckx[1,2] & Hans Van Dyck[1]

[1] Behavioural Ecology & Conservation Group, Earth & Life Institute, UCLouvain, Louvain-la-Neuve, Belgium

[2] Department of Ecology and Genetics, University of Oulu, Oulu, Finland

ORCID Aurélien Kaiser: 0000-0001-7552-8748

ORCID Thomas Merckx: 0000-0002-6195-3302

ORCID Hans Van Dyck: 0000-0002-2013-6824

Corresponding author: Aurélien Kaiser; E-mail: kaiser.aurelien@gmail.com; Tel: +32 (0) 10 47 34 95

Acknowledgements

AK was supported by an Aspirant grant from the Belgian Fund of Scientific Research F.S.R.-FNRS. The research was supported by PDR grant FNRS PDR T.0188.14 and an ARC research grant of Fédération Wallonie-Bruxelles and UCLouvain (ARC-grant 17/22-086) to HVD. This is publication number BRC 364 of the Biodiversity Research Centre (ELI/ELIB, UCLouvain).

Abstract

Most behavioural traits show plastic responses to changes in internal or external conditions. Similarly, animal personality is not necessarily fixed during an individual's lifetime, leading to age-related changes. Both individual plasticity (i.e. within-individual effect) and non-random selective (dis)appearance of behavioural types (i.e. between-individual effect) may contribute to age-related changes observed at the population level. Here, we investigated how boldness changes with age in a woodland population of the Speckled wood butterfly (*Pararge aegeria* L.) using a capture-mark-recapture approach. We used wing wear as an index for age and we show that fresh individuals are bolder than worn individuals. Using the subsample of recaptured butterflies, we found that this pattern is most likely driven by individual plasticity. Our design also allowed us to explore some aspects of the species' spatial ecology and how it relates to personality. We found no relationship between boldness and net displacement between successive captures, a finding which we discuss within a movement ecology framework.

Keywords

Animal personality – Behavioural syndrome – Lepidoptera – Movement ecology – *Pararge aegeria* – Routine movements – Thermal ecology

Introduction

Phenotypic plasticity is an important trait that enables organisms to cope with changing environmental conditions. Virtually all phenotypic traits can show plastic responses at some point, either triggered by external or internal cues. In this context, ageing can be a major driver of within-individual changes. For example, reproductive senescence, the age-related decline in reproductive output, is widely documented in wild animals (Lemaître and Gaillard 2017) and is thought to occur mainly as a consequence of within-individual changes (Nussey et al. 2008). Yet, between-individual changes such as selective (dis-)appearance of certain phenotypes may also explain trait differences between age classes. At the population level, reduced reproductive performance in older individuals may well be due to good reproducers trading off reproductive output with lifespan, and hence dying earlier than poor reproducers (i.e. selective disappearance). Alternatively, poor reproducers may be excluded from reproducing at a young age by more competitive individuals and they may gain access to reproduction only late in life (i.e. selective appearance) (van de Pol and Verhulst 2006). Obviously, a wide array of morphological, physiological and behavioural traits may differ between age classes. Consequently, a proper evaluation of within- versus between-individual changes is needed to fully understand such dynamics.

The study of personalities has gained ground within behavioural ecology (David and Dall 2016). While in humans there is a long tradition of quantifying personality traits, only during the last twenty years or so a lot of evidence has been accumulating on conceptually similar variation in non-human animals (Gosling 2008). Personality refers to inter-individual, intraspecific behavioural variation that is stable over time and in different contexts (Stamps and Groothuis 2010). Nonetheless, the concept of personality does not imply that personality traits are necessarily fixed within an individual's lifetime. Just as with other behaviours, individuals may display plastic adjustments to personality traits. Recent studies have shown that personality

changes with age (e.g. Humphries et al. 2015; Favati et al. 2016; Polverino et al. 2016; Stanley et al. 2017). Although only few studies have tried to tease apart within- and between-individual changes, age-related changes in personality traits appear so far to be mainly caused by within-individual changes (i.e. individual plasticity) (e.g. Class and Brommer 2016; Fisher et al. 2018).

The Speckled wood butterfly (*Pararge aegeria*) is a long-established study system in behavioural ecology. Particularly the mate location behaviour in males has attracted much attention since the game theory-inspired pioneer field study by Nick Davies (Davies 1978). Males try to monopolize a sunlit patch on the forest floor as a rendezvous site for receptive females, which they aggressively defend against other males (reviewed in Van Dyck 2003). More recently, consistent inter-individual variation in behavioural traits has been documented in *P. aegeria*, providing evidence for personality traits in this butterfly (Ducatez et al. 2014; Kaiser et al. 2018). Here, within the same species, we tested the repeatability of a boldness measure under variable field conditions and we investigated age-related changes in this personality trait in a wild woodland population. In a recent study, we reported on boldness senescence in laboratory-reared Speckled woods (Kaiser et al. 2019a). While between-individual changes were an unlikely cause of age-related decline in boldness in this F1 captive-reared population, different mechanisms may be at work in natural populations. Indeed, maintaining animals in laboratories often involves providing *ad-libitum* food and ensuring the absence of predators and pathogens, but such conditions may disrupt the relationship between behaviour and survival (Moiron et al. 2020). In this context, typically harsher natural conditions provide useful set-ups to investigate the role of between-individual patterns. Because bold individuals are supposed to take more risks when exposed to threats (Réale et al. 2007), one may expect bold phenotypes to be removed first form a population (i.e. selective disappearance of bold types). However, since the boldness test we developed mimics a butterfly being stuck in, for example, a spider web or a bird's beak (see below), we can actually expect the opposite

pattern as bold individuals may be able to escape their predators more easily by moving more under stressful conditions. Whatever their direction, such between-individual changes may be reinforced (or instead, counteracted) by within-individual changes, as both mechanisms may play a role in shaping age-related changes in boldness in natural populations (van de Pol and Verhulst 2006). As such, a proper evaluation of the underlying processes is required.

Our capture-mark-recapture study also enabled us to test hypotheses on species-specific thermal ecology and to evaluate whether boldness relates to space use in male Speckled woods. Recently, Spiegel et al. (2017) proposed a framework that links personality traits with some aspects of spatial dynamics in animal populations. While several studies have shown that dispersal is often non-random and biased towards some behavioural types (i.e. personality-dependent dispersal – Cote et al. 2010), few studies have investigated the co-variation between personality traits and aspects of daily space use such as home range size (Spiegel et al. 2017). As an attempt to investigate this in an invertebrate species, we used the net displacement between successive captures as a proxy for space use. While some personality traits, such as activity and exploration, are inherently linked to movements, the relationship between boldness and daily movements is less straightforward. Yet, we expect such a co-variation to arise because shy individuals are expected to take less risks and to more strongly avoid risky environments. We thus expect shy butterflies to settle with high fidelity on territories that they perceive as safe, while we expect bolder individuals to move longer distances between successive captures, indicating larger home ranges.

Material and methods

Study species

113 The Speckled wood (*P. aegeria* L.) is a satyrine butterfly occurring principally in woodland
114 and along woodland edges across its European range (Settele et al. 2008). In Belgium and The
115 Netherlands, it is a very common and widespread species (Van Dyck et al. 2009). The species
116 has a monandrous mating system (Svärd 1985) and males typically emerge before females (i.e.
117 protandry – Gotthard et al. 1994). Under woodland habitat conditions males locate females by
118 either territorial perching or by patrolling (Van Dyck et al. 1997).

119 *Study site*

120 Our study was conducted in a 231 ha woodland, called 'Bois de Lauzelle'. This mixed
121 woodland is locally dominated by oak trees (*Quercus robur*). It is owned by the UCLouvain
122 university and situated near the university campus. Data were collected in the summer of 2018,
123 from July to September, within the period corresponding to the cohort of directly developed
124 summer generation adults (i.e. development without a larval or pupal diapause – Wiklund et al.
125 1983). A map of the study area showing the location of all capture events is shown in Figure
126 S1.

127 *Personality trait, temperature, body size and net displacement*

128 We collected data on Speckled wood males adopting a territorial perching mate location
129 strategy. If a male was spotted during the walking route across the study area, its behaviour was
130 observed to confirm the status of being a territorial male on a sunlit patch at the forest floor (see
131 Van Dyck et al. 1997). Next, the male was captured with a butterfly net and relative adult age
132 was estimated based on the level of wing wear. We used a four-level scale (1 = freshly emerged
133 adult, to 4 = heavily damaged wings, i.e. lost wing scales and a significant loss of wing
134 fragments) (cf. Kemp and Alcock 2003; Takeuchi 2006).

135 Then, the butterfly was gently placed with closed wings in a semi-transparent glassine envelope
136 (63 x 97 mm) and we counted the number of movements the butterfly made either with the

137 head, the wings or the legs during one minute under these stressful conditions. For further
138 details about this boldness test and its significance, we refer to our previous work (Kaiser et al.
139 2018, 2019a, b). After the test, the butterfly was marked by writing a unique number on the left
140 ventral hind wing and we measured body temperature by making contact at the level of the
141 thorax with an infrared thermometer (IBP TS 402). We took three measures within a minute
142 and calculated the average value. Simultaneously, ambient temperature was also measured in
143 the shade close to the sunlit patch (HOBO Pendant Temperature/Light 64K data logger). Before
144 we released the butterfly, we measured the right forewing length from the wing base (i.e. close
145 to the thorax) to the wing tip with a digital calliper (precision: ± 0.1 mm). Wing length was
146 measured three times and we calculated the average as a measure of body size for each
147 individual male. We also recorded capture coordinates (World Geodetic System – WGS84)
148 using the free smartphone app "My GPS Coordinates". Coordinates were written down when
149 the spatial accuracy provided by the app was at least 15 m. A single observer (ME) conducted
150 all behavioural observations and carried out all measurements.

151 For recaptured individuals, we also calculated the net displacement (i.e. Euclidean distance)
152 between the first and the second capture site, based on capture coordinates imported into QGIS
153 (version 3.6). As very few individuals (N = 4) were captured more than two times, we calculated
154 the net displacement between the first and second capture only.

155 *Statistical analysis*

156 All statistical analyses were performed with R 3.5.1 (R Core Team 2020) and all continuous
157 variables were scaled prior to analysis. We analysed the relationship between thorax
158 temperature and ambient temperature using a linear mixed model (*lme4* package). Thorax
159 temperature was the response variable and ambient temperature and forewing length (used as a
160 proxy for body size) were the explanatory variables. Because some individuals were measured
161 several times, we included individual ID as a random effect (i.e. random intercept). We also

included date as a random effect (random intercept). For the analysis of thorax temperature, we used the complete dataset (N observations = 212; N individuals = 183). Boldness was analysed with a generalized linear model, with a Poisson error structure and log link function. We fitted two models. The first model included wing wear (treated as a factor), thorax temperature and forewing length as explanatory variables. Again, we included individual ID and date as random effects in the model. This first model included all behavioural observations (i.e. complete dataset, see above). Based on this model, we estimated boldness repeatability (using the *rptR* package).

Because we found that boldness decreased with wing wear (i.e. a proxy for age) at the population level and we aimed to unravel whether this was due to within- or between-individual effects, we fitted a second model for boldness using a within-individual centring approach (see van de Pol and Wright 2009). The general idea is to decompose age effects into two variables that would reflect within- and between-individual changes. Because the precise age of wild-caught individuals could not be known with precision, we used the number of days since the first capture (to which we applied within-individual centring) as a proxy for within-individual effects. We used individual's maximum wing wear values as a proxy for between-individual effects (treated as a factor) because we aimed at testing the effect of selective disappearance (van de Pol and Verhulst 2006). The model again included thorax temperature and forewing length as explanatory variables and individual ID and date as random effects. The dataset used for this model only contained individuals which were assessed multiple times for boldness (N observations = 54; N individuals = 25).

Net displacement (in meters) between the first and second captures was log-transformed prior to analysis to achieve normality of the residuals. Using data collected from recaptured males (N = 25), we fitted a linear model with forewing length and the number of days elapsed between the two captures as explanatory variables.

187 Finally, we assessed the correlation between boldness and net displacement in recaptured males
188 using a bivariate Bayesian model (*MCMCglmm* package). The model contained boldness and
189 net displacement (log-transformed) as response variables. Similar to the univariate models
190 presented above, we included trait-specific fixed effects: wing wear, thorax temperature and
191 forewing length for boldness; forewing length and the number of days elapsed between the two
192 captures for net displacement. Random effects included individual ID and date. Boldness and
193 net displacement were fitted with a Poisson and a Gaussian error structure, respectively. We
194 used a non-informative prior, with 300,000 iterations, from which we discarded the first 30,000
195 (burn-in), while using a thinning interval of 100. These model specifications resulted in low
196 autocorrelation and we performed three runs to confirm the robustness of the outputs. Then, we
197 computed the between-individual correlation between boldness and net displacement. The
198 correlation was considered significant when the 95% credible interval did not overlap with zero.

199

200 **Results**

201 A total of 183 territorial males were captured at least once. Among those, 158 were captured
202 once, 21 twice and four individuals were captured three times (i.e. overall recapture rate of
203 13.7%). Their thorax temperatures varied between 24.5 and 35.1°C, with 74.5% of the values
204 in the 28 to 32°C range. Thorax temperature increased with ambient temperature ($F_{1,165.3}$ =
205 187.54, $P < 0.001$; Fig. 1), being on average 3.6 ± 1.4°C (mean ± SD) above ambient
206 temperatures, and was not related to body size ($F_{1,165.7} = 0.0001$, $P = 0.99$).

207 During the one-minute test the boldness score varied considerably among males, from no
208 movement at all in 45.8% (97/212) of the assessments to 32 movements (Fig. S2). Boldness
209 shows a significant repeatability of 0.65 (95% confidence interval: 0.27 – 0.88). Overall,
210 boldness scores increased with increasing thorax temperature ($\chi^2_1 = 5.15$; $P = 0.023$) and older

males, as measured by their level of wing wear, had lower boldness scores than young males ($\chi^2_3 = 13.39$; $P = 0.004$; Fig. 2). Pairwise comparisons (Tukey post-hoc test) showed that young individuals had on average higher boldness scores than worn individuals (wing wear 1 versus 3: P = 0.004; wing wear 1 versus 4: P = 0.024; wing wear 2 versus 3: P = 0.010; P-values obtained after applying Benjamini & Hochberg adjustment for multiple testing) (Fig. 2). Boldness was independent of body size ($\chi^2_1 = 1.19$; $P = 0.28$).

Using the subset of individuals that were assessed multiple times for boldness, we explored whether the observed age-related decline in mean boldness occurred because of individual plasticity *versus* selective disappearance. Maximum wing wear (used as a proxy for between-individual effects) was unrelated to boldness ($\chi^2_3 = 4.53$; $P = 0.21$), while boldness tended to decrease with time since the first capture (after applying within-individual centring – used as a proxy for within-individual effects) ($\chi^2_1 = 2.78$; $P = 0.095$) (Fig. 3).

The distance moved between the first and second capture varied between 2 and 445 m. However, more than half of the recaptures (14/25) occurred within 50 m (Fig. S3). Net displacement was neither related to forewing length ($F_{1,22} = 0.29$, $P = 0.59$), nor to the number of days elapsed between both captures ($F_{1,22} = 1.89$, $P = 0.18$). We found no significant correlation between boldness and net displacement in recaptured males (between-individual correlation: -0.09 – credible interval: -0.59; 0.42).

Discussion

Using a capture-recapture design, we assessed variation in boldness in a woodland population of the Speckled wood butterfly. Our field study showed that, at the population level, boldness declined with increasing age, and we show that this is probably due to within- rather than between-individual changes. We also investigated whether boldness correlated with net

displacement between captures as a simple measure of space use, but we did not find evidence for such a relationship. In the following sections, we will discuss these results in the light of the ecology of the study species and integrate our current findings with the existing knowledge on butterfly personality.

Body temperature

While the age dynamic of boldness and its co-variation with space use were the primary focus of our study, our experimental design also allowed us to study some aspects of the thermal ecology of the Speckled wood in woodland habitat and to compare our results and methods to previously published studies on this species. Thoracic temperature scaled with ambient temperature and about 75% of the butterflies had a thoracic temperature in the range of 28 to 32°C. While our results are overall similar to those obtained by Van Dyck & Matthysen (1998), some slight discrepancies can be pointed out that probably relate to methodological differences. For instance, the latter study reported slightly higher (i.e. +2°C) thoracic temperatures, which may be due to the use of a thermocouple sensor inserted inside the thorax while we measured surface temperature. In the butterfly *Heteronympha merope*, internal thorax temperature measured with such a probe was indeed 1.3°C higher than surface temperature (Nève and Hall 2016). Also, Van Dyck & Matthysen (1998) measured internal temperature shortly after capture (within 5 seconds) while in our study at least 1 minute (i.e. the duration of the boldness assessment and butterfly marking) systematically elapsed before we recorded thoracic temperature. Accounting for those methodological aspects may well explain the difference between both studies.

Age-related change in boldness: within- versus *between-individual changes*

Here, we tested for personality under field conditions in the Speckled wood. By far most personality studies make use of laboratory settings, often with reared individuals as we did

previously too (Kaiser et al. 2019a, b). It is therefore interesting to underscore that despite the increased environmental variation under woodland conditions (e.g. in temperature, humidity level or solar radiation), we were still able to show a significant repeatability for boldness. While our simple test proved to be useful to assess boldness under controlled, laboratory conditions, our current results show that the envelope test can also be used to quantify boldness variation in wild populations.

Using wing wear as a proxy for age, we detected an age-related decline in boldness at the population level. Based on the subset of individuals that were captured and assessed for boldness multiple times, we aimed to unravel the underlying mechanisms responsible for the observed decline in boldness with age. Although our ability to discriminate within- and between-individual changes was ultimately limited by the relatively low recapture rate, our results suggest that individual plasticity (i.e. within-individual changes) was the main cause of boldness senescence in the study population. Moiron et al. (2020) found that shy individuals generally have a reduced lifespan under natural conditions (i.e. wild populations as opposed to laboratory conditions). Thus, one could expect selective disappearance to play an important role in shaping age-related changes in personality traits at the population level, but we found no evidence for such an effect in our study. Rather, we found a trend for within-individual changes in boldness. Interestingly, a similar decline of boldness at the population level occurred in laboratory-reared Speckled wood butterflies (Kaiser et al. 2019a) which also appeared to be driven by within-individual changes as mortality was typically very low in the laboratory and selective disappearance of bold types was unlikely to cause the observed pattern. This strongly suggests that individual plasticity is the underlying mechanism explaining the age-related decline in the mean level of boldness, and this in both wild and laboratory populations of the Speckled wood butterfly.

283 Individual plasticity of personality traits as individuals get older has been reported a few times,
284 but the direction of those changes appears to vary among species. For example, handling
285 aggression decreased with age in a wild population of Great tits (*Parus major*) (Class and
286 Brommer 2016), while activity and the tendency to leave a tube both increased as individual
287 Field crickets (*Gryllus campestris*) get older (Fisher et al. 2015, 2018). The direction of the
288 change probably depends on the trait itself, how it relates to fitness components and the
289 proximate mechanism underlying this change. Identifying the proximate cause of the decline in
290 boldness observed here was beyond the scope of this study. We can only speculate about
291 potential proximate mechanisms, which for instance include age-related decline in brain size
292 (Pasquet et al. 2018) and energy depletion, both of which could negatively affect the expression
293 of personality traits. This now warrants further research.

294 *Within-site movements and boldness*

295 In his pioneer work on the Speckled wood butterfly, Davies (1978) noted that males tend to be
296 very sedentary, with as much as 90% of the marked individuals spending their entire life within
297 50 m of the point of first capture. Similarly, Bergerot et al. (2012) found that recapture
298 probability of Speckled woods was highest at 25m from the initial capture location. Males in
299 our study population display a high site fidelity too, with 56% of the recaptures occurring within
300 50 m, and 80% within 100 m.

301 Many personality traits are (in-) directly linked to information gathering and spatial processes,
302 and thus they may be expected to influence daily movements occurring at relatively small
303 spatial scales (Spiegel et al. 2017). Recent empirical studies confirmed that personality indeed
304 plays a role in shaping such movements (e.g. Minderman et al. 2010; Spiegel et al. 2015;
305 Villegas-Ríos et al. 2018; Schirmer et al. 2019). Yet, our current knowledge on animal
306 personality, and how it relates to other biological processes, remains heavily biased towards
307 vertebrates (Kralj-Fišer and Schuett 2014). Here, as a first attempt to explore the relationship

between a personality trait and space use in a territorial butterfly species, we investigated under natural conditions whether boldness related to net displacement in the Speckled wood. However, neither boldness nor forewing length affected local movements within a sunlit patch or between sunlit patches. Thus, net displacement appears to be independent of personality and flight morphology in our study. Perhaps this pattern is not surprising as we focused on territorial males whose mate-location strategy mainly involves routine movements biased towards their territories. Here we should stress that there are clear differences between routine movements and large-scale movements such as dispersal. The former include movements associated with daily activities like foraging and mate-location (Van Dyck and Baguette 2005), while the latter involve between-population movements underpinned by a decision to leave a habitat patch for another (Stevens et al. 2010, 2014). Large-scale movements associated with dispersal likely require a special set of behavioural, physiological and morphological traits, integrated within dispersal syndromes (Stevens et al. 2014). In butterflies, dispersal syndromes typically include long wings (Stevens et al. 2012) and strong flight performance (as measured under stressful conditions; Legrand et al. 2015). Routine movements, however, may not require such a special design (Van Dyck and Baguette 2005), while we can expect the spatial distribution and dynamics of resources to play an important role in shaping daily movements. This has been shown, for example, in two related satyrine butterfly species, where local flight activity at both the individual and species level was not linked to forewing length – and hence dispersal capacity – but where local occurrence was closely linked to the presence of habitat resources (Merckx and Van Dyck 2002).

Yet, we acknowledge that our study does not provide final, clear-cut evidence for the absence of a personality/space use relationship in the Speckled wood. Here, we considered only one aspect of local space use that relates to territoriality. It would now be interesting to monitor a set of males more closely and more frequently than we did in our current study to obtain precise

333 estimates of their realized home range. It could well be that bold perchers are able to hold larger
334 territories and to cover larger home ranges than perchers that are less bold. Additionally, we
335 only considered territorial males, but the mate-locating behaviour system in the Speckled wood
336 butterfly involves both territorial and patrolling males within the same population (Van Dyck
337 2003 and references therein). Both patrollers and females (i.e. the dispersive sex in *P. aegeria*
338 – Hill et al. 1999) are by nature less constrained to a spot like a particular sunlit territory and
339 even their routine movements are likely to occur at wider spatial scales compared to territorial
340 males. Consequently, for females and patrolling males we do expect a relationship between
341 personality and space use. As such, we hope our work will inspire and stimulate further research
342 on the interplay between personality traits and spatial ecology in the Speckled wood, and more
343 generally in other invertebrate taxa. Invertebrates play important roles in ecosystems and
344 understanding how personality shapes their daily movements relative to other factors is
345 therefore important. In this regard, the further miniaturization of (GPS) tracking devices (see
346 for example Woodgate et al. 2016) will benefit this field of research. Indeed, obtaining long-
347 term data on individual animal movements is currently challenging, but of prime importance to
348 accurately explore personality and space use relationships (Spiegel et al. 2017).

349

350 **References**

351 Bergerot B, Merckx T, Van Dyck H, Baguette M (2012) Habitat fragmentation impacts
352 mobility in a common and widespread woodland butterfly: do sexes respond differently?
353 BMC Ecol 12:5. doi: 10.1186/1472-6785-12-5

354 Class B, Brommer JE (2016) Senescence of personality in a wild bird. Behav Ecol Sociobiol
355 70:733–744. doi: 10.1007/s00265-016-2096-0

356 Cote J, Clobert J, Brodin T, et al (2010) Personality-dependent dispersal: characterization,

357 ontogeny and consequences for spatially structured populations. Philos Trans R Soc
358 Lond B Biol Sci 365:4065–76. doi: 10.1098/rstb.2010.0176

359 David M, Dall SRX (2016) Unravelling the philosophies underlying 'Animal Personality'
360 studies: a brief re-appraisal of the field. Ethology 122:1–9. doi: 10.1111/eth.12445

361 Davies NB (1978) Territorial defence in the speckled wood butterfly (*Pararge aegeria*): The
362 resident always wins. Anim Behav 26:138–147. doi: 10.1016/0003-3472(78)90013-1

363 Ducatez S, Humeau A, Congretel M, et al (2014) Butterfly species differing in mobility show
364 different structures of dispersal-related syndromes in the same fragmented landscape.
365 Ecography (Cop) 37:378–389. doi: 10.1111/j.1600-0587.2013.00365.x

366 Favati A, Zidar J, Thorpe H, et al (2016) The ontogeny of personality traits in the red
367 junglefowl, *Gallus gallus*. Behav Ecol 27:484–493. doi: 10.1093/beheco/arv177

368 Fisher DN, David M, Rodríguez-Muñoz R, Tregenza T (2018) Lifespan and age, but not
369 residual reproductive value or condition, are related to behaviour in wild field crickets.
370 Ethology 124:338–346. doi: 10.1111/eth.12735

371 Fisher DN, David M, Tregenza T, Rodríguez-Muñoz R (2015) Dynamics of among-individual
372 behavioral variation over adult lifespan in a wild insect. Behav Ecol 26:975–985. doi:
373 10.1093/beheco/arv048

374 Gosling SD (2008) Personality in non-human animals. Soc Personal Psychol Compass 2:985–
375 1001. doi: 10.1111/j.1751-9004.2008.00087.x

376 Gotthard K, Nylin S, Wiklund C (1994) Adaptive variation in growth rate: life history costs
377 and consequences in the speckled wood butterfly, *Pararge aegeria*. Oecologia 99:281–
378 289. doi: 10.1007/BF00627740

379 Hill JK, Thomas CD, Blakeley DS (1999) Evolution of flight morphology in a butterfly that

has recently expanded its geographic range. Oecologia 121:165–170. doi: 10.1007/s004420050918

Humphries MM, McAdam AG, Boutin S, Kelley AD (2015) Changes in wild red squirrel personality across ontogeny: activity and aggression regress towards the mean. Behaviour 152:1291–1306. doi: 10.1163/1568539X-00003279

Kaiser A, Merckx T, Van Dyck H (2018) Urbanisation and sex affect the consistency of butterfly personality across metamorphosis. Behav Ecol Sociobiol 72:188. doi: 10.1007/s00265-018-2616-1

Kaiser A, Merckx T, Van Dyck H (2019a) Personality traits influence contest outcome, and vice versa, in a territorial butterfly. Sci Rep 9:2778. doi: 10.1038/s41598-019-39155-9

Kaiser A, Merckx T, Van Dyck H (2019b) Behavioural repeatability is affected by early developmental conditions in a butterfly. Anim Behav 157:219–226. doi: 10.1016/j.anbehav.2019.08.006

Kemp DJ, Alcock J (2003) Lifetime resource utilization, flight physiology, and the evolution of contest competition in territorial insects. Am Nat 162:290–301. doi: 10.1086/376890

Kralj-Fišer S, Schuett W (2014) Studying personality variation in invertebrates: why bother? Anim Behav 91:41–52. doi: 10.1016/j.anbehav.2014.02.016

Legrand D, Trochet A, Moulherat S, et al (2015) Ranking the ecological causes of dispersal in a butterfly. Ecography (Cop) 38:822–831. doi: 10.1111/ecog.01283

Lemaître J-F, Gaillard J-M (2017) Reproductive senescence: new perspectives in the wild. Biol Rev 92:2182–2199. doi: 10.1111/brv.12328

Merckx T, Van Dyck H (2002) Interrelations among habitat use, behavior, and flight-related morphology in two cooccurring satyrine butterflies, *Maniola jurtina* and *Pyronia*

403 *tithonus*. J Insect Behav 15:541–561. doi: 10.1023/A:1016385301634

404 Minderman J, Reid JM, Hughes M, et al (2010) Novel environment exploration and home
405 range size in starlings *Sturnus vulgaris*. Behav Ecol 21:1321–1329. doi:
406 10.1093/beheco/arq151

407 Moiron M, Laskowski KL, Niemelä PT (2020) Individual differences in behaviour explain
408 variation in survival: a meta-analysis. Ecol Lett 23:399–408. doi: 10.1111/ele.13438

409 Nève G, Hall C (2016) Variation of thorax flight temperature among twenty Australian
410 butterflies (Lepidoptera: Papilionidae, Nymphalidae, Pieridae, Hesperiidae, Lycaenidae).
411 Eur J Entomol 113:571–578. doi: 10.14411/eje.2016.077

412 Nussey DH, Coulson T, Festa-Bianchet M, Gaillard J-M (2008) Measuring senescence in wild
413 animal populations: towards a longitudinal approach. Funct Ecol 22:393–406. doi:
414 10.1111/j.1365-2435.2008.01408.x

415 Pasquet A, Toscani C, Anotaux M (2018) Influence of aging on brain and web characteristics
416 of an orb web spider. J Ethol 36:85–91. doi: 10.1007/s10164-017-0530-z

417 Polverino G, Cigliano C, Nakayama S, Mehner T (2016) Emergence and development of
418 personality over the ontogeny of fish in absence of environmental stress factors. Behav
419 Ecol Sociobiol 70:2027–2037. doi: 10.1007/s00265-016-2206-z

420 R Core Team (2020) R: A language and environment for statistical computing. R Foundation
421 for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/

422 Réale D, Reader SM, Sol D, et al (2007) Integrating animal temperament within ecology and
423 evolution. Biol Rev 82:291–318. doi: 10.1111/j.1469-185X.2007.00010.x

424 Schirmer A, Herde A, Eccard JA, Dammhahn M (2019) Individuals in space: personality-
425 dependent space use, movement and microhabitat use facilitate individual spatial niche

426 specialization. Oecologia 189:647–660. doi: 10.1007/s00442-019-04365-5

427 Settele J, Kudrna O, Harpke A, et al (2008) Climatic risk atlas of European butterflies.
428 Pensoft, Sofia-Moscow

429 Spiegel O, Leu ST, Bull CM, Sih A (2017) What's your move? Movement as a link between
430 personality and spatial dynamics in animal populations. Ecol Lett 20:3–18. doi:
431 10.1111/ele.12708

432 Spiegel O, Leu ST, Sih A, et al (2015) When the going gets tough: behavioural type-
433 dependent space use in the sleepy lizard changes as the season dries. Proc R Soc B Biol
434 Sci 282:20151768. doi: 10.1098/rspb.2015.1768

435 Stamps JA, Groothuis TGG (2010) Developmental perspectives on personality: implications
436 for ecological and evolutionary studies of individual differences. Philos Trans R Soc B
437 Biol Sci 365:4029–4041. doi: 10.1098/rstb.2010.0218

438 Stanley CR, Mettke-Hofmann C, Preziosi RF (2017) Personality in the cockroach *Diploptera*
439 *punctata*: Evidence for stability across developmental stages despite age effects on
440 boldness. PLoS One 12:e0176564. doi: 10.1371/journal.pone.0176564

441 Stevens VM, Trochet A, Van Dyck H, et al (2012) How is dispersal integrated in life
442 histories: a quantitative analysis using butterflies. Ecol Lett 15:74–86. doi:
443 10.1111/j.1461-0248.2011.01709.x

444 Stevens VM, Turlure C, Baguette M (2010) A meta-analysis of dispersal in butterflies. Biol
445 Rev 85:625–642. doi: 10.1111/j.1469-185X.2009.00119.x

446 Stevens VM, Whitmee S, Le Galliard J-F, et al (2014) A comparative analysis of dispersal
447 syndromes in terrestrial and semi-terrestrial animals. Ecol Lett 17:1039–1052. doi:
448 10.1111/ele.12303

449 Svärd L (1985) Paternal investment in a monandrous butterfly, *Pararge aegeria*. Oikos
450 45:66–70. doi: 10.2307/3565223

451 Takeuchi T (2006) The effect of morphology and physiology on butterfly territoriality.
452 Behaviour 143:393–403. doi: 10.1163/156853906775897879

453 van de Pol M, Verhulst S (2006) Age-dependent traits: A new statistical model to separate
454 within- and between-individual effects. Am Nat 167:766–773. doi: 10.1086/503331

455 van de Pol M, Wright J (2009) A simple method for distinguishing within- versus between-
456 subject effects using mixed models. Anim Behav 77:753–758. doi:
457 10.1016/j.anbehav.2008.11.006

458 Van Dyck H (2003) Mate location, a matter of design? Adaptive morphological variation in
459 the speckled wood butterfly. In: Boggs CL, Watt WB, Ehrlich PR (eds) Butterflies:
460 Ecology and Evolution Taking Flight. University of Chicago Press, Chicago, pp 353–366

461 Van Dyck H, Baguette M (2005) Dispersal behaviour in fragmented landscapes: Routine or
462 special movements? Basic Appl Ecol 6:535–545. doi: 10.1016/j.baae.2005.03.005

463 Van Dyck H, Matthysen E (1998) Thermoregulatory differences between phenotypes in the
464 speckled wood butterfly: hot perchers and cold patrollers? Oecologia 114:326–334. doi:
465 10.1007/s004420050454

466 Van Dyck H, Matthysen E, Dhondt A (1997) Mate-locating strategies are related to relative
467 body length and wing colour in the speckled wood butterfly *Pararge aegeria*. Ecol
468 Entomol 22:116–120. doi: 10.1046/j.1365-2311.1997.00041.x

469 Van Dyck H, van Strien AJ, Maes D, van Swaay CAM (2009) Declines in common,
470 widespread butterflies in a landscape under intense human use. Conserv Biol 23:957–
471 965. doi: 10.1111/j.1523-1739.2009.01175.x

Villegas-Ríos D, Réale D, Freitas C, et al (2018) Personalities influence spatial responses to environmental fluctuations in wild fish. J Anim Ecol 87:1309–1319. doi: 10.1111/1365-2656.12872

Wiklund C, Persson A, Wickman P-O (1983) Larval aestivation and direct development as alternative strategies in the speckled wood butterfly, *Pararge aegeria*, in Sweden. Ecol Entomol 8:233–238. doi: 10.1111/j.1365-2311.1983.tb00503.x

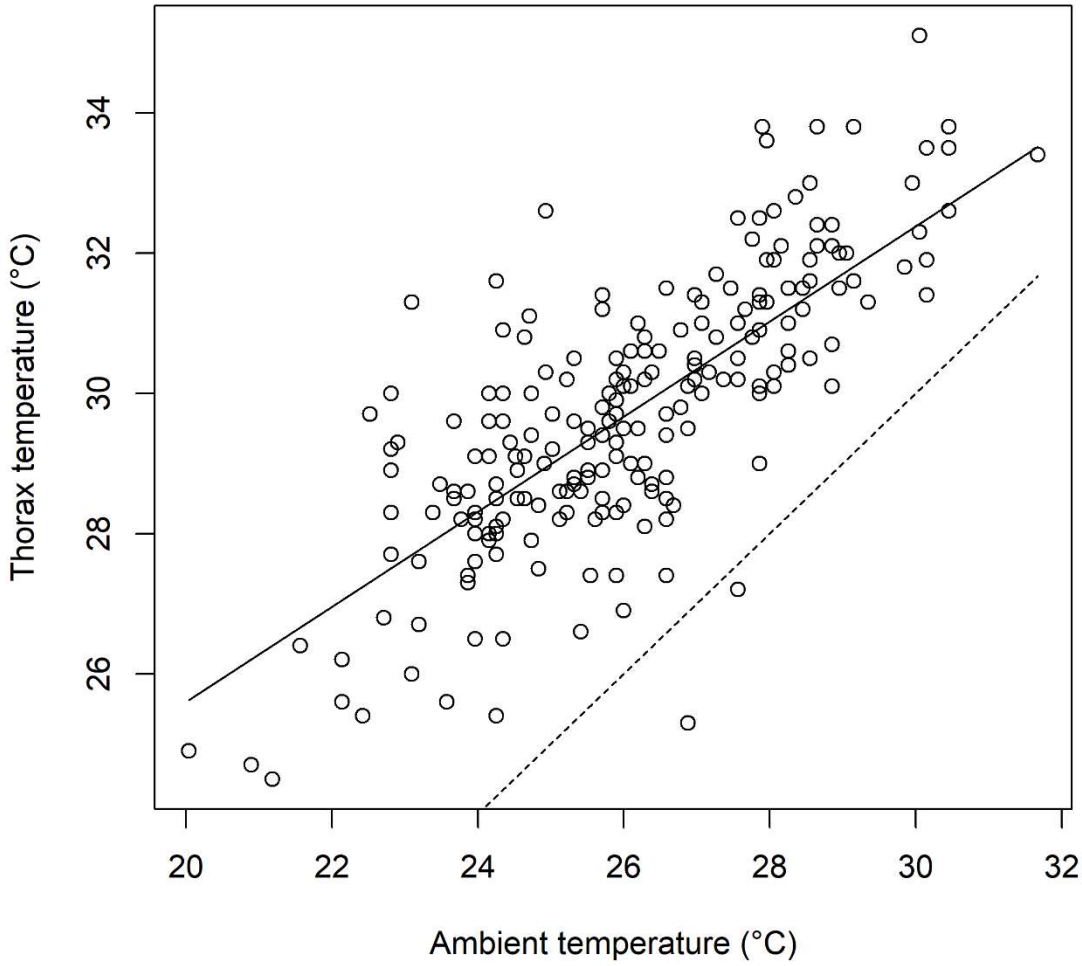
Woodgate JL, Makinson JC, Lim KS, et al (2016) Life-long radar tracking of bumblebees. PLoS One 11:e0160333. doi: 10.1371/journal.pone.0160333

Figure legends

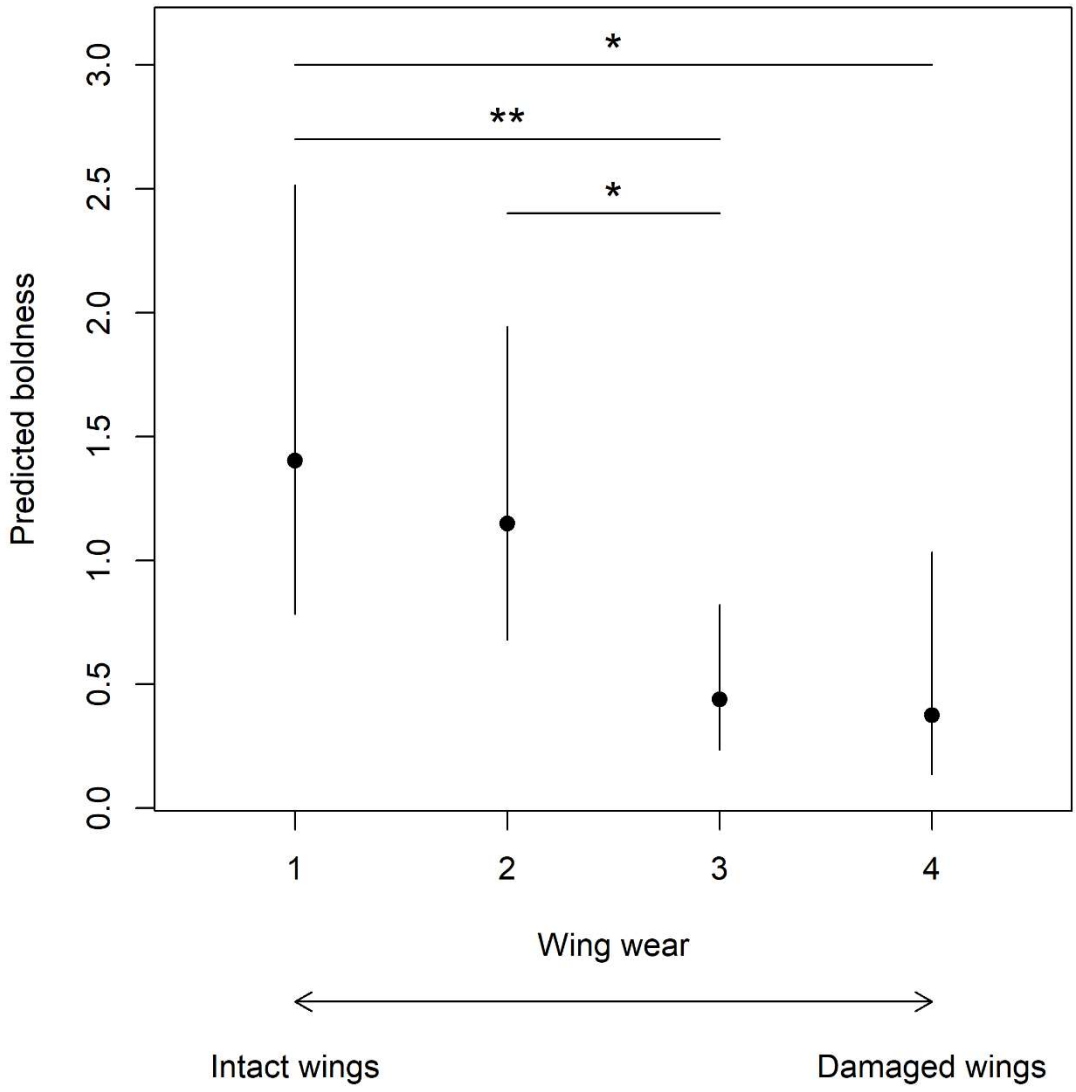
Figure 1: Relation between ambient and thoracic temperatures. The dotted line refers to the isothermal condition (1:1 relationship); the solid line represents the regression line based on the model output.

Figure 2: Effect of wing wear (used as a proxy for age) on butterfly boldness. We show the predicted values (means and 95% confidence intervals) based on the model output. Asterisks show significant differences among age classes (after adjustment for multiple comparisons; *P < 0.05; **P < 0.01).

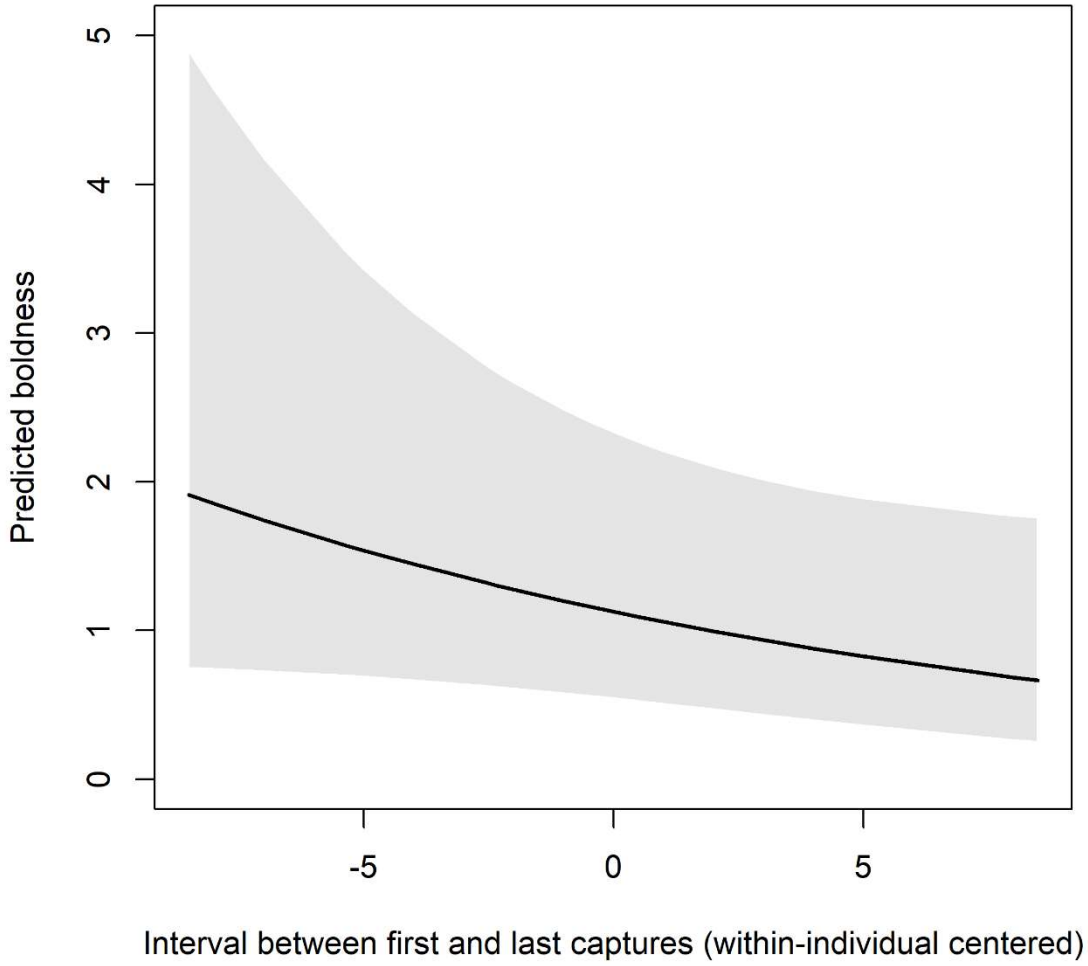
Figure 3: Within-individual change in boldness for recaptured males. The solid line shows the predicted boldness based on the model output; the grey area shows the credible interval.


Thorax temperature (°C)
26
28
30
32
34
20
22
24
26
28
30
32
Ambient temperature (°C)

492


*
**
*
Predicted boldness
0.0
0.5
1.0
1.5
2.0
2.5
3.0
1
2
3
4
Wing wear
Intact wings
Damaged wings

493


Predicted boldness
0
1
2
3
4
5
-5
0
5
Interval between first and last captures (within-individual centered)

494

Electronic supplementary material

Ethology

Individual plasticity drives boldness senescence in a territorial butterfly

Aurélien Kaiser[1], Manon Eymard[1], Thomas Merckx[1,2] & Hans Van Dyck[1]

[1] Behavioural Ecology & Conservation Group, Earth & Life Institute, UCLouvain, Louvain-la-Neuve, Belgium

[2] Department of Ecology and Genetics, University of Oulu, Oulu, Finland

Corresponding author: Aurélien Kaiser; E-mail: kaiser.aurelien@gmail.com

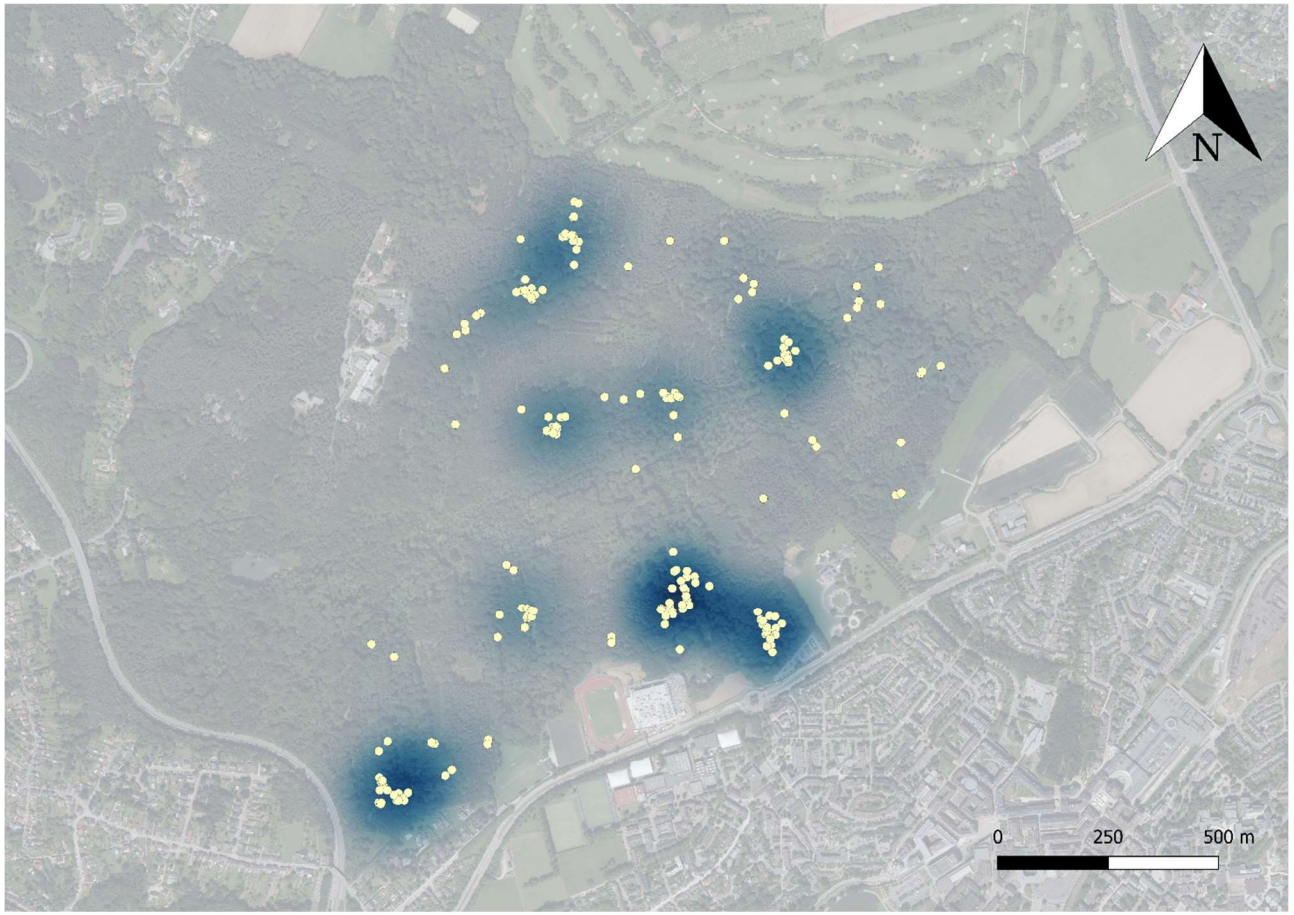
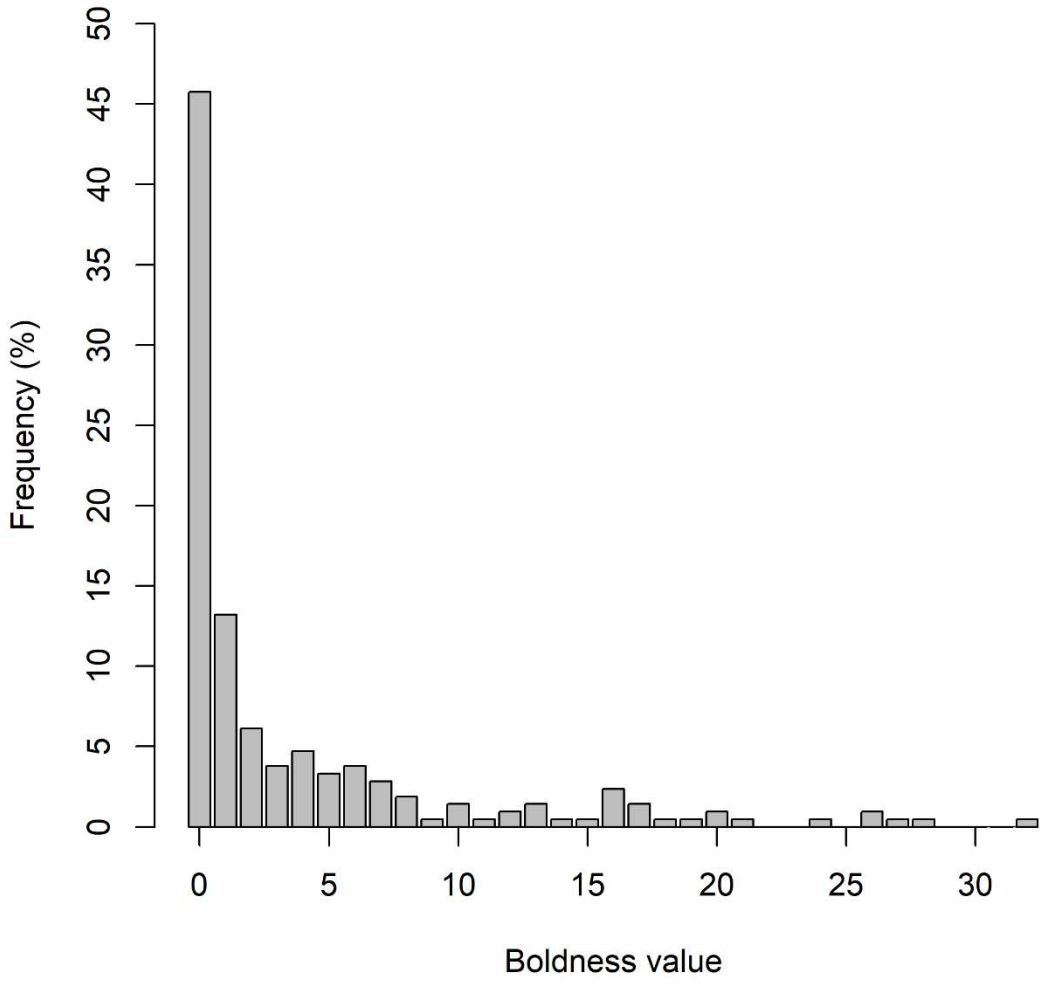
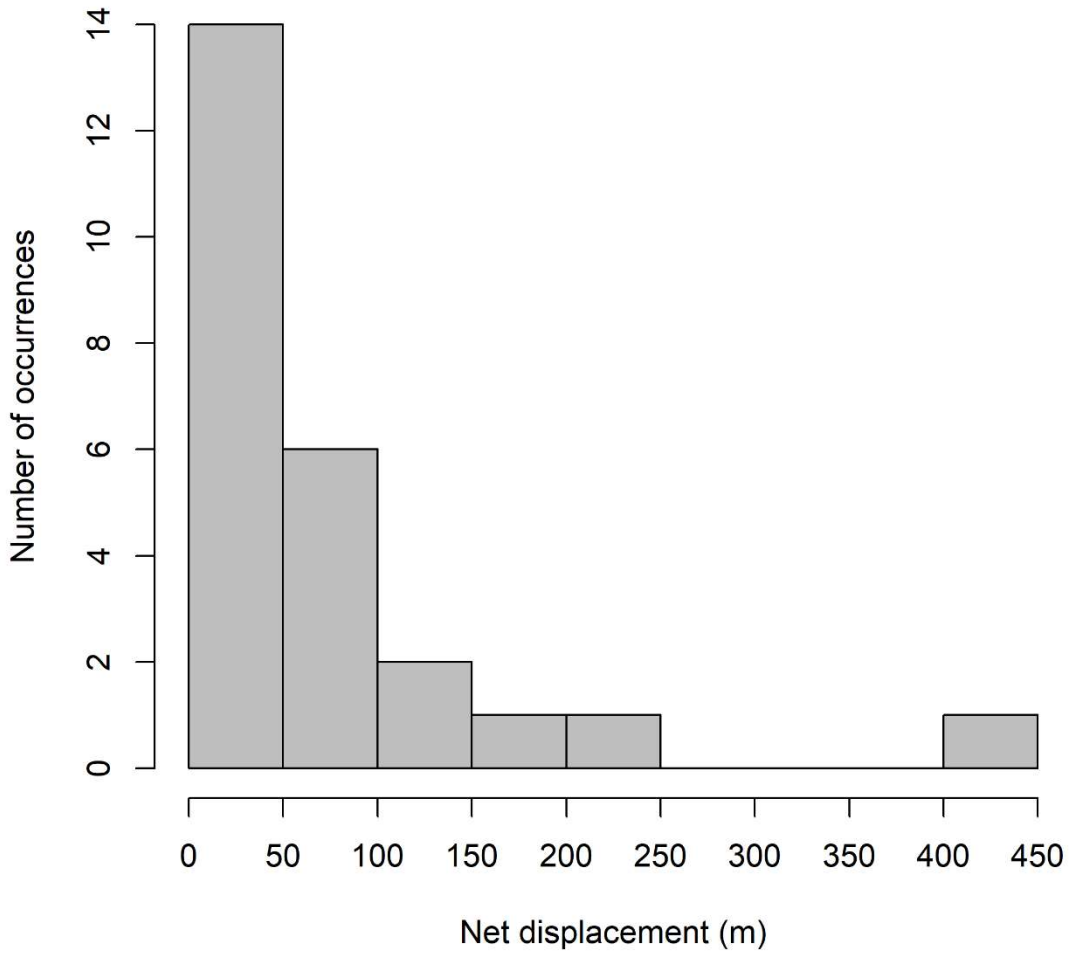


Figure S1: Map of the study area (‘Bois de Lauzelle’). Each yellow point refers to a capture event and subsequent thorax temperature and behavioural assessments. The intensity of the blue hue is proportional to the number of captures, highlighting areas where encounters with territorial speckled wood males were most frequent.



511

512 Figure S2: Distribution of boldness values among territorial males in the study population.



513

514 Figure S3: Distribution of net displacement, measured as the distance (in m) between the first

515 and second captures. Note that the y-axis refers to the number of occurrences (N_{tot} = 25).

516