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Open field behavior in the house cricket (*Acheta domesticus*): effect of illumination, sex differences and individual consistency

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RESEARCH ARTICLE

Abstract

Edible insects are worldwide promoted as an alternative protein, trace mineral and lipid source in animal feed and human food. The house cricket (*Acheta domesticus*) is already being reared at an industrial scale, yet current mass-rearing practices and facility design may still leave room for improvement. Behavioral tests have been suggested as an important assessment tool at the whole-organism level that can be used to find optimal housing conditions (e.g. density, diet, temperature). Here, we adapt the widely used open field test to the house cricket. We tested 16 male and 16 female house crickets four times under two different light intensities. Videos were analysed with Ethovision™ tracking software and variables distance moved, velocity, and duration and frequency in zone were extracted. Results showed that house crickets, like vertebrate model species, spent most time close to the walls of the arena, and crossed the center zone with high velocity. Brighter illumination was associated with increased velocity, in particular in the center zone during the first test occasion, suggesting avoidance of this zone. Male crickets had higher locomotory activity than females. Consistency repeatabilities and intraclass correlation coefficients (ICCs) were moderate to high, and the correlation between subsequent occasions became stronger over the four occasions at day 1, 2, 3 and 7. The first test occasion differed from subsequent occasions, therefore repeated testing may be necessary when analysing experimental manipulations of small effect size. Overall, the results are promising for use of the open field test as a precise phenotyping tool.

Keywords: behaviour, thigmotaxis, animal welfare, repeatability

1. Introduction

Edible insects are worldwide being promoted as an alternative protein, trace mineral and lipid source in animal feed and human food (Van Huis *et al.*, 2013). The house cricket (*Acheta domesticus*) is already being reared at an industrial scale (Van Huis, 2020), yet current mass-rearing practices and facility design may still leave room for improvement. Behavioral tests have been suggested as an important assessment tool at the whole-organism level that can be used to assess animal health and welfare under different conditions, e.g. different crate size, diets and temperatures (Berggren *et al.*, 2019). Here, we report how we designed and adapted a classical behavioral test, the open field test, to house crickets.

The open field is a behavioral test that was originally developed for rats (Hall, 1934). The procedure consists of placing the animal in a novel, empty arena, which can be square, rectangular or circular in shape and from which escape is prevented by the surrounding walls. When the size of the arena is large compared to the size of the animal, many species of animals move along the walls and seemingly avoid the open space in the center of the arena. This behavior, termed 'thigmotaxis', is considered a form of shelter seeking behavior. In fact, shelter seeking behavior may be one of the reasons why house crickets thrive being reared in egg card boxes (Vaga *et al.*, 2018). Treatment with pharmaceuticals that have anxiolytic effects in humans often reduce thigmotaxis in animals in the open field (Prut and Belzung, 2003). This provides pharmacological

validation for the open field test, although not all anxiolytic substances reduce thigmotaxis (Prut and Belzung, 2003; Rodgers, 1997; Walsh and Cummins, 1976). Nevertheless, the open field remains widely used and has been translated to a broad range of species.

Open arenas are regularly used in insect behavioral research, for example for the fruit fly *Drosophila melanogaster* (Kmetova *et al.*, 2021) and in field crickets *Gryllus campestris* (Santostefano *et al.*, 2016). Most studies quantify locomotory activity while thigmotaxis is not always analysed. This may be a reasonable choice when the arena is small compared to the body size of the animal, in which case only a small percentage of the arena can be considered an open space (Stewart *et al.*, 2012). In order to maximise the precision and accuracy of the open field test, the physical characteristics of the arena need to be adjusted to the species, including the dimensions of the arena, temperature and illumination. It is well recognised for rodents that high levels of illumination diminish locomotory behavior and increase thigmotaxis, while rearing and grooming are increased in dim light (Bouwknecht *et al.*, 2007). Hence, under too bright light, ceiling effects in thigmotaxis may make differences between conditions difficult to quantify. If, on the other hand, changes in behavior can be quantified in response to different light intensities, then this is reassuring for the ability to detect differences induced by rearing conditions. A further reduction in residual error can be achieved by taking into account individual traits that affect the mean of a subgroup. Sex differences in open field behavior are regularly reported in other species, e.g. rats (Blizard *et al.*, 1975), mice (Kvist and Selander, 1987) and zebrafish (Thomson *et al.*, 2020; Vossen *et al.*, 2016), although effects are not always in the same direction across species and strains. Finally, the ability to measure a small effect of treatment on open field behavior critically depends on the amount of between- and within-individual variation (Dingemanse and Dochtermann, 2013). If individuals do not behave consistently over testing occasions and show overlap in test outcome, then smaller treatment effects are more difficult to detect.

In this study, we aimed to quantify the behavior of house crickets in the open field test. Specific aims were to quantify: (1) the effect of bright versus dim illumination; (2) sex differences between males and females; and (3) individual behavioral consistency over four testing occasions. Considering the behavior of other vertebrate model species in the open field, we hypothesised that: (1) crickets would reduce locomotory activity and increase thigmotaxis under high illumination; (2) males would be more active and show reduced thigmotaxis compared to females; and (3) animals would show reduced activity and increased thigmotaxis on the first test occasion compared to the second, third and fourth occasion. We discuss how the open field test can be used to evaluate the effect of mass-rearing practices and facility design on house cricket welfare.

2. Materials and methods

Animals and housing

The experiment was carried out in September 2021. We used 32 adult house crickets from an outbred population that is regularly backcrossed with wild-caught individuals from Sweden (Vaga *et al.*, 2020) and tested negative for the *Acheta domesticus* densovirus (Semberg *et al.*, 2019). The animals were reared in transparent plastic containers (dimensions 33×21×29.5 cm L×B×D) in groups of 5-50 individuals in a climate controlled room at 30±1 °C, 45-50% relative humidity, 450-500 lux illumination and a 12 hour light/dark cycle with lights on at 07:00 CET. Animals had *ad libitum* access to standard feed (Vaga *et al.*, 2020), tap water (in 10 ml cotton-plugged tubes) and a shelter (Vaga *et al.*, 2018) consisting of a black plastic tube, 6 × Ø 2.5 cm with plastic straws Ø 0.5 or 0.8 cm.

Behavioural testing

Seven days before the first test, crickets were randomly distributed over two different housing containers (8 males and 8 females per container; dimensions container 33×21×29.5 cm L×B×D). Animals within a container were to be tested under the same illumination. All animals were naïve to behavioral testing. Individuals were tested on four occasions in the open field test, on experimental days 1, 2, 3 and 7 between 09:00 AM and 15:00 PM. Open field arenas had high, slightly inclined walls and were constructed in 7.5 mm thick Perspex, made opaque with sandpaper (dimensions floor plane: 20×20 cm, top plane: 26×26 cm, height: 30 cm). This design ensured that the animal could not jump out of the arena while the high walls did not cover the view for the camera. Transparent tape strips prevented the crickets from climbing the highest parts of the walls. For each trial, four open field arenas were placed in a portable ministudio (80×80×80 cm; LSD80, Godox, Shenzhen, China) inside the climate room. Using a light meter (MT-901, Clas Ohlson, Insjön, Sweden), the LED strips inside the ministudio were adjusted to low illumination (169±1 lux) or high illumination (508±5 lux). Low illumination corresponded to the minimum level of illumination that still provided even lighting, while high illumination reflected the level of illumination in the housing container.

At the start of a test, one cricket was released in each open field arena, after which the ministudio was closed and the trial was video recorded for 15 minutes using a monochrome GigE camera (Basler ace acA1300-60gm, Ahrensburg, Germany) with a 4.4-11 mm lens (Kowa, Düsseldorf, Germany) mounted to the ceiling of the ministudio. After the test, animals were put back in their home containers, except after the first test occasion when animals were individually marked with a unique colour on the pronotum using a Uniball Paint Marker PX-21

(Mitsubichi Pencil, Tokyo, Japan), before being returned to their home container. Between trials, testing arenas were rinsed with ethanol (70% v/v).

Automated video tracking

Videos were recorded with MediaRecorder4 (Noldus, Wageningen, the Netherlands) and tracked with Ethovision XT15 (Noldus). All tracks were manually assessed for any tracking errors. The arena was virtually divided into three zones; a wall zone (the walls of the arena which the crickets sometimes climbed), the floor border (2 cm wide) and the floor center (18×18 cm). For each zone, we extracted four variables: total distance moved (cm), mean velocity (cm/s), mean duration (s) in zone and the frequency of zone visits. These same variables were extracted from Ethovision per minute, allowing for analyses over time. Total distance moved and mean velocity were disregarded for the wall zone since movement in the vertical plane cannot be reliably estimated with a ceiling mounted camera.

Statistical analyses

Statistical analyses were performed using R version 4.1.1 (R Core Team, 2021) with added packages 'lme4' (Bates *et al.*, 2015), 'emmeans' (Lenth, 2020) and 'ggplot2' (Wickham, 2016). Distance moved, velocity and duration in zone were analysed with linear mixed-effects models (LMMs). Duration in zone was recalculated into proportion of time in zone (by dividing duration (s) by total duration in arena (s)) and logit transformed. Frequency of zone entries was analysed with a generalised linear mixed-effects model (GLMM) with negative binomial error distribution. For all four models, we entered fixed effects of zone, illumination, sex and occasion and all interactions, and random intercepts of individual ID and arena.

Repeatability is the proportion of between-individual variation relative to the total phenotypic variance (Nakagawa and Schielzeth, 2010). Between-individual variation is an important requirement for within-individual consistency (Nakagawa and Schielzeth, 2010). We calculated 'consistency repeatability' (sensu (Biro and Stamps, 2015)) over test occasions for each response variable and zone, by adding a main effect of occasion (as numeric) and random intercept effects of individual ID and arena. For variable duration, a logit transformation was applied. We used the functions 'rptGaussian' (for variables distance moved, velocity and duration) and 'rptPoisson' (for frequency) from the 'rptR' package (Stoffel *et al.*, 2017). Within-individual consistency was estimated as the intra-class correlation coefficient (ICC, *R*), i.e. the Pearson's correlation coefficient between sequential occasions (occasion 2 vs 1, 3 vs 2 and 4 vs 3) per variable and zone (Nakagawa and Schielzeth, 2010; Sokal and Rohlf, 1995).

3. Results

Overall, crickets moved longer distances in the floor border compared to the floor center zone (LMM, $F_{1,183}$=338.253, P<0.001; Figure 1A) and crossed the floor center with higher velocity (LMM, $F_{1,181}$=428.105, P<0.001, Figure 1B). Duration in zone was highest for the floor border (LMM contrasts, border vs center: t=12.274, P<0.001; border vs wall: t=18.707, P<0.001, Figure 1E-G), animals spent less time in the floor center and least time climbing the wall (LMM contrast, center vs wall: t=6.495, P<0.001, Figure 1C). The floor border was also entered most times, followed by the floor center (GLMM contrast, z=3.761, P=0.001, Figure 1H-I), while the walls received fewest entries (GLMM contrasts, border vs wall: z=35.724, P<0.001; center vs wall: z=32.170, P<0.001, Figure 1J).

Effect of illumination

The animals tested under high illumination had overall higher velocity (LMM, $F_{1,181}$=5.253, P=0.031, Figure 1C-D). The significant interaction between illumination and zone (LMM, $F_{1,181}$=4.679, P=0.031) indicated that animals tested under brighter lighting conditions selectively increased velocity in the floor center (LMM contrast, t=-3.066, P=0.004, Figure 1D) but not in the floor border (LMM contrast, t=-0.896, P=0.375, Figure 1C). Furthermore, the effect of illumination was dependent on occasion (LMM, $F_{3,184}$=4.776, P=0.003), and was only detected on the first test occasion (LMM contrasts; occasion 1: t=-4.366, P<0.001; occasion 2: t=-0.615, P=0.540; occasion 3: t=-0.664, P=0.508; occasion 4: t=-0.920, P=0.360, Figure 1C-D). Illumination did not influence the duration that crickets spent in each zone (LMM, $F_{1,24}$=0.103, P=0.751, Figure 1E-G), but subjects changed more often between zones under high illumination (GLMM, $F_{1,24}$=3.838, P=0.032, Figure 1H-J).

Sex differences

Males moved at higher velocity than females (LMM, $F_{1,24}$=9.216, P=0.006, Figure 1C-D), but also this effect was only significant for the first test occasion (LMM contrasts; occasion 1: t=-4.388, P<0.001; occasion 2: t=-1.521, P=0.131; occasion 3: t=-1.506, P=0.136; occasion 4: t=-1.204, P=0.232, Figure 1C-D). Males spent longer durations in the wall zone across all occasions (LMM contrast, t=-6.888, P<0.001, Figure 1G), while the sexes did not differ in duration spent in the other zones (LMM contrasts; floor center: t=-0.298, P=0.766; floor border: t=0.702, P=0.484, Figure 1E-G). There was a significant interaction between zone and sex (GLMM, $F_{2,285}$=14.852, P<0.001, Figure 1H-J) on the number of zone entries. Males entered the wall zone more often (GLMM contrast, z=-4.778, P<0.001, Figure 1J) but there were no sex differences in number of entries into the floor border (GLMM contrast, z=-0.542, P=0.588, Figure 1H) nor to the floor center (GLMM contrast, z=0.276, P=0.783, Figure 1I).

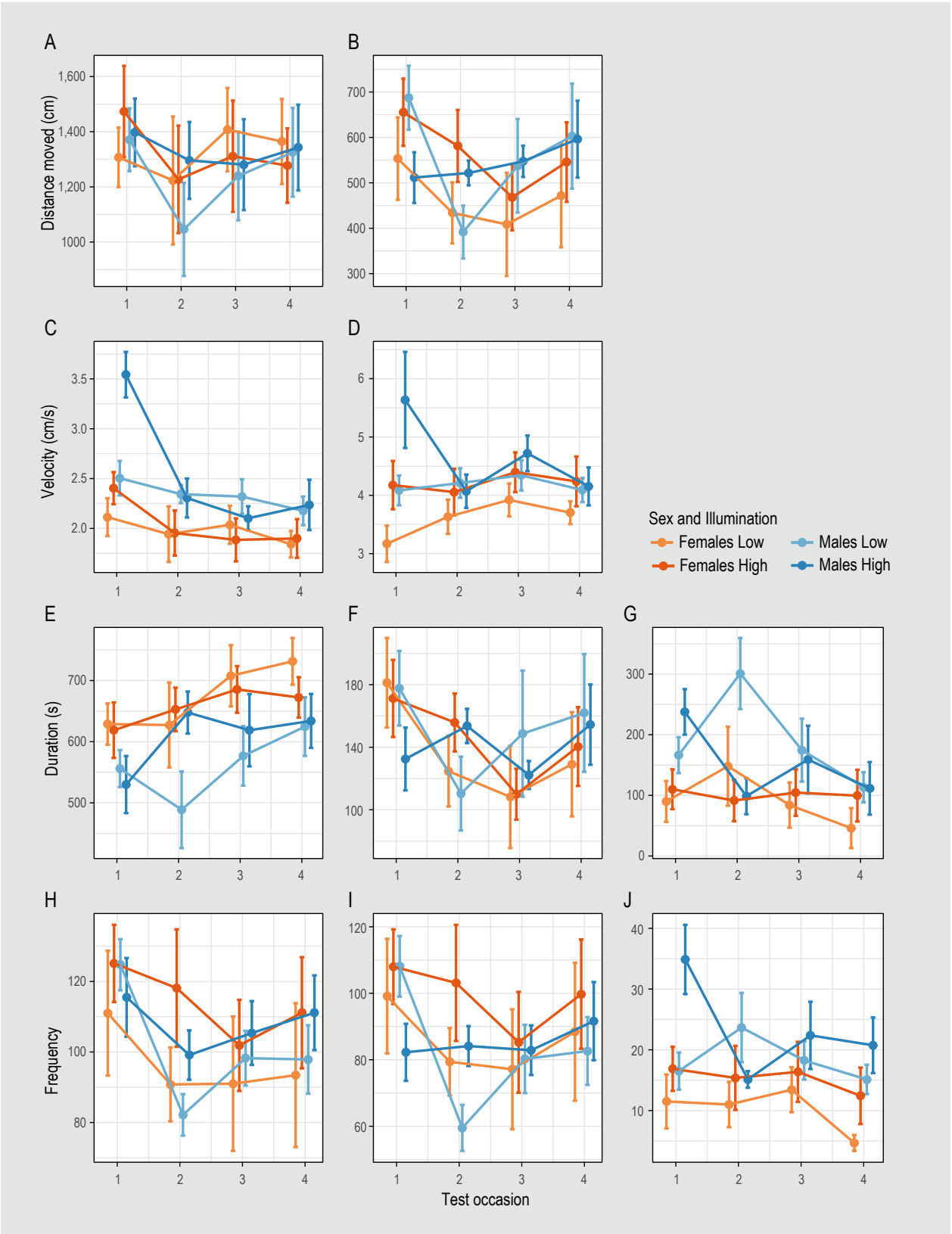

Figure 1. Results of the open field test. Total distance moved: (A) border, (B) center; mean velocity: (C) border, (D) center; duration in zone: (E) border, (F) center, (G) wall; and frequency in zone: (H) border, (I) center, (J) wall over test occasion, faceted by zone. Tests lasted for 15 minutes and test occasion 1-4 was conducted on experimental day 1, 2, 3 and 7, respectively. Colours refer to sex/illumination group (light orange: females low illumination; dark orange: females high illumination; light blue: males low illumination; blue: males high illumination). Points indicate means ± SEM.

Effect of test occasion

There was a main effect of test occasion on velocity (LMM, $F_{3,184}$=4.765, *P*=0.003, Figure 1C-D). Crickets had higher velocity in the first test as compared to the second (LMM contrast, t=3.163, *P*=0.011) and fourth (LMM contrast, t=3.296, *P*=0.007) test occasion. Duration in the wall zone was lower in the fourth test as compared to the first (LMM contrasts; t=4.106, *P*<0.001) and second test occasion (LMM contrasts; t=3.380, *P*=0.005, Figure 1G). Crickets changed more often between zones on the first test occasion compared to the second, third and fourth test (GLMM, F=5.706, *P*<0.001; Contrasts: 1 vs 2: z=3.338, *P*=0.005; 1 vs 3: z=2.780, *P*=0.033; 1 vs 4: z=4.072, *P*<0.001, Figure 1H-J).

Individual consistency

Consistency repeatabilities were moderate to high for distance moved, duration and frequency per zone (0.334–0.668), while velocity had lower repeatability (0.208-0.272; Table 1). Consistency repeatability was particularly high for the duration in the floor border (0.668). Over test occasions, intraclass correlation coefficients (ICCs, *R*) increased and became more statistically significant (Figure 2). For the correlation between occasion 3 and 4, ICCs for distance moved, duration and frequency in zone were 'good' (*R*=0.76, 0.75 and 0.73, respectively) while the ICC for velocity was 'moderate' (*R*=0.49, Figure 2) (Koo and Li, 2016).

4. Discussion

The house crickets displayed high levels of thigmotaxis behaviour and spent approximately 70% of their time in the floor border zone. The animals seemingly avoided the exposed center of the arena, since they payed fewer visits of shorter duration to the center, and crossed it with high velocity. Hence, the behaviour of house crickets in the open field test was similar to other species tested so far, suggesting good predictive validity (Willner, 1984). This general pattern was influenced by all three factors investigated here, i.e. by the lighting conditions during testing, by the sex of the animal and by repeating the open field test.

Under bright illumination crickets moved with a higher velocity and they changed more between zones, suggesting heightened locomotory activity. Level of illumination did not affect the standard measure of thigmotaxis, i.e. the duration spent in the floor border, although the increased velocity in the center may also be interpreted as an escape response. This effect was subtle as it was mostly detected in combination with other potential stressors, i.e. in the exposed center zone and under novelty stress in the first test occasion. In rodents, bright illumination reduces locomotory activity and has been associated with a shorter duration in the center of an open field (Bouwknecht *et al.*, 2007; Dixon and DeFries, 1968; Momeni *et al.*, 2014). Hence it appears that bright illumination resulted in avoidance of the center in all species, while the response in locomotory activity was more species-specific.

We found significant differences in open field behaviour between male and female house crickets. Males showed higher levels of locomotory activity than females, which has also been reported for fruit flies (Bath *et al.*, 2020), mice (Kvist and Selander, 1987) and zebrafish (Thomson *et al.*, 2020; Vossen *et al.*, 2016, 2022), while in rats, females were more active (Blizard *et al.*, 1975). We found no sex differences in thigmotaxis behaviour, i.e. the duration spent in the border zone. For fruit flies, some studies have reported no sex differences in thigmotaxis behaviour (Bath *et al.*, 2020; Lebreton and Martin, 2009; Martin, 2004) while others found higher thigmotaxis in females (Besson and Martin, 2005; Liu *et al.*, 2007). Male crickets climbed the walls of the arena more than females. This behaviour is an

Table 1. Individual consistency in behaviour. Consistency repeatability (R_C) and associated 95% confidence interval (between brackets) and *P*-value (based on permutation) of each response variable per zone and across all four testing occasions. Values were calculated using the 'rptR' package (Stoffel *et al.*, 2017) following the definition by (Biro and Stamps, 2015), see methods section.

Zone	Response variable	R_C	*P*-value
Floor border	Distance moved (cm)	0.518 (0.282-0.665)	0.001
	Velocity (cm/s)	0.272 (0.059-0.450)	0.002
	Duration in zone (s)	0.668 (0.431-0.798)	0.001
	Frequency in zone	0.519 (0.302-0.672)	0.001
Floor center	Distance moved (cm)	0.499 (0.261-0.691)	0.001
	Velocity (cm/s)	0.208 (0.017-0.396)	0.010
	Duration in zone (s)	0.334 (0.132-0.546)	0.001
	Frequency in zone	0.449 (0.234-0.643)	0.002
Wall	Duration in zone (s)	0.462 (0.218-0.690)	<0.001
	Frequency in zone	0.434 (0.211-0.599)	0.003

obvious peculiarity for the cricket, and is therefore difficult to interpret in terms of risk avoidance or escape behaviour. Rather than placing a lid on the arena, we decided to quantify this species-specific behaviour while ensuring that the animals could not leave the arena. However, adding an extra zone to the open field may influence the measures from other zones. Further experimental manipulations are

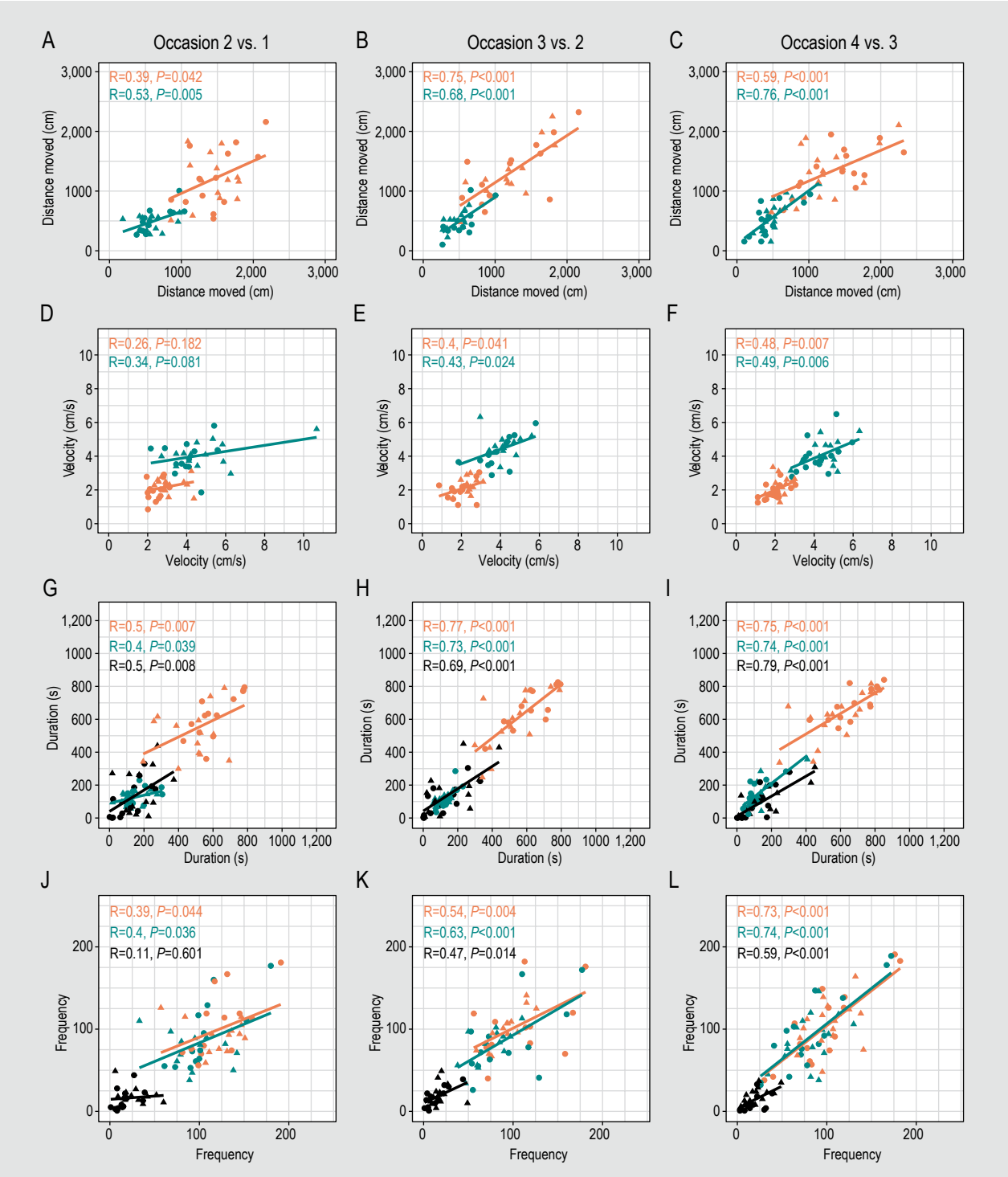

Figure 2. Correlations between test occasions. Intraclass correlation coefficients (*R*, Pearson's correlation coefficient) and associated *P*-values for correlations between sequential occasions for each response variable and zone. (A-C): total distance moved; (D-F): mean velocity; (G-I): duration in zone; (J-L): frequency in zone. Colours refer to the zone (orange: floor border; cyan: floor center; black: wall). Symbol shape refers to the sex of the individual (round: females; triangular: males).

needed to understand the climbing behaviour of crickets in the open field.

Individuals behaved consistently over test occasions, as indicated by moderate to high consistency repeatability and strong intraclass correlation coefficients between subsequent occasions. Nevertheless, the first test occasion stood out in a number of ways. As noted earlier, crickets moved with higher velocity and changed more often between zones in the first test occasion. Also some of the effects of illumination and sex were only detected in the first test. Interestingly, correlations between the third and fourth test were strongest, despite the interval between these test occasions being the longest (4 days). Our results are in line with two studies that repeatedly measured zebrafish behaviour in an open field arena. Using either a intersession interval of days (Tran and Gerlai, 2013) or weeks (Thomson *et al.*, 2020), these studies also reported increased activity during the first test occasion. Repeatabilities increased when the first session was excluded (Thomson *et al.*, 2020). These authors suggested to include an initial 'tank experience' session prior to the main phenotyping session (Thomson *et al.*, 2020; Tran and Gerlai, 2013). Multiple testing likely also improves the repeatability of the open field test in crickets. Nevertheless, the added novelty stress in the first test session may also reveal effects that otherwise remain concealed.

5. Conclusions

Open field behaviour of house crickets was similar to most species studied so far. Animals mostly moved along the walls of the arena and avoided the center and individuals behaved consistently on subsequent testing occasions. In contrast to vertebrate model species, crickets responded to mild stressors (brighter illumination, novelty stress) with increased rather than decreased locomotory activity while thigmotaxis behaviour was seen in all species. Male house crickets had higher locomotory activity than females, although thigmotaxis behaviour did not differ between the sexes, contrary to our hypothesis. We therefore recommend sex to be included as a factor in behavioural analyses of house crickets. Our results are promising for use of the open field test as a precise phenotyping tool, that can inform mass-rearing practices and facility design for crickets.

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Conflict of interest

The authors declare no conflict of interest.

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