Behavioral Ecology and Sociobiology (2023) 77:54
https://doi.org/10.1007/s00265-023-03325-7

ORIGINAL ARTICLE

Spatial scale-dependent effects of urbanisation on phenotypic traits in a thermophilous grasshopper

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Received: 7 July 2022 / Revised: 3 March 2023 / Accepted: 12 April 2023 / Published online: 9 May 2023

Abstract

Urban environments are characterised by different microclimates, ecological resources and habitat connectivity compared to nonurban environments. Hence, urban environments may affect phenotypic variation of local populations relative to populations in adjacent nonurban environments through different mechanisms (phenotypic plasticity, natural selection, phenotypic sorting). We analysed the effects of urbanisation on phenotypic traits in adults of the grasshopper *Chorthippus brunneus* at three spatial scales (0.5, 3, and 5 km). Traits included functional morphology, personality-related behaviours (i.e. boldness and exploration), metabolic rate and song parameters. Our experimental tests pointed to several lines of behavioural divergence between individuals of urban and nonurban populations. Grasshoppers of urban population origin were on average shyer and less explorative, but they were more rapid to reach the edges of an artificial environment deprived of any food or conspecifics compared to individuals of nonurban population origin. Songs of urban grasshoppers were characterised by a narrower frequency range than songs of nonurban grasshoppers. Their constitutive bursts of sound (echemes) were more spaced out than songs of nonurban conspecifics. Interestingly, we found more autotomized grasshoppers (i.e. self-amputated individuals) in the urban environment, and these individuals tended to be bolder, while also having more spaced echemes in comparison to uninjured conspecifics. We also demonstrate that the spatial scale at which urbanisation showed the most pronounced effects varied considerably for behavioural and song traits. The detectability and size effects of urbanisation on traits may vary with the spatial scale at which urbanisation is studied around focal populations or habitat patches. Our study motivates for a documented characterisation of the multiple and complex effects of urbanisation on functional phenotypic trait values at the intraspecific level.

Significance statement

We analysed the impact of urbanisation on multiple phenotypic traits of a thermophilous grasshopper species. We integrated information on morphology, physiology, behaviour and song characteristics, and we addressed the relationships of those traits with urbanisation at several spatial scales. Our study points at phenotypic differences between urban and nonurban environments, and we show that such relationships are spatial-scale dependent or sensitive. We provide evidence for spatial-scale dependent effects of urbanisation on boldness and explorative behaviour. Our study contributes to our understanding of how urban areas shape behavioural variation across anthropogenic landscapes. We also observed a high incidence of autotomized grasshoppers in highly urbanised environments. Autotomy refers to the extreme escape tactic where the grasshopper sheds a limb to escape predation.

Keywords Urbanisation · Spatial Scale · Dispersal Syndrome · Exploration · Boldness · Autotomy

Communicated by K. Shaw.

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Introduction

Understanding the ecological and evolutionary processes by which phenotypes of organisms differentiate relative to spatial and temporal environmental heterogeneity is key both to fundamental studies in behavioural ecology and to applied studies in conservation and global change biology.

As fast-changing urban environments expand more and more across the globe (Seto et al. 2011; Gao and O'Neill 2020), they offer exciting opportunities to study the impact of rapid environmental changes on organisms and their behavioural repertoires (Hendry et al. 2008; Alberti 2015; Alberti et al. 2017a).

Urban environments differ from nearby nonurban environments in several ways, including the high degree of habitat fragmentation within a hostile landscape matrix (Gibb and Hochuli 2002), altered thermal profiles (i.e., urban heat island effect, Arnfield 2003), different chemical pollution regimes (Parris 2016), artificial light at night (Parris 2016), and noise pollution (Farina and Gage 2017), amongst other direct or indirect human-activity related impacts. Anthropogenic environments, like cities, have been shown to be challenging for a range of species. Urban areas provide habitat for some species, but they lack suitable resources for several others (e.g. Magura et al. 2010; Chamberlain et al. 2018), resulting both in changes in communities (e.g. McIntyre et al. 2001) and in differentiation between populations (Thompson et al. 2022).

At the community level, urbanisation often results in biodiversity loss and homogenisation of several taxonomic groups (McKinney 2008). Urban communities typically show functional homogenisation at spatial scales that are linked to taxon-specific mobility (e.g., Rochat et al. 2017; Merckx and Van Dyck 2019). Urban invertebrate communities, which are particularly impacted by urbanisation (McIntyre et al. 2001), are usually biased towards mobile, thermophilous species of generalist ecological profile, both at local and landscape scales (Piano et al. 2017).

At the species level, urban areas may act as a filter on species and species traits (McKinney 2006; Brans et al. 2017). Good dispersal abilities are essential to allow the colonization of cities (Sol et al. 2013; Møller et al. 2014). After arrival, organisms face novel environmental conditions, and establishment may be facilitated by pre-existing coping mechanisms (Møller et al. 2014; Alberti et al. 2017b). Behavioural aspects are of prime significance in this context since behaviour acts as the first interface between an organism and its environment, allowing rapid responses when facing changes in the environment (Sih et al. 2011). Though behavioural plasticity is recognised as an asset to survive under urban conditions, it may be insufficient to cope with human-induced rapid environmental change (Tuomainen and Candolin 2011; van Baaren and Candolin 2018). Species that occur under both high and low levels of urbanisation may therefore enter scenarios of ecological and evolutionary differentiation between urban and nonurban populations as a result of natural selection or of non-random sorting of individuals by behavioural traits that affect dispersal and habitat use (Sol et al. 2013; Caspi et al. 2022). Personality traits like boldness and explorative behaviour have been shown to facilitate dispersal in a risky environment in some species (Fraser et al. 2001; Cote et al. 2010). Boldness also allows urban animals to decrease stress responses and, consequently, increase feeding time (Møller 2008; Lowry et al. 2013; Lapiedra et al. 2017), while a higher tendency for exploration is likely to favour access to food resources in novel environments (Sol et al. 2013). Moreover, correlation between boldness and explorative behaviour have been demonstrated in urban populations of mammals, birds and reptiles (Evans et al. 2010; Atwell et al. 2012; Lapiedra et al. 2017; Dammhahn et al. 2020). However, there is a backlog for personality-related studies in insects and other invertebrates (Kralj-Fišer and Schuett 2014), particularly in an urbanisation context (but see, for example, Kaiser et al. 2020).

Understanding the mechanistic underpinning of variation between populations distributed over an urban gradient is important to analyse species' responses to urbanisation, and more broadly, to analyse their resilience to environmental perturbation (Ouyang et al. 2018). Earlier studies on the effects of habitat fragmentation on population differentiation were often limited to morphological traits that were assumed to reflect selection for, or against, mobility in changing landscapes depending on the spatial configuration of the habitat of the species under study (Van Dyck and Matthysen 1999). In the meantime, knowledge has much improved about the existence of dispersal syndromes (i.e., suites of intercorrelated morphological, physiological, behavioural and life-history traits that are correlated with dispersal, Stevens et al. 2013). But, although dispersal and mobility are important traits for species that thrive in urban areas, cities may also shape other (correlated) life history traits. The pace-of-life syndrome hypothesis predicts variation in behaviour and physiology among individuals to be associated with variation in life history. Hence, populations experiencing different ecological conditions may differ in a suite of metabolic traits that have coevolved with the life-history particularities related to these conditions (Réale et al. 2010). Metabolic rates, for example, have been shown to evolve rapidly and in line with the pace-of-life hypothesis, at least in some study systems (Auer et al. 2018). Urban environments are interesting laboratories for testing both dispersal and pace-of-life syndromes in an integrated way. For example, urban populations of *Daphnia* water fleas showed a pace-of-life syndrome, whereas such a syndrome was absent in rural populations (Brans et al. 2018). However, work on birds (Sol et al. 2018) showed that pace-of-life syndromes may vanish in urbanised environments due to behavioural adjustments. Hence, this highlights: i) the caution to generalise conclusions on urban life styles across taxonomic groups at this stage, and ii) the need to integrate behaviour into life history theory to fully understand how animals deal with urban conditions.

The spatial scale at which urbanisation matters may vary among species and may be the result of complex interaction effects between the organism and the environment (Baguette and Van Dyck 2007). Previous studies on insects (and Orthoptera in particular) have shown that mobile species were more sensitive to urbanisation at larger scales than sedentary species (Penone et al. 2012). However, this important spatial-scale effect is still often being ignored in landscape-scale studies (Moll et al. 2020). Although straightforward predictions for specific spatial scale effects are hard to make at this stage, it is definitely worth exploring whether urbanisation at different distances around local population affects the biological traits under study.

In the present study, we designed a multi-scale approach to test how urbanisation affects intraspecific phenotypic variation. We have chosen to study the grasshopper *Chorthippus brunneus*, which is an abundant and widely distributed ectotherm species that occurs along urbanisation gradients (Benton 2012). However, little is currently known about morphological, physiological, and behavioural changes in relation to dispersal in urban populations of *C. brunneus*. We measured and compared the morphological, physiological and behavioural traits that may facilitate dispersal of individuals originating from distinct populations that differ in their degree of urbanisation (i.e., the percentage of artificially covered surfaces). In our study, we also integrated stridulation behaviour (i.e., the sound production to attract and select mates), as it is of particular significance to Orthoptera and it involves morphological structures that are of prime importance for mobility (i.e., wings and legs).

Methods

Study species

Chorthippus brunneus (Thunberg 1815; Orthoptera, Acrididae) is a thermophilous grasshopper occurring mainly in grassy habitats with sparse vegetation and some bare ground. Due to its efficient behavioural thermoregulation (Willott and Hassall 1998), it can be found in a wider range of open habitats including wastelands in urban areas (Benton 2012). Common garden experiments with laboratory-reared offspring of field-caught specimens of this species showed that individuals of urban populations invested on average more in dispersal-related morphology compared to individuals of rural populations (San Martin y Gomez and Van Dyck 2012). In line with a life-history trade-off between mobility and fecundity (Tigreros and Davidowitz 2019), this increased morphological investment in dispersal ability (i.e., longer wings and relative femur) was traded-off against lower investment in abdominal mass. This species has well-developed dispersal abilities (e.g., long functional wings; Richards and Waloff 1954; Picaud and Petit 2007) and net lifetime track distances vary from very low (7 m to 33 m per generation; Bailey et al. 2003) to more than 1 km (1344 m per generation; Bridle and Butlin 2002). However, such mark-release-recapture studies tend to underestimate dispersal movements (Kindvall 1999). In Belgium, *C. brunneus* is widespread and univoltine. Adult males communicate by stridulation as they scrape the pegs on the hind femurs against the veins on the forewings or tegmina (Elsner 1974). Songs consist of repeated pulses referred to as echemes (Broughton 1976), which each last for 0.25–0.50 s and are separated by intervals of about 3 s (Butlin et al. 1985).

Study areas and sampled populations

Between mid-July and early-August 2015, adult males ($N = 109$) were captured in six Belgian sites spread over three geographic regions (a) Oudenaarde/Galmaarden; $N = 19$ and 17, b) Sint-Niklaas/Bornem; $N = 16$ and 17 and c) Leuven/Oud-Heverlee; $N = 22$ and 18) for which selected plots (3 km × 3 km) and nested subplots (200 m × 200 m) were categorised according to their degree of urbanisation (assessed using a vectorial layer with the precise contours of all building in a GIS, excluding roads and parkings; LRD 2015). (Sub)plots possessing a built-up cover > 15% were classified as urban, while (sub)plots having a built-up cover < 3% were classified as nonurban (Fig. 1). For each geographic region, the sampling site for urban individuals was chosen within one of the urban subplots of the urban plot and the sampling site for nonurban individuals was chosen within one of the nonurban subplots from the nonurban plot. In 2015, average human population density was 557 individuals per km^2, with cities and urban sprawl embedded within an agricultural and semi-natural matrix (IBZ 2015). Degree of urbanisation was calculated in concentric circles around each of the six sample sites at three radii (0.5, 3, and 5 km, i.e. distances covering the range of daily to dispersal movements in *C. brunneus*; Bridle and Butlin 2002; Bailey et al. 2003) as the percentage of the surface covered by artificial surfaces (i.e., human-build structures) using the CORINE Land Cover 2012 database version 18.5.1 (http://land.copernicus.eu/pan-european/corine-land-cover/clc-2012) (see Supplementary Table S1). Calculations were done with ArcGIS (version 10.3; Environmental Systems Research Institute 2014).

In the laboratory, grasshoppers were kept individually in transparent jars (10 cm height; 5 cm and 7.5 cm diameter, at the base and top, respectively), covered with netting. They had *ad libitum* access to daily refreshed leaves of the grass *Poa pratensis* L. To avoid rapid deterioration of food quality by low humidity, a piece of moist cotton was placed in each jar. Jars were placed in an incubator (Sanyo Cooled

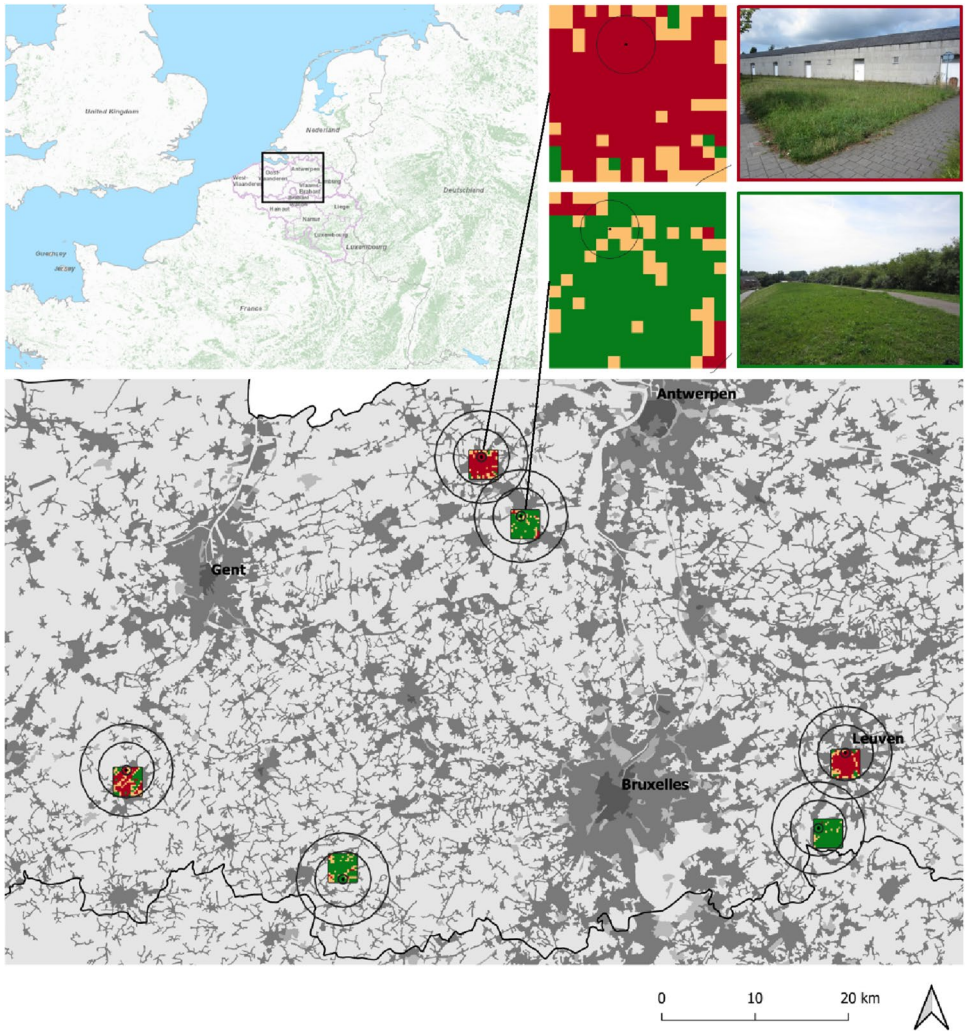

Fig. 1 Location of the six sampling sites on an urbanisation background (CORINE Land Cover European Environment Agency, light to dark gradient corresponding to a gradient from nonurban environment to continuous urban fabric) for northern Belgium (west Europe; Esri, Redlands, California). Urbanisation was quantified as built-up cover (BUC). Urban (sub)plots are depicted in red (BUC > 15%), semi-urban (sub)plots in orange (3% < BUC < 15%) and nonurban (sub)plots in green (BUC < 3%). Solid lines refer to administrative province borders. Subplots allowed sampling using a nested design that covers urbanisation gradients at both the landscape and local scale. Two plots are enlarged and show the within-plot distribution of local subplot types. Urbanisation (surface covered by artificial surfaces) was quantified for each sample site at three spatial scales (500–3,000–5,000 m radii), which on the zoom-in and map are depicted around the sample sites. Photographs depict sites in an urban and nonurban subplot

Incubator MIR-554; thermal regime: 9 h-19 h: 30 °C, 19 h-21 h: 32 °C, 21 h-23 h: 30 °C, 23 h-9 h: 17 °C; Megaman CFL GX53 lights inside the incubator were switched on between 9 and 21 h). After the experiments, individuals were released at the spot of capture in the field.

Morphological measurements

Fresh body mass was measured after one day of starvation (Ohaus Explorer Balance; accuracy ± 0.1 mg). Grasshoppers were individually photographed (Camera Sony HX400) while they were placed inside a Petri dish with cotton wool. Morphological traits were measured on pictures using AutoCAD software (version 21.0; Autodesk, Inc. 2017): pronotum, tegmen (as a proxy for hindwing length; correlation between tegmen and hindwing length: $r = 0.80$, $t = 6.021$, $df = 20$, $P < 0.001$), femur and tibia length. Pronotum length was measured from a picture of the dorsal side of the individual. For tegmen, femur and tibia lengths, we used average values of the left and right side measures.

Standard metabolic rate

Standard metabolic rate (SMR, i.e. the metabolic rate of an ectotherm during inactivity, measured at a particular ambient temperature; Careau et al. 2014) was measured with a respirometry method measuring CO_2-production in a recipient under controlled air (Sables Systems International). Data were visualised with ExpeData software (Sables Systems International). After a 6 h period of fasting and 30 min in a cooler bag, individuals were gently placed in a container, which functioned as metabolic chamber (height: 11.5 cm; diameter: 7 cm). Ambient temperature was set at *c.* 29 °C. The recipient was shielded from direct light to keep the grasshopper at rest. Grasshoppers were allowed to acclimatise further during 10 min. Next, the chamber was closed for 10 min. Produced CO_2 accumulated in the chamber, after which it was flushed and the quantity of CO_2 was measured during 2 min (further details, see: Van Dyck and Holveck 2016).

Behavioural tests

Each individual was submitted to three behavioural tests: i) a boldness test in response to a dummy predator; ii) a test measuring boldness as the willingness to emerge from a dark chamber, together with exploration; and iii) a movement test in a greenhouse. During each test, ambient temperature was recorded every minute (HOBO U23 Pro v2 data logger). Test order was randomised among individuals of different population origin for each behavioural test.

i) Predator attack response test

The threat response test (adapted from Wilson et al. 2010) measured boldness after a simulated predator attack. We used transparent plastic cages (14.5 × 19 × 17 cm) in which a grasshopper was introduced. Each cage was placed in a polystyrene box (60 × 35 × 37 cm; with two cold LED light strips) to minimise interference with simultaneously tested grasshoppers. The behaviour of each individual was filmed (HD webcam Logitech HD Pro C920). After 10 min of acclimatisation, an attack was simulated with a predator dummy (bird shape). Next, the individual's behaviour was recorded during 10 min. The time elapsed between the attack and the first move of the grasshopper was recorded.

ii) Emergence test

A wooden device with six separate corridors (length: 80 cm, width: 10 cm, height: 18 cm) was used. At the beginning of each corridor, there was a dark compartment (15 × 10 × 18 cm), which was connected to a corridor through a small door. A Plexiglas plate covered the corridors preventing the grasshoppers from flying or escaping. During each trial, an individual of each population ($N = 6$) was placed in one of the dark compartments. After 90 s of acclimatisation, the doors to the corridors were opened simultaneously. A video camera (JVC GZ-MG130E) filmed the grasshoppers in the device from a top-down view. Each trial lasted for 10 min. Video recordings were used to quantify i) latency time to emerge from the dark compartment (i.e., time before entering the corridor), and ii) time to reach the end of the corridor. Due to technical issues with some recordings, these variables were available for 82 and 71 individuals, respectively.

iii) Movement test

The movement test investigated the willingness to move in an open, artificial environment deprived of any food and conspecifics. An individual was carefully released from a transparent jar in the middle of an empty greenhouse compartment (dimensions: 16 × 8 m). During 10 min, its trajectory was recorded on a detailed map (i.e., positions of stops and the orientation and lengths of movement steps). In case the individual reached one of the greenhouse edges, the trial was stopped. Movements typically consisted of walks, jumps and flights. Time to move to one of the edges was measured to the nearest second. Outlined trajectories were digitised for further analysis of the movement pathway using ArcGIS (version 9.3; Environmental Systems Research Institute 2008) with the Hawth's Analysis Tools extension (Beyer 2007). Straightness of the trajectory, which could vary based on personality (Spiegel et al. 2017), was calculated by the fractal dimension index (D), with $D = log(n) / (log(n) + log(d/L))$ whereby n is the number of segments that make up the studied trajectory, d is the distance between the start and end points, and L is the total length (Katz 1988). The index theoretically ranges from 1 (straight line) to 2 (Brownian motion search, i.e., equal chances to go straightforward, turn left, etc.). Highly tortuous trajectories had values > 2, which were set to 2 and straight trajectories (with only two landmarks) had zero values, which were set to 1.

Bioacoustical measures

Song analyses were based on fewer individuals ($n = 33$). For song recording, a grasshopper was kept individually in the same plastic jar (see above). An individual was stimulated to sing by exposing it to standard playback recordings of *C. brunneus* male songs. Each time the male responded, the stimulus was stopped and the subsequent song was recorded with a Sony IC Recorder ICD-PX240 (frequency response range: 75—15 000 Hz). The first echeme of a song was not analysed as it may deviate from the following ones (Green 1987). Per individual, at least three (up to 11) echemes were analysed, from up to three recordings. Sound recordings were analysed with R software (version 3.4; R Core Team 2017), the 'warbleR' R package version 1.1.8 (Araya-Salas and Smith-Vidaurre 2017), 'tuneR' version 1.3.2 (Ligges et al. 2016), and 'seewave' version 2.0.5 (Sueur et al. 2008). Recordings were converted to WAV format. Background noise was removed by applying a band-stop filter between 0 and 2 kHz. Echemes within songs were delimited by visual inspection of the spectrograms. Next, six song variables per echeme were calculated using the 'specan' function of the 'warbleR' package: duration, interval between successive individual echemes, peak frequency (i.e., frequency of highest amplitude), mean frequency (i.e., weighted average of frequency by amplitude), mean dominant frequency (i.e., average dominant frequency measured across the acoustic signal), and standard deviation of the frequency weighted by amplitude.

Statistical analyses

Linear mixed models were used to estimate the effect of urbanisation (as a fixed effect) on i) morphological traits, ii) standard metabolic rate, iii) the fractal dimension index of the movement test, and iv) the different song parameters. Analyses were repeated for each of the three spatial scales at which urbanisation was quantified, and *P*-values were corrected for multiple comparisons using the Benjamini–Hochberg procedure (Benjamini and Hochberg 1995). The region of origin was included as a random effect in order to control for possible spatial non-independence between nearby populations. The models of the song analyses included 'individual' as a random effect. When appropriate, covariates were included in the models: i) pronotum length for morphological traits as a control for overall body size, ii) body mass and ambient temperature for metabolic rate; iii) ambient temperature for the trajectory analysis, together with a fixed factor indicating whether or not the individual had reached the edge of the greenhouse; iv) femur length as a body size measure for the analysis of song traits; and v) echeme duration for the standard deviation of the frequency weighted by amplitude. As the models for metabolic rate could not include both body mass and degree of urbanisation due to collinearity, we used residual variation from the linear regression of body mass against degree of urbanisation as a covariate. Covariates were centred to their mean so that the model coefficients were estimated for mean values of the covariates. For the degree of urbanisation, we subtracted the minimum value, so that intercepts were estimated for the minimum degree of urbanisation, and we then rescaled the variable so that an increase of one unit in the estimated slope represented an increase of 10% in urbanisation. Fixed effects were tested with Wald χ^2 tests.

For the analyses of the behavioural tests, we used Cox proportional hazard models for clustered data to analyse time to i) move after the simulated attack in the threat reaction test, ii) leave the dark compartment, and iii) reach the end of the corridor in the emergence test, and (iv) reach the edge of the greenhouse in the movement test. The analyses were repeated for each spatial scale, and hazard ratios (exponentiated β coefficients) were estimated. Again, *P*-values were corrected for multiple comparisons using the Benjamini–Hochberg procedure (Benjamini and Hochberg 1995). Region of origin was used as 'cluster', and ambient temperature at the beginning of the test as a covariate. Fixed effects were tested with Wald χ^2 tests.

As in some other insects (Delnat et al. 2017), grasshoppers may show self-amputation, which is interpreted as a self-defence mechanism (autotomy). Some individuals spontaneously (i.e., not because of direct manipulation) lost one hind leg during their stay in the laboratory. We included this factor as a fixed effect in the relevant models (e.g., for body mass, time to reach the end of the corridor in the emergence test, song variables). As total body mass is affected by the loss of a hind leg, we used residual variation from the linear regression of body mass against hind leg mass as a covariate, instead of using body mass per se, in the relevant analyses. Analyses of behaviours from the emergence test were conducted with the same proportion of individuals with a missing hind leg as observed in each population. To control for the influence of the autotomized grasshoppers, we also reran the analyses only taking the non-amputated individuals into account.

Verification of model assumptions (i.e., homoscedasticity and normality of the residuals for mixed-effect linear models; hazard proportionality and log-linear effect of covariates for Cox models) was done by visual inspection of the distribution of model residuals. Model residuals for femur and tegmen length were somewhat skewed (left and right, respectively), but the applied models are known to be robust for some deviation from normality (Jacqmin-Gadda et al. 2007). All analyses were done with R software (version 3.4). We used the 'lme4' package version 1.1–14 (Bates et al. 2015) for linear mixed-effect models, and the 'survival' package version 2.41–3 (Therneau and Grambsch 2000; Therneau 2015) for Cox models. Correlations between morphological or bioacoustical measures were estimated by Pearson's correlation coefficients and tested with Student tests. For correlations amongst behavioural measures, we used the 'SurvCorr' package version 1.0 (Ploner et al. 2015) applying default options to deal with these censored time-to-event data.

Results

We summarised the results of the models testing the effects of the degree of urbanisation at 3 distances around the sampling site on phenotypic traits of *C. brunneus* males including functional morphology (Table 1), energy metabolism (Table 2), behaviour (personality traits; Tables 2, 3 and 4) and song variables (Table 5). The differences reported below as multiplicative factors are model estimates for the difference between the lowest and highest degree of urbanisation at the corresponding spatial scale.

Morphology

On average, pronotum length was 3.1 ± 0.2 mm, femur length 9.9 ± 0.4 mm, tibia length 8.7 ± 0.4 mm, tegmen length 14.1 ± 0.8 mm, and body mass 99.7 ± 10.6 mg. All were significantly correlated with pronotum length (femur: $r = 0.44$, $t = 4.344$, df = 79, $P < 0.001$; tibia: $r = 0.37$, $t = 3.493$, df = 79, $P < 0.001$; tegmen: $r = 0.43$, $t = 4.203$, df = 79, $P < 0.001$; body mass: $r = 0.56$, $t = 5.956$, df = 79,

Table 1 Summary of the fixed effects of the mixed models for morphological traits (corrected for pronotum length, and absence of a hind leg where relevant) in *C. brunneus* males relative to degree of urbanisation at three spatial scales (0.5 to 5 km radius) around sampling sites. Adjusted significative thresholds are represented in the "Adj. Signif." column

Trait	Scale (km)	Intercept		Urbanisation (10%)					Pronotum length (mm)					Missing leg				
		Coeff	SE	Coeff	SE	P-val	Signif	Adj. Signif	Coeff	SE	P-val	Signif	Adj. Signif	Coeff	SE	P-val	Signif	Adj. Signif
Pronotum length (mm)	0.5	3.08	0.03	0.00	0.00	0.370			—	—	—	—	—	—	—	—	—	—
	3	3.07	0.03	0.00	0.01	0.748			—	—	—	—	—	—	—	—	—	—
	5	3.07	0.03	0.00	0.02	0.776			—	—	—	—	—	—	—	—	—	—
Femur length (mm)	0.5	9.86	0.10	0.00	0.01	0.759			1.05	0.22	0.000	***	***	—	—	—	—	—
	3	9.88	0.11	0.00	0.02	0.906			1.04	0.22	0.000	***	***	—	—	—	—	—
	5	9.88	0.10	-0.01	0.04	0.815			1.04	0.22	0.000	***	***	—	—	—	—	—
Tibia length (mm)	0.5	8.76	0.11	0.00	0.01	0.621			0.89	0.23	0.000	***	***	—	—	—	—	—
	3	8.80	0.10	-0.03	0.02	0.241			0.87	0.23	0.000	***	***	—	—	—	—	—
	5	8.80	0.09	-0.05	0.04	0.181			0.87	0.23	0.000	***	***	—	—	—	—	—
Tegmen length (mm)	0.5	14.27	0.11	-0.02	0.02	0.253			1.76	0.42	0.000	***	***	—	—	—	—	—
	3	14.34	0.11	-0.07	0.04	0.072	(*)		1.77	0.41	0.000	***	***	—	—	—	—	—
	5	14.30	0.10	-0.12	0.06	0.059	(*)		1.77	0.41	0.000	***	***	—	—	—	—	—
Fresh body mass (mg)	0.5	103.04	1.94	-0.25	0.21	0.234			23.93	4.93	0.000	***	***	-8.66	2.77	0.002	**	**
	3	104.15	1.63	-1.04	0.51	0.040	*	(*)	23.62	4.88	0.000	***	***	-7.92	2.82	0.005	**	**
	5	103.68	1.41	-1.85	0.84	0.027	*	(*)	23.62	4.87	0.000	***	***	-7.61	2.86	0.008	**	**

Model structure takes the region of origin as random effect into account. Degree of urbanisation was rescaled so that an increase of one unit in the estimated slope represented an increase of 10% in urbanisation. Pronotum, femur and tibia lengths: $n=91$; tegmen length: $n=90$; fresh body mass: $n=91$

P-values: (*) $P<0.1$, * $P<0.05$, ** $P<0.01$, *** $P<0.001$

Table 2 Summary of the fixed effects of the mixed models for standard metabolic rate and fractal dimension of trajectories in the movement experiment, relative to degree of urbanisation at three spatial scales (0.5 to 5 km radius) around the sampling sites, and relative to temperature. Adjusted significative thresholds are represented in the "Adj. Signif." column

Trait	Scale (km)	Intercept		Urbanisation (10%)					Temperature (°C)					Fresh body mass (mg)				
		Coeff	SE	Coeff	SE	P-val	Signif	Adj. Signif	Coeff	SE	P-val	Signif	Adj. Signif	Coeff	SE	P-val	Signif	Adj. Signif
Standard metabolic rate (µl CO_2 / min)	0.5	1.20	0.04	-0.01	0.01	0.418			0.11	0.06	0.093	(*)		0.01	0.00	0.003	**	**
	3	1.20	0.04	-0.01	0.01	0.753			0.11	0.06	0.100	(*)		0.01	0.00	0.002	**	**
	5	1.18	0.04	-0.01	0.02	0.901			0.10	0.06	0.115			0.01	0.00	0.002	**	**
Trait	Scale (km)	Intercept		Urbanisation (10%)					Temperature (°C)					End reached				
		Coeff	SE	Coeff	SE	P-val	Signif	Adj. Signif	Coeff	SE	P-val	Signif	Adj. Signif	Coeff	SE	P-val	Signif	Adj. Signif
Fractal dimension of the trajectory (movement test)	0.5	1.18	0.04	-0.01	0.01	0.274			0.00	0.01	0.996			-0.05	0.05	0.314		
	3	1.17	0.04	-0.01	0.01	0.520			0.00	0.01	0.996			-0.05	0.05	0.295		
	5	1.16	0.04	0.00	0.02	0.856			0.00	0.01	0.965			-0.06	0.05	0.243		

Fresh body mass (in mg; after correction for urbanisation and the absence of a hind leg) was used as a covariate for standard metabolic rate, and a factor indicating whether or not individuals reached the edge of the greenhouse for fractal dimension. Model structure takes the zone of origin as random effect into account. Degree of urbanisation was rescaled so that an increase of one unit in the estimated slope represented an increase of 10% in urbanisation. SMR: $n = 98$; fractal dimension: $n = 81$

P-values: (*) $P < 0.1$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

$P < 0.001$). Urbanisation did not affect pronotum, relative femur or tibia length at any spatial scale (Table 1). Relative tegmen lengths tended to be smaller with increasing urbanisation. This was the case at the intermediate scale of 3 km radius, but was the clearest at the largest, 5 km radius scale (-2.6%, $P = 0.059$, Fig. 2a). This trend was lost when controlling for false positives. Urbanisation negatively affected relative body mass. This effect was significant at intermediate and large scales, and was the clearest at 5 km radius scale (-5.7%, $P = 0.027$, Fig. 2b).

Metabolic rate

Standard metabolic rates were on average 1.168 ± 0.269 µL CO_2 per min, and were significantly but not highly correlated to body mass after starvation ($r = 0.23$, $t = 2.229$, df = 86, $P = 0.028$). No relationship was detected between SMR and any of the recorded behaviours. Maximum observed SMR was 2.9 times higher than the minimum observed, after correcting for weight and temperature. Urbanisation, at any spatial scale, did not affect standard metabolic rate (Table 2).

Predator attack response test

Overall, 53% of the individuals moved within 10 min after an attack in the threat reaction test. Urbanisation did not affect the estimated probability to move after an attack at any scale (Table 3), but there was nonetheless a trend for a 2.2 times smaller probability to move after an attack at the 5 km radius scale in the situation where significance thresholds were left uncorrected ($P = 0.059$; Fig. 3a, Supp. Fig. S1A). Individuals that missed a hind leg were on average bolder after a fake predator attack (with a probability to move 2.7 times higher at 5 km, $P < 0.001$). Temperature also had a significant effect (2.3 times higher probability per °C, $P < 0.001$).

Emergence test

Overall, 84.1% of the individuals emerged from the dark compartment within 10 min. The degree of urbanisation significantly decreased the probability to emerge, but only at the smallest spatial scale (probability 1.4 times lower, $P = 0.007$; Table 3, Fig. 3b, Supp. Fig. S1B). Ambient temperature did not affect the trait. Overall, 49.3% of the individuals reached the end of the device within 10 min. The probability to reach the end of the device decreased with degree of urbanisation, but again this effect was only significant at the smallest spatial scale (probability 1.7 times lower, $P < 0.001$, Fig. 3c, Supp. Fig. S1C). Ambient temperature had a significant effect (probability 1.3 times higher per °C, $P < 0.001$).

Table 3 Summary of the fixed effects from Cox models on behavioural traits, relative to degree of urbanisation at three spatial scales (0.5 to 5 km radius) around the sampling sites, and relative to temperature. Adjusted significative thresholds are represented in the "Adj. Signif." column

Trait	Scale (km)	Urbanisation (10%)						Temperature (°C)						Missing leg					
		Coeff	Exp(coeff.)	SE	P-val	Signif	Adj. Signif	Coeff	Exp(coeff.)	SE	P-val	Signif	Adj. Signif	Coeff	Exp(coeff.)	SE	P-val	Signif	Adj. Signif
Move after attack (threat reaction test)	0.5	-0.07	0.93	0.03	0.144			0.84	2.32	0.29	0.000	***	***	0.88	2.42	0.39	0.001	***	**
	3	-0.14	0.87	0.08	0.144			0.83	2.30	0.29	0.000	***	***	0.96	2.62	0.41	0.001	**	**
	5	-0.24	0.79	0.14	0.059	(*)		0.83	2.29	0.29	0.000	***	***	1.01	2.73	0.42	0.000	***	**
Emergence from the dark chamber (emergence test)	0.5	-0.04	0.96	0.03	0.007	**	*	0.04	1.04	0.07	0.221			—	—	—	—	—	
	3	-0.11	0.89	0.08	0.188			0.05	1.05	0.07	0.185			—	—	—	—	—	
	5	-0.14	0.87	0.14	0.315			0.05	1.05	0.07	0.249			—	—	—	—	—	
Reach of the end of the device (emergence test)	0.5	-0.06	0.94	0.04	0.000	***	***	0.26	1.29	0.09	0.001	***	**	2.86	17.52	0.59	0.000	***	***
	3	-0.06	0.95	0.11	0.244			0.24	1.27	0.08	0.001	**	**	2.68	14.56	0.58	0.000	***	***
	5	-0.01	0.99	0.18	0.760			0.23	1.26	0.08	0.001	**	**	2.58	13.24	0.59	0.000	***	***
Reach of the edge (movement test)	0.5	0.04	1.04	0.03	0.383			0.20	1.22	0.07	0.066	(*)	(*)	—	—	—	—	—	—
	3	0.13	1.14	0.07	0.021	*	*	0.20	1.22	0.07	0.058	(*)	(*)	—	—	—	—	—	—
	5	0.23	1.26	0.12	0.000	***	***	0.20	1.22	0.07	0.052	(*)		—	—	—	—	—	—

Absence of a leg was used as a covariate for the analysis of the chance to reach the end of the device in the activity test, and of the chance to move after an attack event in the boldness test. Model structure takes the region of origin as cluster into account. Degree of urbanisation was rescaled so that an increase of one unit in the estimated slope represented an increase of 10% in urbanisation. Move after an attack (threat reaction test): $n=98$; Emergence from the dark chamber (emergence test): $n=82$; Reach of the end of the device (emergence test): $n=71$; Reach of the edge of the greenhouse (movement test): $n=99$

P-values: (*) $P<0.1$, * $P<0.05$, ** $P<0.01$, *** $P<0.001$

Table 4 Correlation matrix of the behavioural traits of the individuals submitted to the four behavioural tests

	Reach of the edge (movement test)				Emergence from the dark chamber (emergence test)				Reach of the end of the device (emergence test)				Move after attack (threat reaction test)			
	Coeff	SE	P-val	Signif	Coeff	SE	P-val	Signif	Coeff	SE	P-val	Signif	Coeff	SE	P-val	Signif
Reach of the edge (movement test)	—	—	—	—												
Emergence from the dark chamber (emergence test)	0.10	0.12	0.414		—	—	—	—								
Reach of the end of the device (emergence test)	0.09	0.16	0.600		0.24	0.14	0.104		—	—	—	—				
Move after attack (threat reaction test)	-0.06	0.13	0.638		0.14	0.13	0.285		0.22	0.15	0.159		—	—	—	—

P-values: (*) $P<0.1$, * $P<0.05$, ** $P<0.01$, *** $P<0.001$

Movement test

Most trajectories showed a high degree of straightness (2nd quantile fractal dimension: 1.05, 3rd quantile: 1.13). The fractal dimension of the trajectories was independent of the degree of urbanisation at any spatial scale (Table 2). Overall, 55.8% of the individuals reached the edge of the greenhouse within 10 min. The impact of urbanisation on the probability to reach the edge of the greenhouse appeared at the 3 km radius scale but was the clearest at the 5 km radius scale (probability 2.1 times higher, $P<0.001$, Table 3, Fig. 3d, Supp. Fig. S1D). Ambient temperature showed a near significant trend (probability 1.2 times higher per °C, $P=0.052$).

Correlation between behaviours

Correlation coefficients were calculated between all pairs of time-to-event behavioural data. None of them was greater than 0.24 or significant (Table 4).

Bioacoustics

On average, echemes lasted for 0.27 ± 0.06 s, and the interval between successive individual echemes was 3.16 ± 0.94 s. The degree of urbanisation did not affect echeme duration at any spatial scale (Table 5). Echemes were nonetheless more spaced in songs of males from urban populations and this effect was the clearest, although marginally significant, at small scale (e.g. + 0.49 s at the 0.5 km radius scale, $P=0.052$; Fig. 4a) and remained so until intermediate scale. These trends were lost when controlling for false positives. The interval between successive echemes was positively related with femur length (+ 0.60 s per mm, $P=0.035$). Mean values for mean, peak and mean dominant frequencies were 9.49 ± 0.52, 8.54 ± 2.19, and 9.12 ± 1.11, respectively. We observed no significant difference amongst those variables. Maximum values for peak frequency were nonetheless smallest in the most urbanised site (i.e., Leuven). Standard deviation of the frequency weighted by amplitude was 3.03 ± 0.22 kHz on average and decreased with urbanisation. This effect was already apparent when testing at small scale, but was the strongest at the intermediate scale (e.g., -0.18 kHz at the 3 km radius scale, $P=0.024$; Fig. 4b).

Peak frequency was positively correlated with mean frequency ($r=0.63$, $t=11.694$, df = 213, $P<0.001$). Standard deviation of the frequency weighted by amplitude was positively correlated with echeme duration ($r=0.55$, $t=9.600$, df = 213, $P<0.001$). Mean frequency was negatively correlated with the interval to the next echeme ($r=-0.35$, $t=-4.752$, df = 158, $P<0.001$).

Table 5 Summary of the fixed variables from the mixed models for bioacoustical traits, relative to the degree of urbanisation at three spatial scales (0.5 to 5 km radius) around the sampling sites. Adjusted significative thresholds are represented in the "Adj. Signif." column

Trait	Scale (km)	Intercept		Urbanisation (10%)					Femur length (mm)					Echeme duration (s)				
		Coeff	SE	Coeff	SE	P-val	Signif	Adj. Signif	Coeff	SE	P-val	Signif	Adj. Signif	Coeff	SE	P-val	Signif	Adj. Signif
Echeme duration (s)	0.5	0.29	0.01	0.00	0.00	0.437			-0.03	0.02	0.130			—	—	—	—	—
	3	0.30	0.01	-0.01	0.01	0.176			-0.04	0.02	0.083	(*)		—	—	—	—	—
	5	0.29	0.01	-0.01	0.01	0.194			-0.04	0.02	0.082	(*)		—	—	—	—	—
Interval between successive echemes (s)	0.5	3.01	0.23	0.05	0.03	0.052	(*)		0.60	0.28	0.035	*	(*)	—	—	—	—	—
	3	2.94	0.23	0.14	0.08	0.076	(*)		0.65	0.30	0.028	*	(*)	—	—	—	—	—
	5	3.05	0.21	0.18	0.14	0.172			0.64	0.31	0.038	*	*	—	—	—	—	—
Mean frequency (kHz)	0.5	9.45	0.11	-0.01	0.02	0.583			0.02	0.19	0.927			—	—	—	—	—
	3	9.46	0.13	-0.03	0.05	0.560			0.00	0.20	0.989			—	—	—	—	—
	5	9.44	0.11	-0.03	0.08	0.712			0.01	0.20	0.956			—	—	—	—	—
Peak frequency (kHz)	0.5	8.32	0.43	0.02	0.07	0.736			0.36	0.67	0.598			—	—	—	—	—
	3	8.39	0.49	0.01	0.17	0.964			0.33	0.69	0.629			—	—	—	—	—
	5	8.40	0.44	0.01	0.29	0.979			0.33	0.70	0.634			—	—	—	—	—
Mean dominant frequency (kHz)	0.5	9.13	0.23	-0.02	0.04	0.574			-0.13	0.40	0.742			—	—	—	—	—
	3	9.21	0.25	-0.08	0.10	0.396			-0.19	0.41	0.645			—	—	—	—	—
	5	9.16	0.22	-0.12	0.16	0.463			-0.18	0.41	0.654			—	—	—	—	—
S.d. of frequency weighted by amplitude (kHz)	0.5	3.08	0.03	-0.01	0.01	0.047	*	*	0.10	0.06	0.099	(*)		1.31	0.21	0.000	***	***
	3	3.10	0.04	-0.03	0.01	0.024	*	(*)	0.08	0.06	0.196			1.30	0.21	0.000	***	***
	5	3.09	0.03	-0.06	0.03	0.026	*	*	0.08	0.06	0.221			1.30	0.21	0.000	***	***

Femur length, echeme duration and absence of a leg were used as covariates. Model structure takes the individual and zone of origin as random effects into account. Degree of urbanisation was rescaled so that an increase of one unit in the estimated slope represented an increase of 10% in urbanisation. Echeme duration: $n_{individuals} = 38$, $n_{echemes} = 215$; interval between successive echemes: $n_{individuals} = 37$, $n_{echemes} = 161$; mean, peak, mean dominant, standard deviation of frequencies: $n_{individuals} = 38$, $n_{echemes} = 215$

P-values: (*) $P < 0.1$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

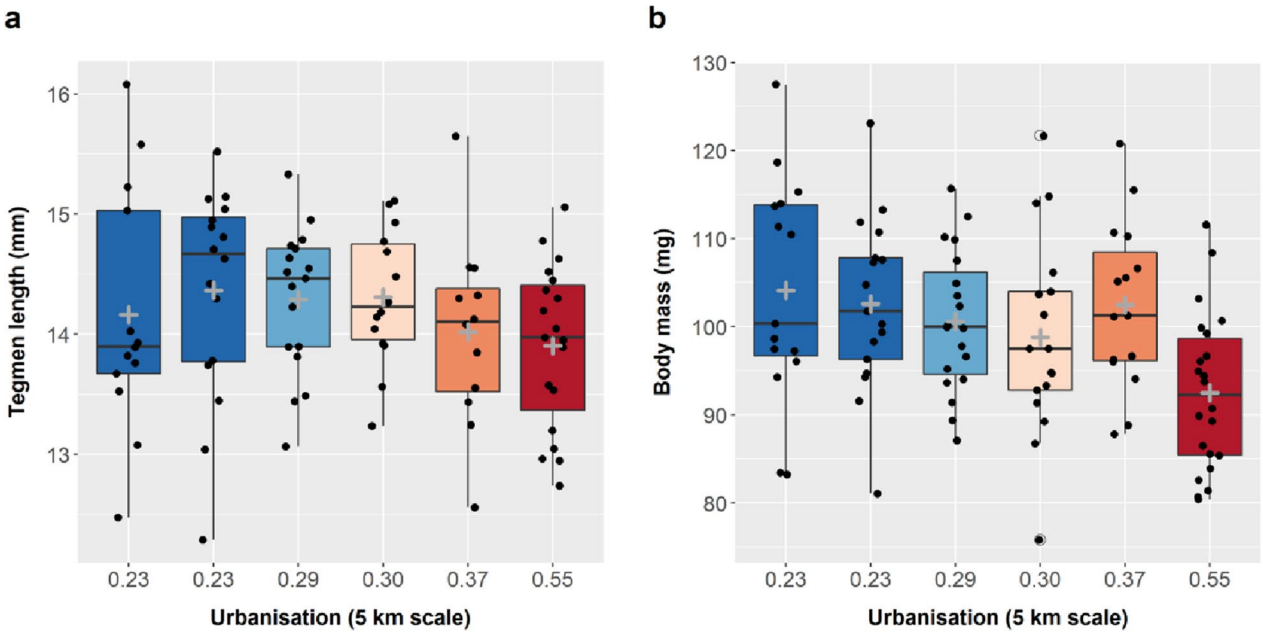

Fig. 2 Tegmen length and fresh body mass of *Chorthippus brunneus* males relative to degree of urbanisation at a 5 km radius scale (**a** and **b**). **(a)** Tegmen length ($n=90$), **(b)** fresh body mass ($n=91$). Boxplots show the median and the upper and lower quartiles. Whiskers indicate the 1.5 × interquartile range. Crosses indicate mean values. Colours range from dark blue for the most rural population to dark red for the most urbanised one

Autotomy

Hind leg loss was absent to rare in the grasshoppers of all populations but Leuven (0–11%). It was however much more common (41%) amongst individuals of the Leuven population, which is the most urbanised site at all considered scales. Redoing the analyses without the self-amputated individuals did not significantly alter our conclusions for a vast majority of tests (Supplementary Table S2). Several morphological (pronotum, femur, and tibia length, and pronotum length on tegmen length and on body mass), physiological (urbanisation and body mass on SMR), behavioural (temperature on the probability to move after an attack, and on the probability to emerge, probability of reaching the end of the device, fractal dimension, and probability of reaching the edge of the greenhouse), and bioacoustical (urbanisation on echeme duration, mean frequency, peak frequency, mean dominant frequency, and echeme duration on standard deviation of the frequency) results were not affected at all. Some results were only slightly affected, either by the appearance (temperature on SMR at all scales, urbanisation on the probability to move after an attack at 0.5 km) or disappearance (urbanisation on tegmen length, femur length on echeme duration and on standard deviation of the frequency at 0.5 km, and urbanisation on interval between successive echemes) of a trend. Finally, a few results were really affected by excluding those individuals: i) appearance of significant differences for the effect of urbanisation on the probability to move after an attack at 3 and 5 km; ii) appearance of significant differences for the effect of urbanisation on the probability to emerge at all scales; and disappearance of previous significant effect for the effects of urbanisation on body mass at 3 and 5 km, femur length on the interval between successive echemes, and urbanisation on standard deviation of the frequency at 0.5 km (this last one becoming a trend).

Discussion

We have tested for differences in functional morphology, personality-related behavioural traits, standard metabolic rate and song production in wild-caught *Chorthippus brunneus* grasshoppers that originated from local populations that varied in the surrounding degree of urbanisation at different spatial scales. Urban environments may filter phenotypic trait variation based on a dispersal syndrome and/or a pace-of-life syndrome rationale by different mechanisms (i.e., phenotypic plasticity, natural selection on standing variation, phenotypic sorting and/or genetic drift). We addressed this issue particularly with this grasshopper species since a previous study pointed to clear life-history differences with higher investment in dispersal in urban compared to rural populations (San Martin y Gomez and Van Dyck 2012), but lacked any direct behavioural data. First, we discuss our different results for dispersal-related morphological investment. Next, we interpret our findings for the series of biological

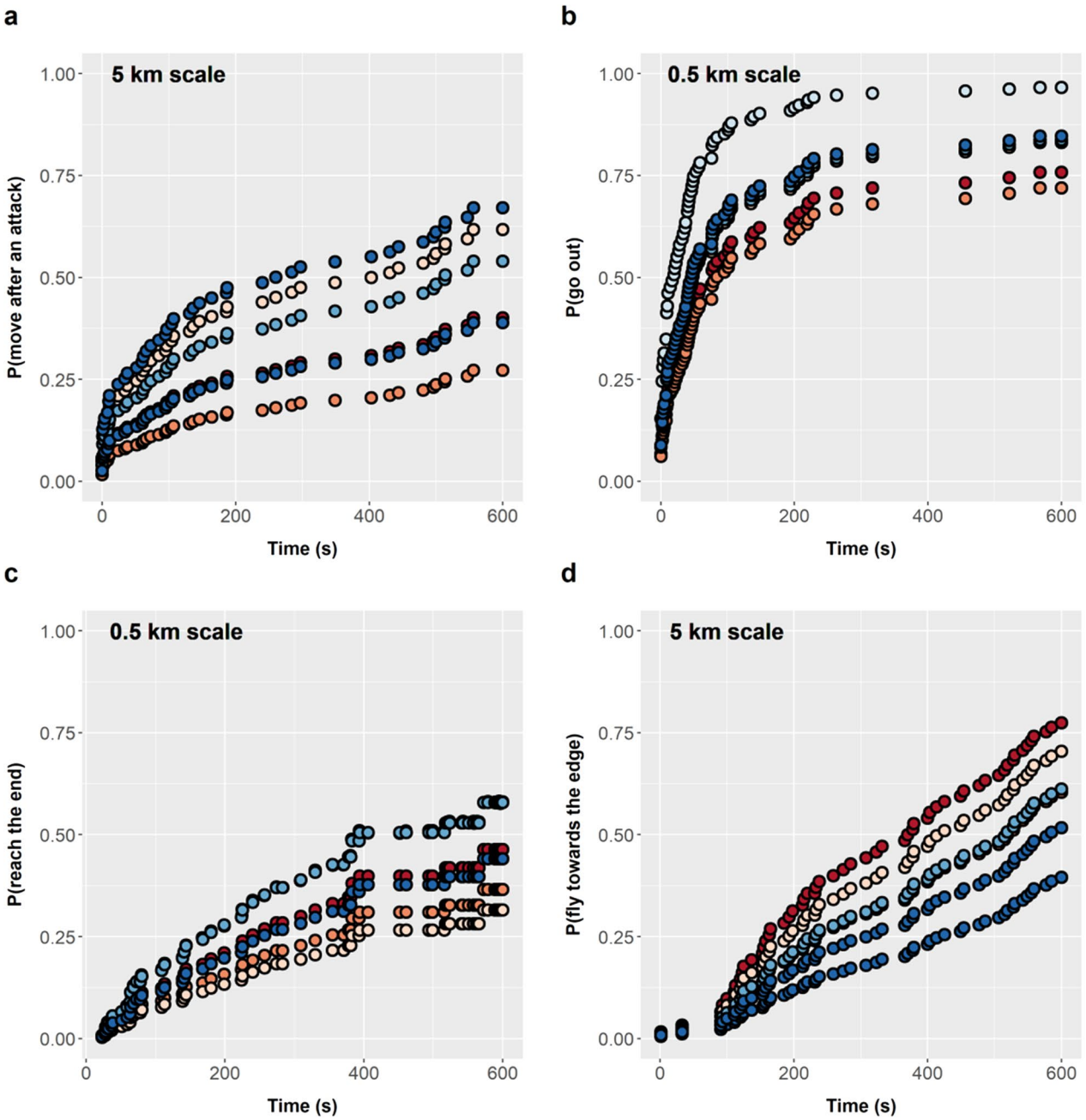

Fig. 3 Behavioural probability models relative to degree of urbanisation at a 5 km radius scale (**a** and **d**) and a 0.5 km radius scale (**b** and **c**). Probability to **(a)** move after an attack (threat reaction test; $n=98$), **(b)** emerge from the dark chamber (emergence test; $n=82$); **(c)** reach the end of the device (emergence test; $n=71$), **(d)** reach the greenhouse edge (movement test; n = 99). Spatial scale is listed on each graph. Colours range from dark blue for the most rural population to dark red for the most urbanised one

traits that were tested and we discuss the idea of a suite of correlated traits (i.e., syndromes). Throughout the discussion, we integrate the significance of the often-ignored issue of addressing urbanisation effects at different spatial scales.

Under common garden conditions, relative wing length was found to be longer in F1-generation offspring of urban grasshopper females compared to conspecifics of rural grasshopper females (San Martin y Gomez and Van Dyck 2012). This pattern was interpreted as a more mobile profile of urban populations. However, we have chosen to work with field-caught individuals from different populations than the ones studied by San Martin y Gomez and Van Dyck (2012), and without common garden developmental conditions. We could not confirm a similar pattern between urbanisation and dispersal-related investment based on morphological measurements. This is puzzling, but interesting at the same

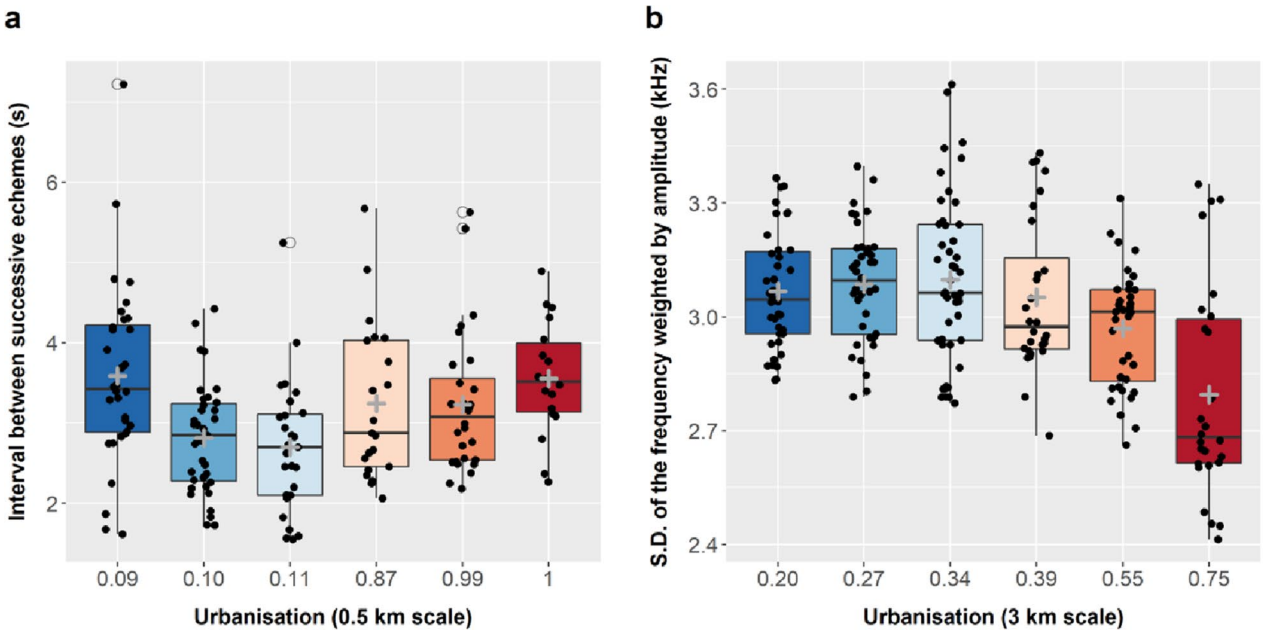

Fig. 4 Song parameters relative to degree of urbanisation at a 0.5 km radius scale (**a**) and 3 km radius scale (**b**). **(a)** Interval between successive echemes: ($n_{individuals} = 37$, $n_{echemes} = 161$), and **(b)** standard deviation of the frequency weighted by amplitude ($n_{individuals} = 38$, $n_{echemes} = 215$). Boxplots show the median and the upper and lower quartiles. Whiskers indicate the 1.5 × interquartile range. Crosses indicate mean values. Colours range from dark blue for the most rural population to dark red for the most urbanised one

time. The magnitude and direction of morphological differentiation amongst rural and urban populations can be site-dependent (Evans et al. 2009), resulting in a more complex mosaic for the distribution of phenotypic variation amongst populations than a clear-cut threshold difference between urban and rural populations. This can arise from parallel evolution or from irregular and asymmetric patterns of dispersal and colonization by divergent phenotypes, which is of particular relevance for areas under ongoing urbanisation (Dufort and Barker 2013). Dispersal-related traits of individuals could also change over time with population age. Cheptou et al. (2008) showed that more dispersive phenotypes of a plant were more likely to colonize urban sites, but once established, there was selection against dispersal with significant effects after only 5 to 12 generations. Similarly, urban grasshoppers may face selection for or against dispersal, depending on the time since colonization and the connectivity amongst local populations. This aspect deserves more attention in future work.

Grasshoppers of more urbanised environments were not shorter than conspecifics of less urbanised environments, but had slightly lower body mass, even when excluding autotomized individuals from the dataset. Such a shift was particularly evident at larger spatial scale. Species for which body size is positively correlated with dispersal ability such as orthopterans, showed larger body size in urbanised areas (Merckx et al. 2018). Under warm developmental conditions, Willott and Hassall (1998) and Walters and Hassall (2006) observed an increase of *C. brunneus* adult body size and mass. However, when reared under identical temperature conditions in the laboratory, urban males were not heavier than rural ones (San Martin y Gomez and Van Dyck 2012). Our results suggest, however, the opposite pattern for warmer urban conditions. Temperature experienced during larval development may not have been sufficiently high in our study sites to induce a significant increase in body size. From other work in Belgian urban and rural habitats, the mean daytime temperature in urban environments was more modest (*c.* 1 °C higher; Kaiser et al. 2016; Merckx et al. 2018) than the 5 °C-treatment difference which was applied in the experiments of Willott and Hassall (1998). Of course, body size is not exclusively affected by thermal conditions during development (Gardner et al. 2011) and there may be confounding factors at play that correlate with urbanisation (e.g., pollution; Hiyama et al. 2012). Relative body mass was negatively affected by urbanisation, which is in agreement with the lower allocation to the abdomen as observed by San Martin y Gomez and Van Dyck (2012). This may be indicative of a trade-off in resource allocation during development, though this was not evident from the other morphological measures, nor from the metabolic measures. However, relative body mass is also a measure reflecting body condition in Orthoptera (Kelly et al. 2014; Stahlschmidt and Chang 2021). Therefore, this negative impact based on our field-caught samples could refer to constraints that were not detectable in the previous common garden study of San

Martin y Gomez and Van Dyck (2012). Moreover, it is also worth considering the possibility of poorer body condition in grasshoppers in urban environments.

Standard metabolic rate was not affected by urbanisation and did not show any correlation with the tested behavioural traits. This result is in line with Royauté et al. (2015) and suggests that a general and simple relationship between personality and basal energy metabolism (Réale et al. 2010) is questionable or at least context-dependent. We only quantified standard and not active metabolic rate, which may have a stronger relationship with locomotion performance (Mattila and Hanski 2014).

Grasshoppers of more urbanised populations were on average shyer, but the magnitude of this effect depended on both the test and the considered spatial scale of urbanisation. Boldness is considered a measure of risk taking (Réale et al. 2007). There are different ways to measure it, such as latency time before emergence into a new environment or flight initiation distance when faced with a danger. Boldness expressed as latency time before emergence from a dark compartment was negatively related to urbanisation. A similar trend was observed when boldness was measured as a response after a simulated predator attack. Excluding self-amputated grasshoppers from the analyses reveals a much stronger effect of urbanisation on boldness. Moreover and intriguingly, this also demonstrates behavioural differences between intact and autotomized individuals, with bolder individuals being more likely to autotomize. While a higher level of activity and a general lack of cautiousness for bolder individuals might explain the observed differences in autotomy, Bateman and Fleming (2006) showed that already autotomized crickets were actually more cautious than intact individuals. Bolder individuals may also present a more acute perception of predation threats, leading to an early expression of antipredator behaviours, such as leg shedding (Poulin 2017). Our study was not designed to study this phenomenon of autotomy but opens novel perspectives for doing so. Although urbanisation has been shown to relate positively to boldness in some taxa (Mccleery 2009; Atwell et al. 2012; Lapiedra et al. 2017), it cannot be considered a general relationship since the relationship can be absent as well (Halpin and Johnson 2014). Moreover, shyer, reactive individuals have been shown to be also more flexible to adjust their behaviour to the environment (Coppens et al. 2010; Harris et al. 2020), which would be of great utility in the highly disturbed urban environment. These results point to the existence of alternative strategies to the frequently documented higher boldness in urban settings, and stress the importance of taking into account the context when selecting sampling sites and behavioural tests.

Urbanisation affected our two movement measures in a novel environment (greenhouse and wooden corridor device) in opposite ways. Urban grasshoppers had a higher probability to reach the greenhouse edge in the movement test (i.e., an artificial environment deprived of any food or conspecifics) at the intermediate and high scales, but a lower probability to reach the end of the corridor in the emergence test (i.e., a novel situation) at the smallest scale. The types of movement differed in the two experiments. In the greenhouse test, movement towards one of the edges always involved flight behaviour, whereas the design of the corridor device prevented grasshoppers from flying. Hence, movement behaviour in the emergence test may rather be considered as a proxy for exploration behaviour, while the movement behaviour in the greenhouse test should rather be seen as a proxy for dispersal (Van Dyck and Baguette 2005).

The effect of urbanisation on dispersal propensity was not clear from our experiments. On the one hand, urban individuals were more prone to leave in the movement test, which is expected to favour dispersal. On the other hand, smaller body mass together with shorter tegmina and being less explorative may be seen as indicative of selection against dispersal in urban areas. Our movement test was designed to measure the willingness of an individual to move in an open, artificial environment. However, willingness or propensity to disperse represents a different component of dispersal than dispersal ability (Cote et al. 2017). There is still much to learn about the way urban environments affect different types of movement. Moreover, movements between sites may be facilitated by higher temperatures (Walters et al. 2006). Temperature positively affected movement in most of our behavioural tests. It is well known in grasshoppers that mean relative daily movement is strongly related to daily temperature recorded within the grass sward, but the effect may vary considerably among the least and the most dispersive individuals in a population (Walters et al. 2006). Our results suggesting that urbanisation is associated with lower levels of boldness and exploration do not agree with most studies in the literature (e.g. Charmantier et al. 2017; Thompson et al. 2018). Like morphology, behavioural differences between rural and urban populations can be site-dependent (Moule et al. 2016). Our results stress the importance of conducting several behavioural assays in parallel, since ecological relevance could vary based on the behavioural test (Beckmann and Biro 2013). Alternatively, test environments can also be perceived differently by individuals (Burns 2008). Based solely on our movement test, one might conclude that *C. brunneus* grasshoppers are more mobile in cities, whereas our emergence test is rather in favour of lower mobility. Despite the fact that some tests were supposed to measure similar behavioural aspects, none of the considered behaviours correlated with each other. In the same vein, Delnat et al. (2017) showed that, although certain aspects of boldness correlated with each other, others did not. Our results thus support this multidimensionality in behaviour-related traits.

Acoustic communication by stridulation is an important behavioural trait of Orthoptera that can be affected by urbanisation-related noise pollution (Lampe et al. 2012, 2014). Urban grasshoppers were found to use narrower frequency ranges, but they did not sing systematically at higher frequency under standardised laboratory conditions. The effect on frequency variance was significant even after accounting for echeme duration. There is no easy explanation for such an effect. Founder effects and drift are likely to be more important in urban populations, which may lead to non-adaptive differences. *C. brunneus* mainly used frequencies > 3 kHz, while urban noise is typically more prevalent at < 2 kHz (Sandberg 2003). Noise levels, however, may vary within urban areas. Lampe et al. (2014) showed an increase in frequency in a closely related grasshopper species after larval development under noisy conditions in the lab, even if the sound recording at the adult stage was done under quiet conditions as we did. Autotomy may also affect acoustic communication in multiple ways, including changes in temporal tuning. The spatial scale influencing the significant longer time between successive echemes for self-amputated individuals is an example that warrants further investigation. Acoustic communication is of primary importance for organisms, as it allows species- and individual-specific recognition through different song parameters (Mathevon et al. 2008). How distance affects these parameters will strongly influence the ability of a species to produce a qualitative long-range advertisement signal (Brumm and Naguib 2009). Since the production of this acoustic signalling is energetically costly (Gillooly and Ophir 2010), one may also search for a trade-off between a qualitative song and other life-history traits in the autotomized grasshoppers.

It is worth noting that our results keep on pointing out the intriguing phenomenon of autotomy. Kuriwada et al. (2023) also observed a higher incidence of limb loss in urban populations of the cricket *Dianemobius nigrofasciatus* compared to rural populations. They linked this difference to a higher predation failure in urban habitat, while seeing no differences in risk-taking behaviour between populations of the two origins. These conclusions do not match with our results, as we do see differences in boldness (i.e., a risk-taking behaviour) between urban and non-urban individuals, and no actual predation could induce leg shedding in our experiments. Whether we included or not the individuals that had lost a leg did not change our conclusions for most traits. For a few tests, there were minor (e.g., tegmen length and body mass) and, for some, significant changes (e.g. behavioural tests). We did not design our study to explore this phenomenon, but it is warranted to do so, as our results could suggest the existence of a particular syndrome for autotomized grasshoppers. Bateman and Fleming (2011) showed behavioural differences in a predation context between autotomized and unharmed individuals of the grasshopper *Paroxya atlantica*. Autotomy has been linked to a reduction in mobility and to an increase in energy expenditure in the cricket *Gryllus bimaculatus*, pointing to a significant cost of this behaviour (Fleming and Bateman 2007). In a gecko species, dispersal characteristics and tail loss have been shown to differ with habitat type (Duckett and Stow 2012), and Talavera et al. (2021) found that autotomy was positively associated with boldness in *Anolis aquaticus*, but all these studies did not consider urban populations.

As we showed throughout our results, the effects of urbanisation on biological trait values can be scale-dependent, but this dependency has received only limited attention at the intraspecific level. We showed phenotypic differences between populations of less and more urbanised environments. Effects on adult dispersal-related morphology were mainly significant when analysed at 3 and 5 km spatial scales – scales at which dispersal takes place in *C. brunneus* –, while behavioural and song differences were observed at various spatial scales. Although the effects remain somewhat puzzling at this stage, our study provides an empirical demonstration of the importance of scale-issues as raised by Martin (2018). Our results stress the importance of a careful thinking about which scales would matter the most when studying phenotypic traits, while also defining precisely the scales which matter for the organisms under study. This can be done by carrying out a preliminary tracking investigation.

In conclusion, our study demonstrates that simple generalisations cannot characterise the multiple, complex effects of urbanisation on phenotypic trait values at the intraspecific level. Moreover, we strongly advocate for considering the spatial scale at which urbanisation is measured, as variation in this metric can profoundly shape the effects of urbanisation on various phenotypic traits and their detectability.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00265-023-03325-7.

Acknowledgements We thank two anonymous reviewers for their constructive comments, and Albittar L, Henneresse T, Kaiser A, Lebeau J, Merckx T, Pirnay M and Turlure C for help with our study. T Merckx also gave feedback on the manuscript. This is publication no BRC 404 of the Biodiversity Research Centre (Earth and Life Institute, UCLouvain).

Author contributions GB, BW and HVD conceptualised the study. Experiments were conducted by GB and BW and analyses by GB and BW in interaction with HVD. All authors contributed to write the manuscript.

Funding The research was done without external funding.

Data availability The datasets supporting this article have been uploaded as part of the electronic supplementary material (Waterschoot—Bataille—Van Dyck—Data.txt and Waterschoot—Bataille—Van Dyck—Data_Bioacoustics.txt).

Code availability Not applicable.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflicts of interest The authors declare that they have no conflict of interest.

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