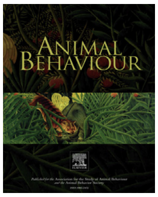
Animal Behaviour 200 (2023) 15–23

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav



Local adaptation does not constrain the expression of behaviour in translocated wild crickets

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ARTICLE INFO

Article history:
Received 28 September 2022
Initial acceptance 5 December 2022
Final acceptance 8 February 2023
Available online 6 April 2023
MS. number: 22-00469R

Keywords:
altitude
basking
plasticity
temperature

Behaviour has the potential to retard evolutionary adaptation by equipping animals with the capacity to radically change their interactions with the environment without evolving. Despite this potential for plasticity, laboratory studies frequently identify among-population differences in responses to identical stimuli, suggesting that genetic adaption often reduces behavioural flexibility. However, laboratory environments are typically far removed from nature, so their relevance to the variation we might expect to see in the wild (either among environments or as a result of changes in climate) is unclear. This is a particularly acute issue in relation to behaviour because behaving in an optimal fashion requires animals to receive and process complex sensory information which may be disrupted by laboratory conditions. We translocated newly adult male field crickets, *Gryllus campestris*, from five high-altitude and five low-altitude populations into a single low-altitude meadow from which we had removed all naturally present males. By tagging every individual and employing a network of 140 video cameras we were able to record comprehensive behavioural information from early adulthood until death. This allowed us to directly compare the behaviour of individuals from populations known to be genetically divergent and adapted to either high or low altitudes. We found very limited evidence for an effect on behaviour of the altitudinal environment in which crickets had evolved and developed, despite the large scale of our study (>20 000 h of observations of 128 males). Our findings suggests that when provided with all the environmental cues present in their natural environment, local adaptation does not lead to substantial constraints on behaviour. This supports the hypothesis that the potential flexibility of behaviour may tend to reduce selection for local adaptation.

Whether behaviour has a special role in evolution is a long-standing question. Behavioural ecologists have devoted most attention to considering whether the capacity for behaviour has contributed to the evolutionary diversification of animals (Bailey et al., 2018; Mayr, 1963). West-Eberhard (1989) proposed that behaviour may accelerate evolution, principally because novel phenotypes that can be expressed behaviourally can then be modified by subsequent genetic changes. Alternatively, it has been suggested that behaviour may tend to retard evolution by allowing genetically similar animals to thrive in a range of environments (Price et al., 2003). In this scenario, the flexibility provided by behaviour reduces selection for genetic differentiation between populations. However, an often-overlooked assumption of this hypothesis is that it requires not only that there is an interaction between behaviour and the environment affecting fitness, but also that behaviours change in response to changes in the environment.

We have only a limited understanding of the extent to which individual animals are able to sense features of their environment and to strategically respond by altering their behaviour (Snell-Rood, 2013). All organisms face constraints in relation to their ability to respond to environmental cues, and these constraints are likely to be particularly acute in small animals with concomitantly small nervous systems. A hypothetical insect might increase the probability of its survival by adjusting its behaviour to the weather, predation risk or food availability. However, to consistently match cues to an optimal behavioural response will likely necessitate integrating multiple sources of information, including adjusting for factors such as diurnal patterns and other interactions between

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https://doi.org/10.1016/j.anbehav.2023.03.010

environmental factors that affect their reliability. Using environmental cues to modulate behaviour requires a nervous system with sensory and processing capabilities requiring investment in terms of energy, development time and other currencies. There may also be direct constraints of insufficient genetic variation to allow the evolution of these capabilities, especially in the relatively simple nervous systems of many invertebrates.

Understanding the extent to which small animals can change their behaviour to cope with environmental variation is a formidable challenge. There is abundant evidence from comparisons of behaviour, either in situ, or in the laboratory, that substantial regional variation in behaviour exists (Foster, 2013; Foster & Endler, 1999). Examples include differences in behaviour and the plasticity of its expression between nine-spined sticklebacks, *Pungitius pungitius*, from four pond habitats (no piscine predation) versus a coastal and a lake habitat (high piscine predation; Herczeg & Välimäki, 2011), variation in boldness in zebrafish, *Danio rerio*, from four environmentally distinct habitats (Roy & Bhat, 2018), differences in hiding behaviour in crickets, *Gryllus integer*, from two populations differing in predation pressure (Hedrick & Kortet, 2006) and numerous others. There is a concern that many studies of this type compare the behaviour in the laboratory of individuals from only two populations and draw conclusions relating to specific environmental differences without having the replication to rule out other possible differences between populations. Nevertheless, the overall picture suggests consistent support for adaptive behavioural differences between populations across studies. The persistence of differences in behaviour in common-garden testing environments indicates that these differences are evolved adaptations. It also suggests that most species that have been tested do not have the capacity to change their behaviour when they are tested in a common environment, despite the potential flexibility of these behaviours. However, firm conclusions on this point cannot be drawn from existing studies because the laboratory or seminatural environments in which behaviours are measured may lack important cues that animals could use to change their behavioural expression in nature, like incident sunlight and the presence of predators.

We set out to test whether an insect that is locally adapted to one environment can change the expression of a range of behavioural traits when tested in a completely natural, but environmentally divergent environment. We used the flightless field cricket, *Gryllus campestris*, as a model species. In Europe its rough grassland habitat is highly fragmented by anthropogenic (intensive agriculture, towns, roads, etc.) and natural (rivers, forests, other unsuitable habitat) barriers. In northern Spain, *G. campestris* are found from sea level to over 1300 m. Substantial differences in climate between altitudes have the potential to impose strong selection on ectotherms. Ectotherms have the potential to use behaviour to modify their temperature (Kearney et al., 2009; May, 1979; Ørskov et al., 2019). This may be particularly important for field crickets since adults and late-stage nymphs are frequently predated if they fail to reach the safety of their burrows when attacked by birds and shrews (Rodríguez-Muñoz et al., 2011). Temperature affects exposure to predation by altering the speed at which crickets can move. We have previously used laboratory experiments to test for local adaptation in this species in relation to development rate and chill-coma recovery times. Comparing crickets from high and low altitudes (Tregenza et al., 2021), we found that crickets from low altitudes grew faster at high temperatures and took longer to recover from chill coma. These studies provide evidence for local adaptation in these developmental and physiological traits, but variation in behaviour as expressed in the natural environment has not been examined. We do not have detailed in situ behavioural observations from the two types of habitats, but the large differences in average temperatures between the two (in the order of 5–7 °C, Tregenza et al., 2021) mean that in situ behavioural differences are inevitable. Our aim here was to standardize the natural environment and compare the behaviour of crickets from high-altitude habitats (where optimal behaviours are expected to be different from those at low altitudes) with the behaviour of crickets native to low altitudes. To do this, we needed to observe both types of crickets simultaneously in a common natural environment.

We translocated adult crickets from populations that were either climatically and ecologically very similar to the natural meadow used as a common-garden testing environment, or that were from ca. 1000 m higher, where climatic conditions and the associated biota were substantially divergent (see Methods). Our previous work on this species has established methods for noninvasive tagging and monitoring of individuals using a network of video cameras (Bretman et al., 2011; Makai et al., 2020; Rodríguez-Muñoz et al., 2010; see Methods). Furthermore, they can be translocated from one burrow to another without any detectable effects on their behaviour, that is, they resumed their normal behaviour when using their burrow and did not immediately try to leave the area (our observations in this study; Niemelä & Dingemanse, 2017).

We assumed that individuals drawn from populations at low altitude will express behaviour that is optimal for this environment. In contrast, we predicted that crickets moved from high altitude would differ in their behaviour as a result of being adapted to an environment that is very different along multiple axes. It was difficult to make very specific predictions, but we could speculate about how environmental differences between high and low altitudes might be expected to lead to differences in behaviour between the two groups. (1) The species and density of predatory birds differ substantially with altitude (for example, chough, *Pyrrhocorax* sp., flocks occur at high altitude but not in the lowlands), making it likely that there will be differences in behaviours related to predator avoidance. These may include how readily individuals retreat into their burrows in response to perceived threats (burrows are predominantly a refuge from predation), how rapidly they subsequently re-emerge (‘Fleeing’) and how much time they spend outside their burrows (‘Outside’; see Table 1 for descriptions of these traits). (2) Because the breeding season at high altitudes is significantly shorter (spring arrives later and winter comes earlier), high-altitude crickets are expected to live their life at a faster pace (Ricklefs & Wikelski, 2002; Wikelski et al., 2003); this may make them less risk averse (affecting a range of traits, ‘Outside’, ‘Feeding’, ‘Basking’, ‘Movements’ and ‘Fleeing’). It will also alter trade-offs between somatic maintenance and senescence (Kirkwood, 1977; Rodríguez-Muñoz, Boonekamp, Fisher, et al., 2019; Rodríguez-Muñoz, Boonekamp, Liu, et al., 2019). This may lead to high-altitude males spending more time singing (‘Calling’) and may also mean that optimal body temperatures are higher at higher altitude affecting propensity to sun-bask (‘Basking’). (3) Much lower air temperatures mean that gaining heat from solar radiation will be more important at higher altitudes. This may lead to crickets from higher altitudes spending more time basking (‘Basking’). (4) The longer breeding season at low altitudes may mean that feeding as an adult is more important than it is at high altitudes, leading to more time being spent feeding at lower altitudes (‘Feeding’).

Furthermore, as well as genetic adaptation, the behaviour of translocated crickets that have developed and overwintered at high altitude may be expected to have been affected by the environment they experienced during development (Pigliucci, 2001). Hence, we predicted differences in behaviour between the altitude groups both because of genetic differences and because of differences in rearing environment. Our prediction was that when adult crickets

Table 1
Description of six behavioural traits analysed for the field cricket, *G. campestris*

Trait	Description	Analysis distribution
Outside	Time spent outside the burrow, i.e. when any portion of the cricket's body is visible	Binomial
Feeding	Time spent feeding as seen in the video	Binomial
Calling	Time spent calling	Binomial
Basking	Time basking in the sun from periods when the burrow entrance is in sunlight	Beta-binomial
Movements	Number of times the cricket has moved from inside the burrow to outside, or vice versa	Negative binomial
Fleeing	Time taken by the cricket to move outside the burrow after fleeing inside to escape a perceived threat	Gaussian

Description of the six *G. campestris* behavioural traits compared between wild adult males collected at high- (above 1100 m) versus low- (below 160 m) altitude populations. The last column shows the type of distribution used for analysis of each trait.

from higher altitudes are translocated to a low-altitude environment, they will be unable to modify their behaviour despite having all the appropriate environmental cues available to them. As a result, their behaviour was expected to differ systematically from that of low-altitude individuals.

METHODS

Study Site and Species

We extracted data from video recordings of crickets released at the WildCrickets meadow (see www.wildcrickets.org), during the breeding season in the spring of 2019 (see below). We collected adult male crickets from 10 different sites in Asturias (north Spain), five sites at altitudes below 160 m and five at over 1100 m. The five populations in each altitude group came from independent river catchments and gene flow between them is likely to be very limited (see details of site locations and alititudes in Tregenza et al., 2021), an assertion that is supported by our observation of genetic differentiation among these populations (Tregenza et al., 2021). The replicate populations within the altitude treatments were included solely to provide independent replication of high- and low-altitude conditions and we were not interested in differences between populations per se. Additional adult females to be used for the study were collected from three different meadows at altitudes of 60–80 m, within a radius of 4 km around the WildCrickets meadow. A weather station located at the study site provided us with temperature data at ground level at 10 min intervals.

G. campestris lives in and around burrows excavated in the ground in grassland habitats. Burrows are used as shelter from predation and bad weather. Both sexes are very territorial, and burrows are only shared with a single individual of the opposite sex during the breeding season. The species has discrete annual generations. Adults emerge in the spring, mating and laying eggs until mid-late summer. Adult males sing from the entrance of their burrows to attract females for mating, and both sexes move around frequently during the breeding season in search of mates, using multiple different burrows. When two adults of the same sex meet at a burrow, one of them immediately leaves, or else they fight until one of them takes over the burrow and the other one leaves. Females lay eggs in the ground at or around the entrance to their burrow. Eggs hatch over the spring and summer and nymphs develop until early autumn, when they retreat into their burrow and enter diapause at their penultimate instar. Early in the following spring they resume activity, completing their development and emerging as adults.

Experimental Protocol

In March–early April 2019, we trapped all the nymphs living in the WildCrickets meadow, using a Flipper trap (https://crickettrapping.wordpress.com/), removing males and retaining females to be used in the experiment. Over the first half of April 2019, we collected crickets from the sites reported above. After collection, all individuals were taken to an unheated building at the WildCrickets meadow and reared in 10-litre plastic boxes, with food and water. We reared males from different populations in separate boxes. There were not enough females present in the experimental meadow, so we added females from populations close to one another (see above) and kept in three boxes without attempting to retain population identities. Between 22 and 29 April, we released 132 females and 130 males into our study site. Of the 130 males, 65 were from high- and 64 from low-altitude populations. The mean number of males per population was 12.9 (SD = 0.99), ranging between 11 and 14. The earliest adult crickets in the study area emerge in early April so we can be confident that the crickets used in our experiment were within the range of 2–3 weeks old at the time of release.

On the day of release, we marked each cricket with a plastic ID tag printed with a unique two-character code to allow individual identification. Before releasing the crickets, we made artificial burrows distributed around the meadow based on the positions of the natural burrows found in the previous 10 years. We released each individual inside a randomly chosen burrow (one cricket per burrow) and covered the burrow with a metal cage to prevent the cricket moving away immediately after release. We removed the cages 2–4 days later, and prior to that, we installed a high-resolution IR video camera (Vivotek ip8332) over each of the burrows where we released males and over a sample of the burrows with females (140 cameras). We continuously recorded cricket activity (day and night) at the burrow, using digital video recording software (iCatcher, iCode Ltd, www.icatchercctv.com), until the end of the breeding season (when the last adult died). Over the breeding season, we kept searching for new burrows at least once a week. Burrows are conspicuous at the beginning of the season, becoming more difficult to find as the grass grows. However, we usually quickly detected any new, untagged cricket that showed up in the meadow when it visited any of the video-monitored burrows, and that allowed us to catch and tag it. In any case, the possibility of an untagged cricket being present in the meadow does not affect the analyses in this study.

We recorded a cricket as dead either when it was observed being predated or on the day after it was last observed alive. To extract data from these videos, they were randomly assigned to observers who were blind to the provenance of the individuals they were watching and such that all observers watched both high- and low-altitude crickets.

Behavioural Traits and Data Collection

We analysed six behavioural traits as described in Table 1. These traits were chosen as they represent key components of all the behaviours that we could observe and that occur more than a few times in each cricket's lifetime, that is, we did not pre-screen to identify potentially informative behaviours; we recorded everything that it was practical to record. We only collected data from periods when males were alone at a burrow, ignoring periods when males shared a burrow with a female or another male; this excludes direct effects of the presence of other crickets on the behaviour of the target male. To extract the data from the video, we followed a two-step protocol. First, all the video from each camera was watched at high speed for the first 10 min of every hour, to find the portions where a male was occupying the burrow under that camera on its own. To improve efficiency, observers carried out this first step on groups of nine cameras at once. The second step consisted of watching the portions of video of single males at a slower speed, one camera at a time, recording all the behavioural events included in Table 1. Observations were recorded with a resolution of 1 min, with the only exception being Fleeing, where the time when the cricket fled into the burrow and the time when he came out later were recorded to the nearest second. For each period when any target behaviour was performed, we recorded the mean temperature at ground level. Overall, we analysed 20 505 h of cricket activity for 128 males, with a mean of 160 h of observation per male (SD = 126).

For all the traits other than Fleeing, we grouped all records per individual according to temperature bins of 1 °C (range 12–27). Thus, the final data set for each of these behaviours included altitude of origin and temperature bin as fixed effects and cricket identity and population of origin as random effects. The response variable for each behaviour was the time spent performing that behaviour and total time under observation. The exception was Movements, where the response variable was number of movements per hour inside/outside the burrow. We analysed Fleeing as the time taken to leave the burrow after fleeing inside, with each Fleeing event being a separate record in the data set.

Data Analyses

We ran all statistical analyses as generalized linear mixed models (GLMMs) in R (version 4.0.3) using lme4 (Bates et al., 2015; version 1.1.26) and glmmTMB (Brooks et al., 2017; version 1.1.2.2). Both packages provide *P* values in their outputs, based on the Wald *Z* test. For Fleeing, analysed with a linear mixed model, we used the package lmerTest (Kuznetsova et al., 2017) to calculate *P* values (using the default Satterthwaite's method). Four of the traits were binomial (these traits were quantified as the number of minutes performing the target behaviour versus the number of minutes not performing it within the time under observation, over the whole life of the cricket for each of the temperature bins). One trait was a count variable (the number of times the cricket moved in and out of the burrow during the observation period), and another was a Gaussian variable (the time taken to move outside the burrow after fleeing inside). Data on the four binomial traits had strong overdispersion; to deal with this in our analyses, we followed the recommendations of Harrison (2015). We compared the estimates of the parameters and the overdispersion (ratio of the residual deviance by residual degrees of freedom) obtained with the same model when running it under three different approaches (distributions), binomial, binomial with OLRE (object level random effect)

Table 2
Model comparison for the effect of temperature and altitude on six behavioural traits

Trait	Model		*df*	ΔAIC
Outside	Binomial with OLRE	cbind(Y,N)~Tem*Alt+(Tem|Tag)+(1|OLRE)	8	0
		cbind(Y,N)~Tem*Alt+I(Tem^2)*Alt+(1|Tag)+(1|OLRE)	8	−179
		cbind(Y,N)~Tem*Alt+I(Tem^2)*Alt+(Tem|Tag)+(1|OLRE)	10	−212
		cbind(Y,N)~Tem*Alt+I(Tem^2)*Alt+(Tem|Tag)+(1|Pop)+(1|OLRE)	11	−210
		cbind(Y,N)~Tem*Alt+I(Tem^2)*Alt+(1+Tem+I(Tem^2)|Tag)+(1|OLRE)	13	**−260**
Feeding	Binomial with OLRE	cbind(Y,N)~Tem*Alt+(Tem|Tag)+(1|OLRE)	8	–
		cbind(Y,N)~Tem*Alt+I(Tem^2)*Alt+(1|Tag)+(1|OLRE)	8	0
		cbind(Y,N)~Tem*Alt+I(Tem^2)*Alt+(Tem|Tag)+(1|OLRE)	10	−41.5
		cbind(Y,N)~Tem*Alt+I(Tem^2)*Alt+(Tem|Tag)+(1|Pop)+(1|OLRE)	11	−39.5
		cbind(Y,N)~Tem*Alt+I(Tem^2)*Alt+(1+Tem+I(Tem^2)|Tag)+(1|OLRE)	13	**−64.1**
Calling	Binomial with OLRE	cbind(Y,N)~Tem*Alt+(Tem|Tag)+(1|OLRE)	8	0
		cbind(Y,N)~Tem*Alt+I(Tem^2)*Alt+(1|Tag)+(1|OLRE)	8	−605
		cbind(Y,N)~Tem*Alt+I(Tem^2)*Alt+(Tem|Tag)+(1|OLRE)	10	−667
		cbind(Y,N)~Tem*Alt+I(Tem^2)*Alt+(Tem|Tag)+(1|Pop)+(1|OLRE)	11	−665
		cbind(Y,N)~Tem*Alt+I(Tem^2)*Alt+(1+Tem+I(Tem^2)|Tag)+(1|OLRE)	13	**−694**
Basking	Beta-binomial	cbind(Y,N)~Tem*Alt+(Tem|Tag)	8	−11
		cbind(Y,N)~Tem*Alt+I(Tem^2)*Alt+(1|Tag)	8	0
		cbind(Y,N)~Tem*Alt+I(Tem^2)*Alt+(Tem|Tag)	10	−24
		cbind(Y,N)~Tem*Alt+I(Tem^2)*Alt+(Tem|Tag)+(1|Pop)	11	−22
		cbind(Y,N)~Tem*Alt+I(Tem^2)*Alt+(1+Tem+I(Tem^2)|Tag)	13	**−54**
Moving	Negative binomial	Moved~Tem*Alt+(Tem|Tag)	8	–
		Moved~Tem*Alt+I(Tem^2)*Alt+(1|Tag)	8	0
		Moved~Tem*Alt+I(Tem^2)*Alt+(Tem|Tag)	10	−48.2
		Moved~Tem*Alt+I(Tem^2)*Alt+(Tem|Tag)+(1|Pop)	11	−46.2
		Moved~Tem*Alt+I(Tem^2)*Alt+(1+Tem+I(Tem^2)|Tag)	13	–
		Moved~Tem*Alt+I(Tem^2)*Alt+I(Tem^3)*Alt +(1+Tem+I(Tem^2)|Tag)	19	**−119.4**
Fleeing	Gaussian	sqrt(AfterFleeing+1)~Alt	3	0
		sqrt(AfterFleeing+1)~Alt+(1|Tag)+(1|Pop)	4	**−20.16**
		sqrt(AfterFleeing+1)~Tem*Alt+(1|Tag)	6	−17.12
		sqrt(AfterFleeing+1)~Tem*Alt+(Tem|Tag)	8	−16.94
		sqrt(AfterFleeing+1)~Tem*Alt+(Tem|Tag)+(1|Pop)	9	−16.94
		sqrt(AfterFleeing+1)~Tem*Alt+I(Tem^2)*Alt+(Tem|Tag)+(1|Pop)	11	−14.43

Model comparison for the effect of temperature (Tem) and altitude of origin (Alt), of six behavioural traits (time spent outside their burrow, time spent calling, time spent feeding, time spent basking in the sun, number of movements in and out of the burrow per hour and time taken to leave the burrow after fleeing inside) in adult male *G. campestris*. Crickets were collected from 10 independent populations, five from low altitude (<160 m) and five from high altitude (>1100 m) and monitored in the low-altitude WildCrickets meadow. We coded binomial response variables as 'Y' for the time (min) spent doing the target behaviour and '*N*', for the time spent not performing it. Random effects include unique individual identity (Tag) and population of origin (Pop). The table shows the difference in AIC for each model as compared to the simplest one. Differences in AIC <7 are considered 'nonsignificant' (Burnham et al., 2011); among models with nonsignificant differences, we selected the simplest one. Selected models are highlighted in bold. No value indicates no convergence.

and beta binomial. To calculate overdispersion, we used bootstrapping with the code provided by Harrison (2014). After the comparison, we selected the most suitable approach to account for overdispersion (Appendix Table A1), under the assumption that ratio values between 0.5 and 1.5 are acceptable. For the trait time spent outside (Outside) the beta binomial distribution was the best at dealing with the overdispersion but not all the models converged, so we used the second-best alternative, the binomial with OLRE which also dealt with the overdispersion appropriately. To analyse the number of movements in and out of the burrow, we standardized them as movements per hour, and rounded the values as integers so that we could analyse them as count data using a negative binomial distribution. For each of the variables, we compared a number of models with different levels of complexity for each behaviour, based on their Akaike information criterion (AIC), and chose the one with the smallest AIC value following the recommendation of Burnham et al. (2011). The fixed effects of the full model included an interaction between temperature at ground level and altitude of origin of the population, both as linear and quadratic terms. The only exception was number of movements, where we also included a cubic term after an exploratory look at the raw data in relation to temperature suggested a likely cubic relationship. Random effects included individual identity (as random intercepts or random slopes, including a quadratic term) and population of origin. Including random effects in model selection procedures is becoming increasingly common (Buscemi & Plaia, 2020). In our case, this model selection procedure is the only available approach to identifying the existence of relevant variation in random slopes.

Ethical Note

The crickets used in this study were collected from natural meadows and retained for only a few days. At the end of that period, we took a small haemolymph sample and a small portion of the tip of one of the hindlegs for future DNA analysis and attached a plastic tag by gluing it to the pronotum. After that, the crickets were released into our study meadow. Observations of individuals immediately after these procedures indicate that they exhibited normal behaviours within a few minutes of being released, and as far as we know, crickets have never died as a result of any of the procedures used in this study. Our tagged crickets live out their natural lives in the meadow.

RESULTS

Models including the quadratic term of temperature in both the fixed and random effects showed the smallest AIC for all the behaviours, except the time to leave the burrow after fleeing (Table 2). Movements in and out of the burrow were better described by a model that also included a cubic term in the fixed effects. None of these models showing the smallest AIC included population (Table 2). To confirm that population was not relevant, we checked the variance associated with it in the full model and found that it was zero or very close to zero for all traits but Fleeing. Including it in the model for this last trait did not change the results anyway. The models with the best fit also included an interaction between temperature and cricket identity, showing that it explained a relevant portion of the variance among individuals. The inclusion of cricket identity in the best model for the time taken to move out of the burrow after fleeing, indicates that this also varied among males. However, there was no difference between altitudes in how long males took to move out of their burrow after fleeing (Fig. 1). All variables, except Fleeing, showed a significant relationship with temperature. This relationship did not differ between males from different altitudes, with the only exception being that there was a significant interaction between the linear term of temperature and altitude of origin affecting the time crickets spent outside (Figs. 2 and 3, Table 3).

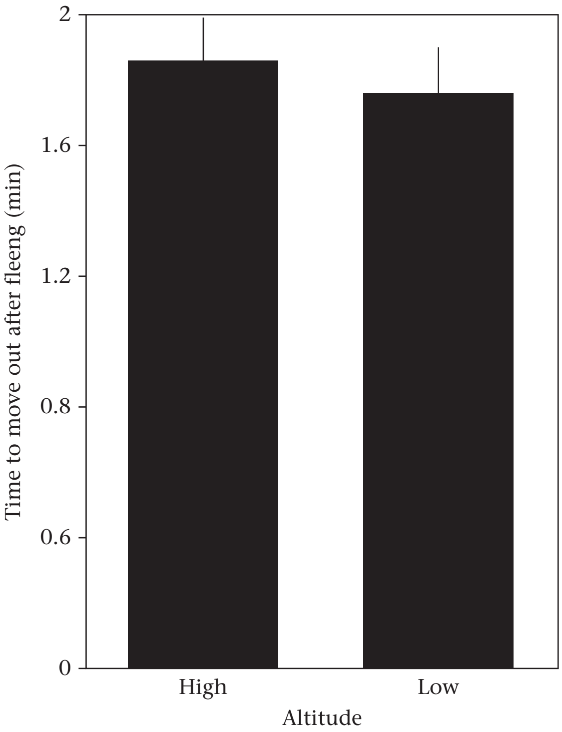

Figure 1. The time interval before wild *G. campestris* males from either high or low altitude came back out of their burrows after fleeing inside to escape a potential threat (mean + SE). Crickets were collected from five locations at over 1100 m and five locations under 160 m. All observations were made in the low-altitude WildCrickets meadow in spring 2019.

There is evidence from our analyses that crickets have an optimal operating temperature that is similar across different types of behaviour. Visual inspection of Fig. 2 indicates that the proportion of time crickets spent outside increased to a temperature of around 19 °C, then plateaued at close to 100% of the time outside until the temperature was over 24 °C, when it declined again. Similarly, calling activity peaked at around 20 °C. Fig. 3 shows that movements in and out of the burrow were consistently infrequent, until the temperature rose above 22 °C at which point movements started to become more frequent.

DISCUSSION

Whether behaviour has a special role in evolution remains a contentious subject (Bailey et al., 2018; Mayr, 1963; Price et al., 2003; West-Eberhard, 1989).

One of the questions at the heart of this debate is whether the capacity to thrive in a range of environmental conditions, which behaviour has the potential to provide, is actually realized (Beever et al., 2017; Snell-Rood, 2013). Invertebrates have an enormous repertoire of behaviours. However, the extent to which their expression of behaviour is genetically hard-wired (‘developmental plasticity’, Snell-Rood, 2013), as opposed to being dependent upon environmental cues (‘activational plasticity’, Snell-Rood, 2013) is rarely examined. Our aim was to determine whether crickets derived from very different environments show differences in behaviour in a common natural environment. Our main finding was that crickets derived from, and reared in, climatically very distinct

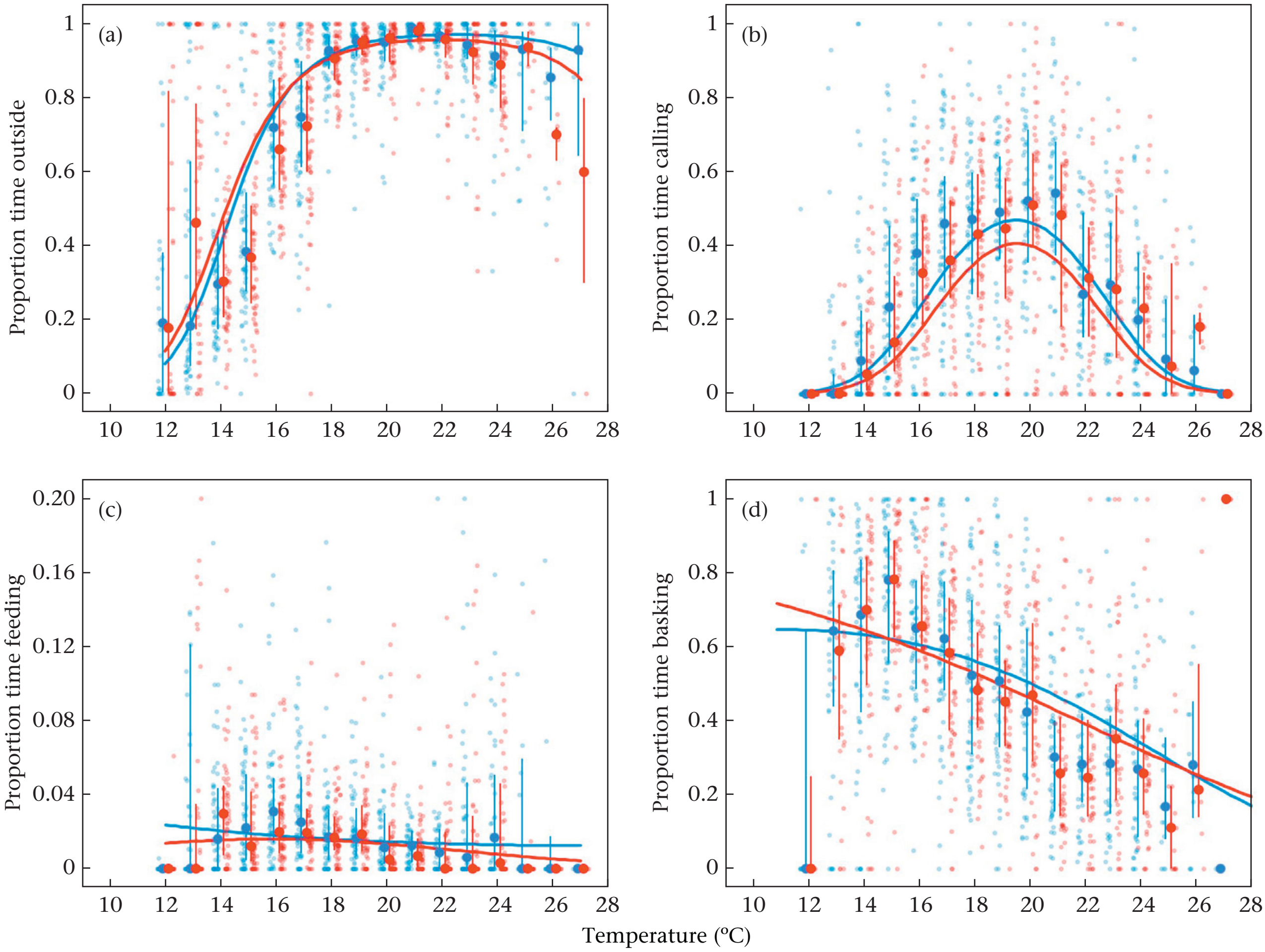

Figure 2. The relationship between temperature at ground level and the proportion of time spent performing four different behaviours in wild *G. campestris* males from five locations at over 1100 m (blue) and five locations under 160 m (red). (a) Time outside their burrow, (b) time calling, (c) time feeding and (d) time basking. All observations were made in the low-altitude WildCrickets meadow in spring 2019. Small dots show data. Large dots show medians (plus quartiles). Lines show the relationship estimated in *R* from mixed models analysed with the lme4 package (Bates et al., 2015) for Outside, Calling and Feeding, using a binomial distribution including an oriented-level random effect to remove the effect of overdispersion (Harrison, 2015). The fourth variable (Basking) was analysed with a beta-binomial distribution using the glmmTMB package (Brooks et al., 2017).

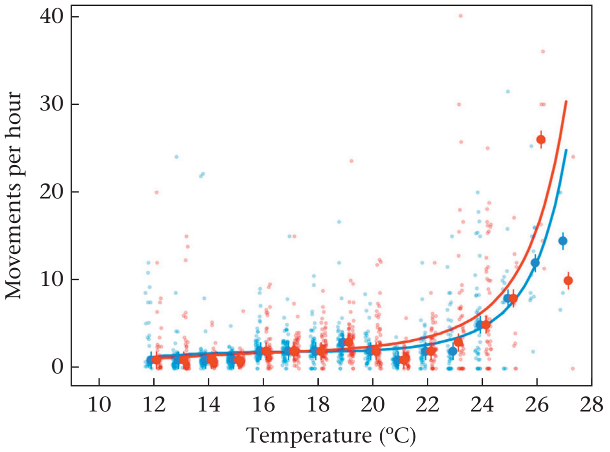

Figure 3. The relationship between temperature at ground level and the number of times that wild *G. campestris* males from five locations at over 1100 m (blue) and five locations under 160 m (red) moved in and out of their burrow. All observations were made in the low-altitude WildCrickets meadow in spring 2019. Small dots show data. Large dots show medians (plus quartiles). Lines show the relationship estimated from a mixed model in R using the lme4 package (Bates et al., 2015), with a negative binomial distribution.

environments nevertheless behaved in a broadly similar fashion. The only difference we were able to identify was that there was an interaction between ambient temperature and altitude affecting the time that crickets spent outside. This effect can be seen in Table 3 and Fig. 2a, where the most obvious altitudinal difference is that crickets from cooler, higher altitudes appeared to continue to spend more time outside at high temperatures, whereas crickets from warmer, lower altitudes showed a steeper tendency to reduce their time outside as the temperature rose. This observation fits with an interpretation that high-altitude individuals are more willing to tolerate high temperatures to find a mate, in line with a faster pace of life strategy (Ricklefs & Wikelski, 2002) which might be selected for by the shorter breeding season at high altitudes. It is not appropriate to attempt to calculate statistical power post hoc (Hoenig & Heisey, 2001). However, the relatively small SD associated with each of our estimated parameters suggests that we should be able to detect small differences in behaviour between altitudes. This indicates that for those traits where we did not find any effect of altitude, any differences that do exist are not very large.

Our previous work on crickets from the same high- and low-altitude environments (Tregenza et al., 2021) found differences in physiological and life history traits between them. We

Table 3
Effect of temperature and altitude of origin on six behavioural traits in wild *G. campestris* males

Fixed effects	Random effects (σ^2)		Outside	Calling	Feeding	Basking	Movements	Fleeing
(Intercept)		Est	2.518	−0.219	−4.147	0.135	0.716	1.613
		SD	0.104	0.187	0.095	0.093	0.079	0.074
		P	**<0.001**	0.239	**<0.001**	0.146	**<0.001**	**<0.001**
Tem		Est	1.710	0.753	−0.190	0.534	0.071	–
		SD	0.099	0.105	0.092	0.069	0.098	–
		P	**<0.001**	**<0.001**	**0.038**	**<0.001**	0.469	–
AltL		Est	−0.116	−0.296	−0.040	−0.020	0.063	−0.042
		SD	0.145	0.264	0.134	0.091	0.111	0.106
		P	0.422	0.262	0.763	0.825	0.569	0.702
I(Tem^2)		Est	−0.739	−1.199	0.035	−0.156	0.054	–
		SD	0.092	0.089	0.089	0.062	0.064	–
		P	**<0.001**	**<0.001**	0.696	**0.013**	0.396	–
I(Tem^3)		Est	–	–	–	–	0.162	–
		SD	–	–	–	–	0.044	–
		P	–	–	–	–	**<0.001**	–
Tem:AltL		Est	−0.282	0.108	−0.012	−0.058	0.185	–
		SD	0.136	0.147	0.129	0.096	0.137	–
		P	**0.037**	0.463	0.928	0.549	0.174	–
Alt*I(Tem^2)		Est	0.018	−0.098	−0.185	0.111	−0.010	–
		SD	0.126	0.122	0.126	0.088	0.088	–
		P	0.883	0.422	0.143	0.208	0.906	–
Alt*I(Tem^3)		Est	–	–	–	–	−0.008	–
		SD	–	–	–	–	0.062	–
		P	–	–	–	–	0.896	–
	OLRE		2.008	1.069	1.090	–	–	–
	Pop		–	–	–	–	–	0.015
	Tag		0.228	1.931	0.245	0.351	0.236	0.044
	Tem		0.282	0.421	0.235	0.150	0.212	–
	I(Tem^2)		0.211	0.214	0.181	0.101	0.116	–
	I(Tem^3)		–	–	–	–	0.042	–
	Residual		–	–	–	–	–	0.210
	N populations		–	–	–	–	–	10
	N records		1372	1350	1350	1272	1372	378
	N individuals		128	128	128	126	128	87

Relation of temperature (Tem) and altitude (Alt) of origin to six behavioural traits in wild *G. campestris* males collected from 10 independent populations, five from low altitude (L, <160 m) and five from high altitude (>1100 m) and monitored in the low-altitude WildCrickets meadow. Outside, Calling, Feeding and Basking represent the proportion of time spent doing those behaviours. Movements is the number of movements in or out of the burrow per hour. Fleeing is the time taken to move out the burrow after fleeing inside to escape from a perceived threat. The table shows the results of mixed models in R. All traits except Basking were analysed with the lme4 package (Bates et al., 2015) using a binomial distribution including an oriented-level random effect (the first three), to remove the effect of overdispersion (Harrison, 2015). Movements and Fleeing were analysed with negative binomial and Gaussian distributions, respectively. Basking was analysed with a beta-binomial distribution using the glmmTMB package (Brooks et al., 2017). Est: coefficient estimation. Significant *P* values are highlighted in bold.

interpreted this as evidence for local adaptation, particularly in relation to life history traits since individuals were reared in a common laboratory environment. Our present findings, in a design that allowed both local adaptation and influences of early rearing environments to express themselves, nevertheless found limited behavioural differences in relation to the provenance of individuals. We interpret this as evidence supporting the hypothesis that animals have a lot of flexibility over the expression of behaviour, a capacity that may tend to retard adaptive evolution. It also suggests that the early life experiences of individuals do not have large effects on the expression of the types of behaviour that we monitored. There are, of course, a list of caveats that apply to this interpretation. These include the fact that we only studied behaviour in adult males. Differences might still exist in some of the studied behaviours in females, or even in both sexes during nymph development and growth. For example, differences in feeding behaviour between altitudes might exist in adult females which need to continuously produce eggs. Also, those differences could be more important in postdiapause nymphs, as these need to feed a lot to become adults early in the spring, and thermal differences between altitudes are far more pronounced at that time of year.

Aside from testing our prediction about the flexibility of expression of behaviour, our study provides some insights into the adult behaviour of these insects which may turn out to be common in other species. (1) Most adult males spent less than 4% of their time feeding (Fig. 2). This suggests that at this stage they are mainly focused on finding mates fuelled by stored reserves, but it might also reveal that at this time of year food is so abundant and nutritious that they do not need to spend much time feeding. (2) Activity seemed to peak at around 21 °C (Figs, 2 and 3). The increase in the frequency of movements in and out of the burrow above this temperature (Fig. 3) suggests the crickets might control their body temperature by alternately warming up outside and cooling down inside. This thermoregulatory behaviour is well known in vertebrate ectotherms.

In the longer term, being able to measure the magnitude of fitness costs and benefits associated with expression of behaviours will clearly be extremely valuable (Keller et al., 2013). Our previous work on this species has demonstrated that they can be individually phenotyped and genotyped in their natural habitat and fitness measures extracted (Bretman et al., 2011; Rodríguez-Muñoz et al., 2010; Rodríguez-Muñoz, Hopwood, et al., 2019). This creates the potential to conduct experiments in which individuals from putatively divergent populations are reared in a common-garden environment or reciprocally translocated in order to isolate genetic and environmental effects. Subsequent behavioural observations of the type described in this study could be combined with genotyping of the released individuals and of their offspring in the subsequent generation. This would allow researchers to partition the contributions to fitness of local adaptation and phenotypic plasticity in behaviour more precisely.

Author Contributions

R.R.M., P.H. and T.T. designed the research; R.R.M., P.H., J.J.B., S.A.E., T.W.P., J.R. and T.T. performed the research; R.R.M. and J.J.B. performed all statistical analyses; R.R.M. and T.T. wrote the article and P.H. and J.J.B. contributed substantially to revisions.

Data Availability

Data will be made available on request.

Declaration of Interest

None.

Acknowledgments

The following people contributed to video processing and data recording: Tiffany Tsui, Jasmine Thomas-Campbell, Trinity King, Tilly Evans and Shae Clements. We also thank www.icode.co.uk for developing their i-catcher video recording package to optimize it for behavioural research. Jon Slate has been a constant partner in the development of the project. We thank Xavier Harrison and Tom Houslay for advice on statistical analyses. This work was supported by Natural Environment Research Council standard grants: NE/E005403/1, NE/H02364X/1, NE/L003635/1, NE/R000328/1 and the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 792215 (Boonekamp).

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Appendix

Table A1
Comparison of three different approaches to control overdispersion in behavioural traits of crickets

Factor	Binomial	Binomial OLRE	Beta-binomial
Outside			
(Intercept)	2.511 (0.152)	2.568 (0.110)	1.633 (0.072)
Tem	2.663 (0.191)	1.655 (0.066)	1.012 (0.068)
AltL	−0.434 (0.213)	−0.159 (0.155)	1.542 (0.071)
I(Temˆ2)	−0.206 (0.010)	−0.785 (0.064)	−0.532 (0.046)
Tem*AltL	−0.392 (0.267)	−0.302 (0.093)	−0.156 (0.093)
AltL*I(Temˆ2)	0.257 (0.014)	0.067 (0.091)	0.101 (0.065)
Ratio	283 (238–317)	**1.5** (1.4–1.7)	0.9 (0.8–1.0)
Calling			
(Intercept)	−0.166 (0.178)	−0.278 (0.192)	–
Tem	0.671 (0.153)	0.744 (0.058)	–
AltL	−0.241 (0.252)	−0.297 (0.275)	–
I(Temˆ2)	−0.839 (0.009)	−1.095 (0.059)	–
Tem*AltL	−0.169 (0.215)	0.141 (0.083)	–
AltL*I(Temˆ2)	−0.139 (0.013)	−0.087 (0.086)	–
Ratio	43 (36–47)	**1.5** (1.1–1.7)	–
Feeding			
(Intercept)	−3.875 (0.087)	−4.212 (0.102)	−3.649 (0.079)
Tem	−0.355 (0.099)	−0.222 (0.061)	−0.189 (0.080)
AltL	−0.031 (0.123)	−0.021 (0.144)	−3.646 (0.079)
I(Temˆ2)	0.051 (0.024)	0.133 (0.061)	0.052 (0.052)
Tem*AltL	0.102 (0.140)	−0.026 (0.088)	−0.001 (0.115)
AltL*I(Temˆ2)	−0.257 (0.037)	−0.206 (0.093)	−0.235 (0.079)
Ratio	9 (8–10)	**1.3** (1.1–1.5)	0.6 (0.5–0.7)
Basking			
(Intercept)	0.098 (0.112)	0.183 (0.108)	0.139 (0.084)
Tem	−0.885 (0.104)	−0.629 (0.052)	−0.495 (0.043)
AltL	−0.237 (0.157)	−0.190 (0.156)	−0.006 (0.083)
I(Temˆ2)	−0.188 (0.012)	−0.162 (0.050)	−0.132 (0.042)
Tem*AltL	−0.013 (0.146)	−0.036 (0.074)	−0.035 (0.060)
AltL*I(Temˆ2)	0.148 (0.017)	0.056 (0.073)	0.047 (0.062)
Ratio	27 (25–30)	1.8 (1.6–2)	**0.8** (0.7–0.8)

Comparative coefficient estimations (SD in parentheses) obtained from three different approaches to remove the effect of overdispersion, when analysing the effect of temperature (Tem) and altitude of origin (Alt), on the time spent by wild adult male *G. campestris* performing four different behaviours. Crickets were collected from 10 independent populations, five from low altitude (L, <160 m) and five from high altitude (>1100 m) and monitored in the low-altitude WildCrickets meadow. Estimation of overdispersion (ratio, residual deviance by residual degrees of freedom (95% CI)) obtained after Harrison (2014). The ratio value of the chosen model for each trait is in bold. All analyses were run using R, with lme4 (Bates et al., 2015) and glmmTMB (Brooks et al., 2017) for the binomial-OLRE and beta-binomial, respectively. Note that for the beta-binomial analyses, the term AltL is included as the actual value provided in the output, and not as a value to be added to the intercept. Lack of values means no convergence.