The effects of social cues on the expression and quantitative genetic variation and covariation in animal personality traits

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“The art and science of asking questions is the source of all knowledge.”

Michael Berger

“Fascinating.”

Mr. Spock

“One cannot think well, love well, sleep well, if one has not dined well.”

Virginia Woolf

“Do. Or do not. There is no try.”

Yoda

“I have yet to see any problem, however complicated, which, when you looked at it the right way, did not become still more complicated.”

Paul Alderson
Thesis declaration

I, Fabian Rudin, certify that:

This thesis has been substantially accomplished during enrolment in the degree.

This thesis does not contain material which has been submitted for the award of any other degree or diploma in my name, in any university or other tertiary institution.

No part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of The University of Western Australia and where applicable, any partner institution responsible for the joint-award of this degree.

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The research involving animals reported in this thesis followed The University of Western Australia and national standards for the care and use of laboratory animals.

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This thesis contains published work and/or work prepared for publication, all of which has been co-authored.

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25/01/2018

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Summary

Amongst the many ways in which the environment affects the expression and evolution of behaviour, the social environment has been imputed to have particularly strong effects on behaviour. This is because this aspect of the environment is itself heritable and can lead to coevolutionary dynamics between actions and responses of interacting individuals. A proposed mechanism for these effects is the concept of interacting phenotypes, which occur when the expression of traits is affected by interactions with conspecifics. This in turn leads to indirect genetic effects, which occur when the genes of an individual affect phenotypic expression in another individual. In this thesis, I use male Australian field crickets, *Teleogryllus oceanicus*, to investigate how different aspects of the social environment affect the expression of and quantitative genetic (co)variation in different personality traits. By measuring behaviours twice I was able to calculate the repeatability of behaviour, a measurement often used as an indicator for the presence of ‘animal personalities’.

By experimentally manipulating dominance status, I found that changes in dominance status affect cricket behaviour considerably. More specifically, dominant individuals that became subordinate became bolder, more explorative and more active, while subordinates that became dominant became less bold, explorative and active. Changes in dominance status also reduced the repeatability of and correlations between some personality traits, but not others. I concluded that the social environment needs to be taken into consideration when investigating animal personalities. It would be impossible to know if and for how long changes in the social environment affect the repeatability of, or correlations between behaviours.

To further test the notion that social environmental effects on behaviour are particularly strong I measured cricket behaviour in response to the presence and absence of a social cue (recorded acoustic sexual signals of other males) and a non-social physical cue (mechanical shaking). I found that going from a silent to an acoustic environment resulted in decreased boldness, exploration and activity, while changing from the acoustic to the silent environment led to increases in boldness and activity. Such effects were far less pronounced in response to changes in a physical disturbance cue. While changes in both the social and the non-social environment had effects on correlations between and the repeatability of some of the behaviours measured, the effects of social cues were considerably greater. The notion that social environmental effects may be more important drivers of rapid coevolutionary dynamics than non-social effects was supported by my findings.
Using a pedigreed full-sib/half-sib breeding design, I investigated how the genetic architecture of different behaviours in light of the presence or absence of male acoustic sexual signals relates to the phenotypic expression of those behaviours. I found that the repeatabilities were similar between the two environments, but that there were significant differences in the heritabilities and evolvabilities between environments. Similarly, the phenotypic covariance matrices across environments were similar, while the genetic covariance matrices differed significantly. I also found that, for most behaviours, the repeatable aspect of behaviour (i.e. personality) was more heritable in the acoustic environment than in the silent environment.

As I have found, the effects of the social environment on behavioural expression and the underlying genetic architecture are striking, and the potential for future research in this area intriguing. I discuss the ecological and evolutionary implications of my findings within each chapter and discuss how they address gaps in our understanding of the effects of the social environment on behaviour.
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Authorship declaration

This thesis contains work that has been published, accepted for publication (in press) or prepared for publication. I have outlined the details of the work, their location in the thesis and contributions by authors and people that helped in the lab below. Both of my supervisors, LW Simmons and JL Tomkins, are co-authors on all chapters for contributing significantly to formulating ideas and methods, interpreting and analysing results and revising and improving manuscripts.

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Chapter One

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General introduction

Behaviour allows animals to respond quickly to various environmental stimuli (Relyea 2001; Levitis et al. 2009; Duckworth 2010). Consequently, many aspects of the environment can be expected to have marked impacts on behaviour. Besides the intrinsic properties of individuals such as cognitive ability (Sih and Del Giudice 2012) or metabolism (Wolf and McNamara 2012), external abiotic factors, such as light (e.g. Herberstein and Fleisch 2003) or temperature (e.g. Biro et al. 2010), and biotic factors, such as predation threats (Bell and Sih 2007; Briffa et al. 2008) or food availability (e.g. Heithaus and Dill 2002), shape the way in which behaviours are expressed. Amongst the biotic factors, different aspects of the social environment have been found to influence the behaviour of animals particularly strongly (Bailey et al. 2017). Examples include a study on zebra finches which showed that males exposed to a complex social environment (45 unfamiliar conspecifics of both sexes) sing significantly more than males exposed to a simple environment (one unfamiliar female) (Adar et al. 2008), and a study showing that guppies reared at high densities are less successful foragers than those reared at low densities (Chapman et al. 2008). The three chapters presented in this thesis investigate the effects of the social environment on the expression, repeatability, heritability and evolvability of behaviour. I chose male Australian field crickets (Teleogryllus oceanicus) as my study subjects because they are easy to maintain under laboratory conditions and exhibit suites of behaviours that have been well characterised previously. These behaviours include the emergence from a shelter after a disturbance (‘boldness’), movement in a novel, open environment (‘exploration’) and general activity (e.g. Hedrick 2000; Kortet and Hedrick 2007; Niemelä et al. 2012a).

Individuals in a population or group often differ consistently from each other behaviourally across time and/or situations (Sih et al. 2004a). This phenomenon is often called ‘animal personality’. Statistically, such consistent between-individual differences are quantified using a measure called ‘repeatability’. This measure ranges from 0 to 1, and it increases as the difference between individuals increases and within-individual variance decreases (Nakagawa and Schielzeth 2010). Animal personalities are well documented in the literature and have been found in various species across different phyla (Sinn and Moltschaniwskyj 2005; Wilson et al. 2009; e.g. Bókony et al. 2012; Rudin and Briffa 2012). Between-individual correlations in different behaviours may also exist, a phenomenon sometimes termed ‘behavioural syndromes’ (Sih et al. 2004a; Bell 2007). Such correlations may have critical ecological and evolutionary implications as they suggest that certain behaviours may be linked in some way. Thus, different behaviours may not be expressed and evolve independently, but together as suites of traits (Price and Langen 1992; Sih et al. 2004a), and this can lead to seemingly maladaptive
behaviour (Sih et al. 2004a; Stamps 2007; Smith and Blumstein 2008). One social interaction that has been shown to affect personality traits is dominance status. While dominant individuals appear to be bolder, more explorative and/or active (e.g. Colléter and Brown 2011; Dahlbom et al. 2011) than their subordinate counterparts in general, this is not always the case (Katzir 1983; Dingemanse and de Goede 2004). Dominance status is a relative condition and we currently lack understanding of how fluctuations in dominance status might impact within and among-individual behavioural variation. The first chapter of this thesis describes an experiment in which I manipulated the dominance status of individuals by subjecting them to several rounds of agonistic encounters. The objective was to determine the degree to which dominance status, and changes therein, affect the expression of (both behavioural plasticity and behavioural consistency) and correlations between behaviours. These are aspects of behaviour that have remained largely unexplored.

Besides the social environment, other exogenous factors such as predation risk can shape between-individual variation in behaviour (Sih et al. 2003; e.g. Bell and Sih 2007). Similar to the effects of the social environment, we can expect behavioural plasticity in light of predation risks. However, plasticity in response to such risks may be more costly because of the often lethal outcome of incorrect decisions. Consequently, plasticity may be less pronounced and behaviour optimised to maximise survival in different environments. Additionally, it has been suggested that indirect genetic effects (IGEs) arise when individuals interact with conspecifics in ways that influence their own behaviour (interacting phenotypes; Moore et al. 2009). This means that the genes of interacting individuals affect trait expression in each other (Wolf et al. 1998; Moore et al. 2009). These findings lead to the hypothesis that variation in the social environment should have greater effects on the expression of, and correlations between, behaviours than would non-social environmental cues. This hypothesis forms the basis for the second chapter of this thesis. Here my aim was to further our understanding of the effects relatively short-term environmental variation on behavioural plasticity, correlations among behaviours and behavioural repeatability by investigating the effects of different environmental cues (social vs. non-social) within the same experimental framework. I manipulated two aspects of the environment, the social environment via recorded male acoustic sexual signals and the physical environment via mechanical shaking.

In the third chapter, I expand upon Chapters One and Two by going beyond phenotypic behavioural measurements, and look at quantitative genetic variation and covariation in personality traits under differing social environments. Phenotypic measurements are often used to draw evolutionary inferences because quantitative genetic experimental designs require large sample sizes and/or time
investments. Additionally there is an assumption within evolutionary ecology which states that phenotypic patterns should be indicative of underlying genetic patterns these hypotheses are known as the ‘phenotypic gambit’ (Grafen 1984) or ‘Cheverud’s conjecture’ (Roff 1995). However, it has been suggested that evolutionary trajectories can be affected by IGEs (McGlothlin et al. 2010; Drown and Wade 2014) and we would consequently expect heritabilities and evolvabilities to differ between social environments. If such differences exist on a genetic level but not (or to a lesser degree) on a phenotypic level, the validity of the phenotypic gambit or Cheverud’s conjecture would have to be questioned. Using a combined full-sib/half-sib and pedigree breeding design, I investigated how variation in the social environment (presence vs. absence of male acoustic sexual signals) affects cricket behaviour on the phenotypic level (plasticity, repeatability and correlations) and on the genetic level (heritability, evolvability and genetic correlations). Additionally, because I was able to estimate both the repeatability and the heritability of behaviour, I could employ an approach suggested by Dochtermann et al. (Dochtermann et al. 2014) to assess the relative contribution of genetic variation to personality variation. Little is known about this aspect of behaviour (Dochtermann et al. 2014; Petelle et al. 2015; Edwards et al. 2017) and my hope was to make a contribution to the field using this extensive quantitative genetic dataset.

By employing a number of novel or underused approaches, the aim of this thesis was to address gaps in our knowledge about the impacts that the social environment can have on behaviour, both in terms of phenotypic and genetic patterns. I discuss the implications of my findings from an ecological and evolutionary perspective, I summarize my findings in a general discussion, and make suggestions as to how future research could build upon this research.
Chapter One

Changes in dominance status erode personality and behavioural syndromes
1.1 Abstract

The interplay between consistent individual differences in behaviour (i.e. animal personality) and behavioural plasticity has recently attracted increased interest. We used male Australian field crickets (*Teleogryllus oceanicus*) to investigate how dominance status influences the consistency and plasticity of different personality traits, namely boldness, exploration and activity, by experimentally manipulating dominance status between measuring sessions. We found that dominants that became subordinate when socially challenged, shifted their behaviour, becoming less bold, explorative and active, while subordinates that became dominant, became bolder, more explorative and more active. Individuals that experienced no change in dominance status did not alter their behaviour. Changes in dominance status reduced the repeatability of the putative personality traits of exploration and activity while not affecting the repeatability of boldness. Moreover, changes in dominance status affected the presence of correlations between some personality traits, but not others. Finally, calling behaviour was related to current and future dominance and explorative tendencies. We discuss the broader evolutionary and ecological implications of our findings and propose that changes in social status should be considered when investigating behavioural syndromes and the interplay between animal personality and behavioural plasticity.
1.2 Introduction

Individuals within a group or population often differ consistently from one another behaviourally, and exhibit within-individual stability (i.e. repeatability) of behaviours over time and/or across situations (Sih et al. 2004a). Different terms have been used to describe these consistent between-individual behavioural differences, including temperament, tendencies, strategies, or coping styles, and are comparable to so called personality types in humans (Pervin and John 1999). Therefore the term ‘animal personality’ (or simply ‘personality’) is now commonly used when describing consistent, repeatable behaviours in animals. The presence of personalities is well established in the literature and has been observed in many phyla, including chordata (e.g. Bókony et al. 2012), arthropoda (e.g. Wilson et al. 2010), cnidaria (e.g. Rudin and Briffa 2012), and mollusca (e.g. Sinn and Moltschaniwskyj 2005). Individuals can exhibit a range of behaviours which lie somewhere on a continuum of possible response levels along various axes such as shyness-boldness, exploration-avoidance, or active-docile, leading to ‘behavioural types’ (Bell 2007). Among-individual correlations in behaviours across contexts (functional behavioural categories such as mating, exploration, antipredator or contest contexts) may also exist, leading to ‘behavioural syndromes’ (Sih et al. 2004b; Bell 2007). This has critical evolutionary and ecological implications as behaviour in one context may be linked to the way an individual behaves in another. In a landmark study, Huntingford (1976) found that boldness towards a predator was correlated positively with the degree to which male three-spined sticklebacks (*Gasterosteus aculeatus*) are territorially aggressive. More recently, Sih et al. (2003) found that the time streamside salamander (*Ambystoma barbouri*) larvae spent outside a refuge in the absence of a predator cue was positively correlated with time spent outside a refuge in the presence of the cue. Wilson et al. (2010) found positive correlations across mating, exploration and antipredatory behaviours in European house crickets (*Acheta domesticus*) while Kortet and Hedrick (2007) found that intrasexual aggression in field crickets (*Gryllus integer*) was correlated with activity in a novel environment.

The existence of such behavioural correlations among individuals suggests that behaviours in different contexts may not evolve independently, but as suites (Price and Langen 1992; Sih et al. 2004a). Behavioural syndromes may therefore impose constraints on the evolvability of individual behaviours if those behaviours are underpinned by genetic correlations, as selection on one behaviour in a certain context may affect selection on other behaviours in other contexts. Indeed, a meta-analysis of additive-genetic variance-covariance matrices found that behavioural syndromes potentially constrain evolutionary responses by an average of 33% (as opposed to 13-18% for life-history or morphological syndromes, Dochtermann and Dingemanse 2013). Behaviour is often viewed as a flexible phenotypic trait that allows individuals to react quickly to environmental stimuli (e.g. Relyea 2001; Duckworth 2010). However, the existence of behavioural syndromes may limit behavioural
plasticity to some degree, resulting in individuals only expressing a subset of the full range of behaviours present in a population. Additionally, behaviours that seem maladaptive may arise because individuals may not always be able to react optimally in different contexts as a consequence of genetic correlations. For example, several studies have linked bold, proactive behaviour to the ability of individuals to sire more offspring and gain better access to resources but such behaviours may also result in increased predation risk and lowered survival (Sih et al. 2004b; Stamps 2007; Smith and Blumstein 2008). The interplay between consistent between-individual behavioural differences and behavioural plasticity is attracting increased interest (e.g. Dingemanse et al. 2010; Mathot et al. 2011) but is yet to be fully understood and is the central focus of this study.

Social interactions appear to be important environmental stimuli that shape personality in many species (McNamara et al. 2009; Dahlbom et al. 2011). Here, we focus on one such interaction, namely dominance status. The relationship between dominance and personality traits such as exploratory behaviour or boldness is complex. Generally, dominant individuals appear to be more explorative and/or bold (e.g. Colléter and Brown 2011; Dahlbom et al. 2011), but this is not always the case. In great tits (Parus major), dominant territorial males explore novel environments more quickly than do subordinate males, but this is reversed for non-territorial juveniles (Dingemanse and de Goede 2004). In jackdaws (Corvus monedula), dominant individuals were less likely to enter a novel area (i.e. less bold) than subordinate individuals (Katzir 1983). The use of different foraging tactics has been suggested as an explanation for such observations (Giraldeau and Beauchamp 1999). For example in some species with strict dominance hierarchies, dominant foragers are able to use the scrounger tactic whilst relying on subordinates to find resources (e.g. Zanette and Ratcliffe 1994).

Here, we examine both behavioural plasticity and animal personality by investigating the way in which dominance status affects between-individual behavioural differences in the Australian field cricket Teleogryllus oceanicus. This species provides a good model because field crickets have previously been used in studies investigating personalities and behavioural syndromes, by measuring behaviours such as emergence from a shelter after a disturbance, activity and movement in novel environments, and
aggression towards conspecifics (e.g. Hedrick 2000; Kortet and Hedrick 2007; Niemelä et al. 2012b). Furthermore, it has been shown that short-term changes in dominance status (dominant males losing contests to other dominants) cause dominant *T. oceanicus* males to change their cuticular hydrocarbon profile to more closely resemble subordinate males (Thomas and Simmons 2011). Thus, we examine how changes in dominance status across measuring sessions affect both behavioural plasticity and consistency in between-individual behavioural differences in the short term (e.g. Figure 1). Finally, we use our data to explore the presence of correlations between different personality traits, thus implying the existence of behavioural syndromes.

Figure 1. Simplified representation of the interplay between behavioral plasticity and consistent between-individual behavioral differences (adapted from: Briffa et al. 2008 and Dingemanse et al. 2010). Grey bars represent average measures of behaviors from four individuals and each individual is represented by a line. Here, behaviors are measured across two time-points/social situations/environments (A and B). (a) and (b): Absence of behavioral plasticity as average population response levels across A and B are similar. (c) and (d): Presence of behavioral plasticity as average population response levels change across A and B. (a) and (c): Consistent between-individual differences across A and B, i.e. the rank order between individuals is maintained. (b) and (d): No or little consistency in between-individual differences between A and B, i.e. disruption of rank order between individuals. The strength of animal personalities can be assessed using tests of behavioral consistency such as Pearson’s product-moment correlation (e.g. Johnson and Sih 2007) or the intraclass correlation coefficient (Nakagawa and Schielzeth 2010). Here, (a) and (c) would indicate strong personalities, while (b) and (d) would indicate the absence of or at most the presence of weak personalities.
1.3 Methods

1.3.1 Study population
Experimental animals were from a large (>500) laboratory stock population derived from Carnarvon (Western Australia), with freshly collected individuals from the field added annually. The stock is maintained in a temperature controlled room (26°C) with a 12:12 h light:dark cycle. Food and water were provided ad libitum. Only males were used in these experiments. Males were separated into individual plastic containers (7 × 7 × 5 cm) at the 3rd or 4th instar stage. After their final molt, individuals were given 14 days before being used in experiments to ensure they were sexually mature and would thus display aggressive intra-sexual behaviour. Behavioural experiments were conducted in a temperature controlled room (26°C) under dimmed red light to ensure maximal activity levels and minimal disturbance from the observer.

1.3.2 Initial dominance rank
Male fighting ability was determined using methods similar to those described in Shackleton et al. (2005) and Thomas & Simmons (2009a). Blocks of eight males each (58 blocks in total or 464 individuals) were established so that the age difference (days past adult eclosion) was ± 2 days. In each block, male dominance was assessed in three rounds of fights. One cricket was placed on either side of a cardboard divider placed in the middle of a plastic container (16 × 10 × 9 cm) filled with ca. 0.5 cm of sand. Crickets were then given 1 min to settle before removing the divider which initiated aggressive behaviours. The contest was considered lost by the individual that displayed avoidance behaviour towards the other (Thomas and Simmons 2009a). The first round of contests resulted in four winners (W) and four losers (L) within each block. Winning males and losing males were thus paired in three successive bouts – yielding 8 males with different W-L profiles: WWW, WWL, WLW, WLL, LLL, LLW, LWL, and LWW. Only winners or losers of three consecutive contests (i.e. WWW and LLL) were chosen for the behavioural trials to ensure maximal difference in fighting ability between individuals. Henceforth, WWW individuals will be referred to as dominant (D) and LLL individuals as subordinate (S). Each behavioural trial ran for 3 min and individuals were identified by being marked with acrylic paint.

1.3.3 Behavioural trials
Experimental setup (Figure 2) for the behavioural trials consisted of two shelters cut from PVC pipe (height: 8.5 cm; diameter: 8 cm) located in opposite corners of a 31 cm deep plastic trough (38 × 52 cm at top, 32 × 46 cm at base). One of the shelters was fitted with a movable door that could be
opened from outside the trough by pulling a piece of string. The 2\textsuperscript{nd} shelter had a similar, but constantly open door. The base of the trough was covered with fine sand (approx. 2 cm deep).

A video camera (Panasonic WV-CL930), connected to a desktop PC, was installed 80 cm above the base of the arena to film the crickets while in the arena. EthoVision v8.5 was used to track and analyze cricket movement. In EthoVision, three areas within the arena were defined in order to assess different behaviours: ‘near’ was defined as the area within a 9 cm radius of the shelter in which the cricket was initially placed; ‘far’ was defined as the area within a 9 cm radius of the 2\textsuperscript{nd} shelter, and ‘middle’ was defined as the area between ‘near’ and ‘far’ (see Figure 2). EthoVision enabled the quantification of the following behaviours: latency to emerge from the shelter, initiated once the whole body of the cricket was outside the shelter; distance moved within the arena; time spent within the arena generally as well as each pre-defined area; the latency to reach the farthest area from the

\textbf{Figure 2.} Diagram of arena used for behavioural trials.
shelter; the average velocity of the cricket while outside the shelter; the time spent moving, defined as any movement >0.3 cm/s; the proportion of time spent moving >0.3 cm/s versus not (or very slowly) moving (<0.3 cm/s). Additionally, we recorded whether or not crickets produced calling song at any point during the trials.

Behavioural trials were initiated immediately after determining individual dominance status. Prior to the onset of each trial, an individual was placed inside the shelter with the movable door closed. The shelter was then tapped for 10 s using a plastic rod to startle the crickets. Thirty seconds after tapping stopped, the shelter door was carefully opened, marking the onset of the trial. Individuals were allowed 10 min to emerge from the refuge. If a cricket did not emerge after 10 minutes, the trial was considered concluded for that individual. For crickets that did emerge, EthoVision started tracking their movement on emergence. Tracking was terminated 10 min after crickets first emerged from the shelter. Time spent inside shelters after crickets exited the first shelter for the first time was not included in the analysis because it is the inverse of the time spent in the arena. Crickets rarely entered the 2nd shelter (49 out of 232 trials) and if they did, only for short amounts of time (average of 12.6 seconds).

1.3.4 Social challenge
After obtaining initial dominance status and quantifying behaviour in the arena, males underwent a social challenge in which dominant (D) individuals fought against a dominant individual from another block, while subordinate (S) individuals fought against a subordinate individual. This resulted in half of the dominants remaining dominant (DD), while the other half switched to being subordinate (DS). Likewise, half of the subordinate individuals after the initial round of fights remained subordinate (SS) while the other half switched to being dominant (SD). Behaviour in the arena was then re-measured approx. 15 – 25 min after social challenge as described previously.

1.3.5 Statistical analyses
Behavioural measures were grouped into different behavioural categories as follows: ‘Boldness’ was represented by only one measure: latency to emerge from shelter following disturbance (hereafter referred to as boldness). Boldness, generally defined as an individual’s response to a risky situation (Carter et al. 2013), is sometimes equated to antipredatory behaviour in the literature (e.g. Wilson et al. 2010). Here, we refer to the reaction of crickets to a disturbance as boldness (and not antipredatory behaviour) because we cannot be certain that this stimulus is perceived as a predator cue. All other behavioural measures were taken after crickets had left the shelter and were in an open arena. A principal components analysis (PCA) was performed based on the correlation matrix of the
behavioural measures recorded for each individual in both trials (before and after social challenge). The axes of variation were not rotated, and scores on axes with eigenvalues >1 were extracted (Table 1). This resulted in 2 principal components. Distance moved, latency to ‘far’, time spent ‘middle’, time spent ‘far’, average velocity and time spent moving all loaded most strongly onto the first principal component (PC1) which will be considered as a measure of ‘activity’ henceforth. Time ‘near’ and the proportion of moving vs. not moving loaded most strongly on the second principal component (PC2) and will be considered as a measure of ‘exploration’. Since a high score on PC2 equates to more time

<table>
<thead>
<tr>
<th></th>
<th>PC1</th>
<th>PC2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eigenvalue</td>
<td>3.75</td>
<td>1.67</td>
</tr>
<tr>
<td>Variance explained</td>
<td>40.62</td>
<td>18.48</td>
</tr>
<tr>
<td>Distance moved</td>
<td>0.549</td>
<td>0.031</td>
</tr>
<tr>
<td>Latency to ‘far’</td>
<td>-0.532</td>
<td>0.364</td>
</tr>
<tr>
<td>Total time in arena</td>
<td>0.873</td>
<td>0.436</td>
</tr>
<tr>
<td>Time ‘near’</td>
<td>0.468</td>
<td>0.677</td>
</tr>
<tr>
<td>Time ‘middle’</td>
<td>0.493</td>
<td>0.147</td>
</tr>
<tr>
<td>Time ‘far’</td>
<td>0.688</td>
<td>-0.041</td>
</tr>
<tr>
<td>Velocity</td>
<td>0.417</td>
<td>0.031</td>
</tr>
<tr>
<td>Time spent moving</td>
<td>0.917</td>
<td>-0.220</td>
</tr>
<tr>
<td>Moving/not moving</td>
<td>0.528</td>
<td>-0.714</td>
</tr>
</tbody>
</table>

‘near’ and a lower proportion of time spent moving (Table 1), the score indicates how ‘unexplorative’ a cricket is. We therefore reverse signed PC2 to ease interpretation.

A total of 116 crickets (58 dominants and 58 subordinates) were used and tested in the arena twice (before and after social challenge, resulting in individuals that were DD, DS, SS or SD), yielding 232 trials. Crickets failed to emerge from their shelter in 17 out of 232 trials (7.3%), 9 of them before and 8 after the social challenge. For boldness, all crickets were included in the statistical analyses and those that did not emerge were assigned the maximum time allowed for emergence (600 seconds). Individuals were considered missing values if they did not emerge from the shelter or failed to express a behaviour within ‘exploration’ or ‘activity’ (e.g. failed to move to the ‘far’ area of the arena). This was the case in 55 out of 232 (25.4%) trials (28 before and 27 after the social challenge). Consequently, sample size was lower for exploration and activity (177 overall, 88 before and 89 after the social
challenge) than for boldness (232, 116 before and 116 after the social challenge). Sample sizes of exploration and activity were further reduced whenever differentials between before and after measures were used in the analyses. A differential could only be obtained if a cricket emerged and expressed all behaviours within ‘exploration’ and ‘activity’ both before and after the social challenge. Whenever differentials were used, exploration and activity sample size was 75.

Behavioural differences between dominants and subordinates after the initial dominance ranking were assessed using a series of independent samples t-tests. To investigate the effects of dominance profile after the social challenge (DD, DS, SS and SD) on individual behaviours, we ran univariate GLMs for each behavioural trait (latency to emerge for boldness and principal component scores for exploration and activity). For boldness, the dependent variable was determined by calculating the difference between the latency to emerge before and after the social challenge (after minus before). For exploration and activity the difference was calculated in the same manner using the principal component scores. The dominance profile was defined as the fixed factor and size as the covariate.

Post-hoc comparisons using Fisher’s least significant difference were used to determine whether behaviours differed significantly when comparing individuals that did not switch dominance status (DD and SS respectively) to individuals that experienced a change in dominance status (DS and SD respectively). Equality of variance assumptions were met.

Between-individual correlations across behaviours (i.e., behavioural syndromes), were assessed using nonparametric Spearman’s rank correlation tests ($r_s$) between boldness and activity (PC1) as well as boldness and exploration (PC2) (following suggestions and examples in: Huntingford 1976; Bell 2007). Correlations between activity and exploration cannot be assessed because the PCs that describe these traits are by definition uncorrelated. Before and after social challenge measures were assessed separately, with the after measures split into individuals that did not change dominance status and individuals that did. Fisher r-to-z transformation was used to assess the significance of the difference between the correlation coefficients for individuals that did not change dominance and those that did.

The repeatability (or intraclass correlation coefficient [ICC]) of behaviours before and after the social challenge were calculated from the variance components obtained from linear mixed models using the $R$ package rptR (Nakagawa and Schielzeth 2010). Repeatability of behaviours includes both intra-individual correlations as well as inter-individual variation. An increase in intra-individual correlation and/or inter-individual variation will result in higher repeatability. Individual identities were treated as factorial predictors and thus the mixed model derived ICCs that explain the proportion of total variance accounted for by differences among individuals (Nakagawa and Schielzeth 2010). To compare repeatabilities of individuals that experienced changes in dominance status with individuals that did not, we calculated the 84% confidence intervals around repeatability estimates within the different
behavioural traits. We used 84% confidence intervals because two non-overlapping 95% confidence intervals may not be significantly different at the 0.05 level. When adjusting the confidence levels to 1.39 times the standard errors (SEs), or 84%, the 0.05 significance level can be visualized by the non-overlap criterion (Goldstein and Healy 1995).

To further explore the effect of dominance on behavioural variation we fitted mixed effects models with only random intercepts for individual identity and compared the variance components to a model with dominance fitted as a fixed effect. The amount of among individual differences explained by dominance status was then represented by the reduction in among-individual variance. Similarly, we were able to estimate the amount of within-individual variation explained by dominance status by looking at the residual variances.

1.3.6 Size measures
An estimate of body size was obtained by transferring stills from the video footage to ImageJ 1.49v and measuring the length of each cricket twice. A high degree of repeatability between the two measures was found (average measure ICC: 0.997; $F_{115,115} = 301.71, P < 0.001$) and the average of the two measures was therefore used as the indicator of individual size. Mean ± SE length of all individuals was 3.39 ± 0.02 cm.

1.4 Results

1.4.1 Dominants vs. subordinates before social challenge
Most males (107 of 116 or 92%) emerged from the shelter within 10 min. While size did not differ significantly between dominant and subordinate males, there were significant differences in latency to emerge between dominants and subordinates, with dominants emerging more quickly than subordinates. Additionally, dominants being more explorative and active than subordinates (Figure 3).

1.4.2 Changes in behaviour following a social challenge
There were significant changes in behaviour following a social challenge that depended on the outcome of that challenge (Figure 4). Post-hoc analyses revealed that in all cases, behaviours of individuals that did not experience a change in dominance status (DD and SS) were unaltered after the social challenge while those that switched dominance status (DS and SD) altered their behaviours after the social challenge (Figure 4). A change from subordinate to dominant (SD) resulted in reduced time to emerge from the shelter and increased explorative and active behaviours following the social challenge. A change from dominant to subordinate (DS) resulted in increased time to emerge and
decreased explorative effort and activity following the social challenge. Size had no significant effect in any of these models.

For boldness, exploration and activity, the amount of between-individual variation explained by dominance status was 4.38%, 15.24% and 12.87% respectively while the amount of within-individual variation explained by dominance status was 1.81%, 5.90% and 6.76% respectively.

Figure 3. Differences between dominant (D) and subordinate (S) males before the social challenge using independent samples t-tests. (a) Dominants were no larger ($t_{114} = 1.11; P = 0.27$); but were (b) significantly bolder ($t_{114} = -2.56; P = 0.014$), (c) more active ($t_{86} = 6.125; P < 0.001$) and (d) more explorative ($t_{86} = -2.820; P = 0.006$) than subordinates. The bottom and top of the box represent the first (Q1) and third quantiles (Q3) of the data, respectively (interquartile region, IQR). The horizontal line within the box represents the median. The whiskers end at the largest and smallest non-outliers. Outliers (1.5 x IQR above Q1 and below Q3) are represented by dots.
1.4.3 Calling behaviour

Dominant crickets were significantly more likely to produce calling song during trials prior to the social challenge than were subordinates. A total of 12 out of 116 males called, 10 of which were dominant (chi-square test: $\chi^2 = 5.949$, $df = 1$, $N = 116$, $P = 0.015$). Crickets that called during initial behavioural trials were more likely to be dominant after social challenges than silent crickets (logistic regression: $\chi^2 = 8.608$, $df = 1$, $P = 0.003$). Of the behavioural measures, only explorative tendencies before the social challenge had a significant effect on calling behaviour (logistic regression: $\chi^2 = 4.747$, $df = 1$, $P = 0.026$), indicating that crickets that called during initial behavioural trials were also more explorative during those trials than crickets that did not call. Size did not significantly affect the probability of calling (logistic regression: $\chi^2 = 2.348$, $df = 1$, $P = 0.167$).

1.4.4 Correlations across behaviours

Correlations are summarized in Table 2. We found significant correlations between all three behavioural traits of behavioural variation before the social challenge: boldness (latency to emerge) significantly correlated with both exploration and activity. For individuals that did not change dominance status after the social challenge boldness again correlated with both exploration and activity (Table 2). However, for individuals that changed dominance status after the social challenge, only boldness and activity were still correlated, while boldness and exploration were not (Table 2). Furthermore correlations between boldness and exploration differed significantly when comparing individuals that changed dominance to those that did not (Table 2).

1.4.5 Repeatability

Repeatabilities for behavioural traits pooled across all social challenge outcomes and for the four possible status outcomes (DD, DS, SS and SD) are summarized in Table 3. For boldness, the 84% confidence intervals around the repeatability for individuals that did not change dominance status during the social challenge overlapped with the confidence intervals for individuals that did change dominance status and were thus not significantly different. For exploration, the confidence intervals for DD and SS individuals did not overlap with the confidence intervals for either DS or SD and were therefore significantly different. Likewise, the confidence intervals for DD and SS individuals did not overlap with the confidence intervals of either DS or SD in the activity scores and were therefore significantly different.
Figure 4. Differences in behaviour after social challenge (behavioural score after minus before) for boldness (latency to emerge), exploration and activity. General linear models were significant for all 3 traits (boldness: $F_{3, 115} = 4.374$, $P = 0.006$; activity: $F_{3, 74} = 8.827$, $P < 0.001$; exploration: $F_{3, 74} = 6.450$, $P = 0.001$). Size had no significant effect on either behavioural trait (boldness: $F_{1, 115} = 0.026$, $P = 0.873$; activity: $F_{1, 74} = 0.393$, $P = 0.533$; exploration: $F_{1, 74} = 0.537$, $P = 0.697$). Individuals that did not undergo a dominance status change after a social challenge (DD and SS) did not alter their behaviour over time. Individuals that underwent a dominance status change (DS and SD) shifted their behaviour after the social challenge. Individuals that changed from dominant to subordinate (DS) had significantly increased latency to emerge and decreased explorative effort and activity following the social challenge. Individuals that changed from subordinate to dominant (SD) emerged significantly quicker and were more explorative and active following the social challenge. The bottom and top of the box represent the first (Q1) and third quantiles (Q3) of the data, respectively (interquartile region, IQR). The horizontal line within the box represents the median. The whiskers end at the largest and smallest non-outliers. Outliers (1.5 x IQR above Q1 and below Q3) are represented by dots.
Table 2. Spearman correlations ($r_s \pm SE$) and their significance between the 3 behavioural traits (boldness, exploration and activity), both before and after the social challenge (SC). The correlations after the social challenge were split into individuals that did change dominance status and those that did not; these correlations were compared using a z-test. The 95% confidence intervals around the correlations are also provided.

<table>
<thead>
<tr>
<th>Behavioural traits</th>
<th>$r_s$ before SC</th>
<th>$P$</th>
<th>$r_s$ after SC; no change</th>
<th>$P$</th>
<th>$r_s$ after SC; change</th>
<th>$P$</th>
<th>$z$ between change and no change</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boldness – activity</td>
<td>-0.587 ± 0.063</td>
<td>&lt; 0.001</td>
<td>-0.487 ± 0.173</td>
<td>0.001</td>
<td>-0.404 ± 0.119</td>
<td>0.007</td>
<td>0.47</td>
<td>0.63 (NS)</td>
</tr>
<tr>
<td></td>
<td>[-0.720, -0.374]</td>
<td></td>
<td>[-0.729, -0.191]</td>
<td></td>
<td>[-0.650, -0.183]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boldness – exploration</td>
<td>-0.428 ± 0.094</td>
<td>&lt; 0.001</td>
<td>-0.450 ± 0.139</td>
<td>0.002</td>
<td>0.176 ± 0.151</td>
<td>0.206 (NS)</td>
<td>3</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>[-0.635, -0.207]</td>
<td></td>
<td>[-0.693, -0.146]</td>
<td></td>
<td>[-0.142, 0.464]</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Repeatabilities (mean ± SE) and their significance for boldness, exploration and activity pooled across all social challenge outcomes and for different subsets (DD and SS i.e. no change in dominance status; DS and SD i.e. change in dominance status). The 84% confidence intervals around repeatabilities are also provided.

<table>
<thead>
<tr>
<th>Behavioural trait</th>
<th>Pooled [84% CIs]</th>
<th>DD [84% CIs]</th>
<th>SS [84% CIs]</th>
<th>DS [84% CIs]</th>
<th>SD [84% CIs]</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boldness</td>
<td>0.518 ± 0.068</td>
<td>&lt;0.001</td>
<td>0.607 ± 0.117</td>
<td>&lt;0.001</td>
<td>0.567 ± 0.132</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>[0.374, 0.647]</td>
<td>[0.331, 0.791]</td>
<td>[0.273, 0.762]</td>
<td>[0.184, 0.612]</td>
<td>[0.207, 0.637]</td>
<td></td>
</tr>
<tr>
<td>Exploration</td>
<td>0.439 ± 0.078</td>
<td>&lt;0.001</td>
<td>0.616 ± 0.154</td>
<td>&lt;0.001</td>
<td>0.606 ± 0.134</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>[0.213, 0.576]</td>
<td>[0.368, 0.782]</td>
<td>[0.385, 0.753]</td>
<td>[0.0, 0.307]</td>
<td>[0, 0.271]</td>
<td></td>
</tr>
<tr>
<td>Activity</td>
<td>0.471 ± 0.084</td>
<td>&lt;0.001</td>
<td>0.657 ± 0.101</td>
<td>&lt;0.001</td>
<td>0.645 ± 0.126</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>[0.249, 0.589]</td>
<td>[0.421, 0.736]</td>
<td>[0.430, 0.719]</td>
<td>[0, 0.286]</td>
<td>[0, 0.352]</td>
<td></td>
</tr>
</tbody>
</table>
1.5 Discussion
Here we investigated the effects of dominance status on three different behavioural traits (boldness, exploration and activity) using male field crickets (*Teleogryllus oceanicus*). We looked at short-term behavioural plasticity and rank-order consistency in light of dominance status changes resulting from individuals being socially challenged. Furthermore, we assessed the degree to which between-individual behavioural correlations were present (i.e. behavioural syndromes).

1.5.1 The effect of dominance before a social challenge
Dominants and subordinates differed significantly for all 3 behavioural traits before undergoing the social challenge, i.e. dominant individuals were quicker to emerge from a shelter following a disturbance and they were also more explorative and active in an open environment. A possible explanation for such links between dominance status and boldness, exploration and activity are winner and loser effects. Also called the ‘confidence’ effect (Barnard and Burk 1979), this pertains to the fact that individuals that lose an agonistic encounter are less likely to initiate and more likely to lose subsequent encounters while winners are more likely to both initiate and win subsequent encounters (Simmons 1986). In *T. oceanicus*, for example, the probability of winning a fight after winning five consecutive fights is 87%, while the probability of winning after five consecutive losses is only 18% (Burk 1983). Winner effects may persist for several hours (e.g. 1-6 h in the cricket *Gryllus bimaculatus*; Khazraie and Campan 1999) or even days (e.g. 2-6 days in the rodent *Peromyscus californicus*; Fuxjager et al. 2010), with loser effects generally persisting longer than winner effects (e.g. Kasumovic et al. 2010). Our results are in line with the general observation that socially subordinate individuals tend to behave more reactively or shy than dominant individuals who act more proactively or bold (Verbeek et al. 1996; Koolhaas et al. 1999; Øverli et al. 2004). While male dominance has been linked to fertilization success in *T. oceanicus* (e.g. Simmons 1986; Thomas and Simmons 2009a), long-term fitness may be similar for subordinate individuals that act cautiously and dominant individuals that act boldly (Hedrick and Riechert 1993; Dall et al. 2004; Dingemanse and de Goede 2004) because of the potential costs of bold behaviour. Additionally, it has been found that subordinate male *T. oceanicus* change their cuticular hydrocarbon profile to become more attractive to females, possibly increasing their mating success (Thomas and Simmons 2009a). This further supports the notion that male reproductive success and lifetime fitness are influenced by the interaction of different traits (behavioural or otherwise) in this species.

Dominant crickets were more likely to call than subordinates during the first behavioural trial and crickets that called during the first behavioural trial were more likely to win their social challenge, either becoming or remaining dominant. This is in line with studies showing calling effort to be
positively correlated with competitive ability (e.g. Simmons 1986; Favati et al. 2014). Furthermore, explorative effort (but not boldness or activity) was positively related to calling effort. If calling males have increased reproductive success (as is the case in the field cricket Teleogryllus commodus; Bentsen et al. 2006) dominance and exploration may both be subject to sexual selection.

1.5.2 Behavioural syndromes

When behaviours are inherently linked (for example hormonally or genetically), they can form a behavioural syndrome. For example, Wilson and Whattam (2010) found positive correlations between mating, exploratory and antipredatory behaviour in the European house cricket *Acheta domestica*. While limited behavioural plasticity resulting from behavioural syndromes may constrain the evolvability of certain behaviours (Dochtermann and Dingemanse 2013), natural selection may still favour these limits as it may be costly to produce and maintain behavioural plasticity (e.g. Dewitt et al. 1998). Thus, behavioural syndromes may be able to explain potentially maladaptive or suboptimal behaviours. In this study, we found correlations between boldness, exploration and activity before the social challenge, implying a behavioural syndrome across these behaviours; individuals that emerged quickly after disturbance were more exploratory and active in an open environment. However, we also found that the social challenge reduced some of these correlations for individuals that experienced a change in dominance status. The fact that boldness and activity remained correlated in these individuals implies that this syndrome is unaffected by such social interactions and that the underlying links between these behaviours are strong. The boldness – exploration correlations were reduced in magnitude when dominance changed, despite appearing to be strong before the social challenge or when status did not change during a challenge. Hence short-term social interactions such as dominance status changes can relax apparently strong syndromes. This is of particular significance since syndromes are imputed to have such strong effects on constraining the evolution of behaviour (Dochtermann and Dingemanse 2013) and the evolvability of certain behaviours may therefore be underestimated when excluding social interactions such as changes in dominance status.

1.5.3 The effects of social challenge on behaviour

The way in which individuals altered their behaviour in response to a social challenge depended on whether or not individuals experienced a change in dominance status. Crickets that did not experience a change in dominance (DD, SS) did not alter their behaviour in response to the challenge. Conversely, crickets that did experience a change in dominance status significantly altered their behaviour in response to the challenge. Interestingly, the behavioural differences between dominants, regardless of their previous status, were negligible after the social challenge and the same pattern was observed
in subordinates. The winner/loser effect may again provide an adequate explanation for our findings as individuals that lose a contest may, at least for some time, avoid encounters with other males while winners may become more ‘confident’. Indeed, winning has been found to induce hyper-aggression transiently in the cricket *G. bimaculatus* via activation of the octopaminergic system (Rillich and Stevenson 2011). Such behavioural changes in response to the outcome of contests may indeed be adaptive. Losers may alleviate potential costs of encountering other males or predators by acting more cautiously, thus increasing their long-term fitness. Winners, on the other hand, may increase their short-term reproductive and/or foraging success by behaving less cautiously at the risk of predation or multiple, costly encounters with other males. Interestingly, Thomas and Simmons (2011) showed that dominant male *T. oceanicus* also change their cuticular hydrocarbon profile to more closely resemble subordinates after losing contests to other dominant males, and suggested that males may switch signalling modalities to avoid encounters with dominant males. Our study is the first, to our knowledge, to document short-term behavioural plasticity in response to changes in dominance status. We encourage further research into the way in which changes in dominance status, or other expressions of internal state, affect levels of behavioural expression in other species, both in the short- and long-term.

1.5.4 Repeatability

For this study, we used repeatability as a measure of the consistency of between-individual behavioural differences. Increases in both inter-individual variation and intra-individual correlation will result in higher repeatabilities. This measure therefore adequately represents the animal personality concept as it takes into account both the behavioural differences between individuals as well as the individual consistency of those behaviours. The overall repeatability of behaviours in animals was found to be 0.37 in a meta-analysis conducted by Bell et al. (2009). Here, we report repeatabilities slightly higher than the average for insects (0.36; Bell et al 2009); 0.52 for boldness, 0.44 for explorative behaviour and 0.47 for activity overall. In general, behaviours measured in the wild appear to be more repeatable than in the laboratory (Bell et al. 2009). However, the repeatabilities found here are markedly higher than recent estimates in a wild population of another field cricket, *Gryllus campestris* (Fisher et al. 2015), which were 0.06, 0.12 and 0.21 for boldness, exploration and activity respectively. One possible reason for such relatively high repeatabilities may be the short intervals of roughly 2 – 2.5 hours between observations in this study. In general, repeatability estimates tend to be higher when behaviours are measured close together in time (Bell et al. 2009).
Our results show that there was no change in behavioural expression and considerable repeatability (e.g. Figure 1a) when individuals retained their dominance status after the social challenge. However, changes in dominance status had a disruptive impact on the short-term repeatability of exploration and activity (e.g. Figure 1d; low repeatability and no change in levels of behavioural expression), but not boldness (e.g. Figure 1c; high repeatability and significant changes in levels of behavioural expression). In addition, the amount of variation in boldness explained by dominance status appeared to be lower than that of exploration and activity, both within and between individuals. The relative stability of boldness despite its correlations with the more plastic exploration and activity is intriguing. On the one hand, boldness is likely to be more heritable than explorative behaviour or activity because short term environmental effects did not reduce its repeatability; on the other there is small comfort for those hoping to adopt the ‘phenotypic gambit’ (Brommer 2013; Dochtermann et al. 2014) to estimate heritability from repeatability – since it is impossible to know which short term environmental effects may erode personality.

This study raises questions regarding the long-term effects of changes in dominance, for example how long it would take personalities of individuals that experience a status change to revert to levels similar to individuals that did not change dominance. Our results support the theoretical notion that the consistency of between-individual behavioural differences may depend on past and current social interactions. Consistent individual behavioural differences can be a result of consistent individual variation in how individuals perceive their social environment since individuals respond plastically to their perceived social environment. We suggest that social interactions, such as dominance status, need to be considered when assessing repeatabilities of behaviours (and therefore the strength and/or presence of personality) as they may be subject to fluctuations over time in response to such interactions. Ignoring social interactions may therefore lead to the inference of personality when there is none or to over-/underestimating the strength of personality.

1.5.5 Interacting phenotypes and the evolution of personalities

Little is known of the relationship between short-term changes in social status and personality traits. Here, we investigated the complex interplay between dominance status and the plasticity and consistency of different personality traits. We showed that changes in dominance status disrupt the repeatability and between-individual correlations of some behaviours. We can conclude that wherever stable social hierarchies are present, personalities and syndromes may be inflated. We therefore suggest that dominance status (and possibly other expressions of internal state) need to be considered when investigating animal personalities or using repeatabilities to infer a genetic contribution to personality traits (Dochtermann et al. 2014). Furthermore, we found differences in the
way in which changes in dominance status affected between-individual correlations of certain behaviours and suggest that such social interactions are also considered when investigating behavioural syndromes. Previous studies have often focused on dominance status at a given point in time and its relation to personality or syndromes; including changes in dominance status over time in such studies would be informative of the reliability of these estimates. The negligence of social interactions could explain the lower repeatabilities of behaviours found in some studies on wild populations (e.g. Fisher et al. 2015) as individuals may be subject to frequent changes in dominance status.

Through social interactions with conspecifics, individuals can have effects on the behaviour or level of behavioural expression of other individuals in the population (Wolf et al. 1999; interacting phenotypes; Moore et al. 2009). If those traits affect an individual’s ability to access food or mates or its fecundity, then social interactions may affect fitness and the behavioural traits in question will be subjected to selection. For example, Wilson et al. (2009) found that social behaviours in deer mice (*Peromyscus maniculatus*) emerge from interactions of multiple phenotypes and thus do not belong to any specific individual. Here, we found that boldness, exploration and activity were expressed differently depending on the outcome of social interactions. Thus, an individual’s behavioural phenotype was affected by the individuals it interacted with. Since behavioural traits such as boldness, exploration or activity can influence access to food or mates, social interactions may thus have carry-over fitness consequences for the interacting individuals and result in differential selection. Social interactions are unique in that they can be considered environmental but also genetic. The expression of genes in one individual can influence the phenotype of another, conspecific individual, a phenomenon termed an indirect genetic effect (Wolf et al. 1998). When the social environment gives rise to indirect genetic effects, such effects can provide a source of heritable variation for selection to act upon. Our study does not allow us to identify a genetic component to the social environment. However, future work should use quantitative genetic approaches to allow for a more complete understanding of the evolutionary dynamics of socially induced behavioural plasticity, and its consequences for the evolution of animal personalities.
Chapter Two

The effects of the social environment and physical disturbance on personality traits
2.1 Abstract

The environment can have a considerable impact on behaviour. The social environment is predicted to be a particularly important driver of behavioural variation and evolution through the indirect genetic effects that arise whenever individuals interact with conspecifics. We used male Australian field crickets (Teleogryllus oceanicus) to examine the effects of changes in the social environment (recorded acoustic sexual signals of other males) on the expression and consistency of boldness, activity and exploration, and their among-individual covariation. Switching from a silent environment to being exposed to male acoustic sexual signals resulted in crickets becoming less bold, active and explorative. Switching from an acoustic to a silent environment resulted in increased boldness and activity. We also looked at the effects of changes in the non-social environment via a physical disturbance that mimicked the presence of a potential predator (mechanical shaking). The effects of physical disturbance (and changes thereof) on behaviour were far less pronounced than the effects of changes in the social environment. Neither the repeatability of nor correlations between behaviours were affected by changes in physical disturbance. Only the average level of exploration was affected significantly when crickets were moved from an undisturbed to a disturbed environment, with crickets becoming less explorative. Although changes in the social and the non-social environment affected the repeatability of and correlations between some of the behaviours measured, changes in the social environment had the greater effect. We discuss the ecological and evolutionary implications of our findings and how they relate to our current understanding of social and non-social environmental effects on behaviour.
2.2 Introduction

The effect of environmental factors on animal phenotypes is well established. In particular, the behaviour of an animal can be profoundly influenced by its social environment. In honeybees, for example, brood pheromone has been found to affect the age at which workers start foraging (Le Conte et al. 2001) and in bank voles, male expenditure on the ejaculate can be affected solely by the presence of rival male pheromones in the environment (delBarco-Trillo and Ferkin 2004). Similarly, we have known for some time that different levels of predation risk affect both non-behavioural (Creel et al. 2007; Hawlena and Schmitz 2010) and behavioural traits (Werner et al. 1983; Lima and Dill 1990; Briffa et al. 2008) that serve in predator avoidance in both vertebrate and invertebrate taxa.

Investigating between-individual (animal personality) and within-individual behavioural variation (phenotypic plasticity) together (Dingemanse et al. 2010) has attracted increased interest in recent years. Different explanations for such behavioural variation have been proposed. Besides adaptations to endogenous attributes such as cognitive ability (Sih and Del Giudice 2012) and metabolism (Wolf & McNamara, 2012), between-individual behavioural variation may be shaped by exogenous factors such as predation threats (Sih et al. 2003; e.g. Bell and Sih 2007) or social environments (Montiglio, Ferrari, & Réale, 2013; Wolf & McNamara, 2013). When individuals interact with conspecifics in a way that influences their own behaviour (interacting phenotypes; Moore et al. 2009), indirect genetic effects (IGEs) are predicted to arise, where the genes of interacting individuals affect the expression of traits in each other (Moore et al., 2009; Wolf et al., 1998). A diverse range of selective pressures can therefore result from social interactions which might prove to be especially important drivers of behavioural variation (Bailey et al. 2017). Similarly, we can expect behavioural plasticity in light of predation risks. However, plasticity in response to such risks may be more costly (and therefore lower) because incorrect decisions often lead to death (which is not the case for plasticity in response to social cues). Thus, behaviour may be optimised to maximise survival in different environments. In a recent review, Bailey et al. (2017) suggested that behaviour is particularly prone to variation in the social environment. Such variation should therefore have a greater impact on behavioural plasticity than other aspects of the environment such as the presence of predators. Some recent theoretical papers suggest that there are coevolutionary processes that lead to the existence of both socially responsive and consistent individuals as a result of negative frequency-dependence (Johnstone & Manica, 2011; McNamara, Stephens, Dall, & Houston, 2009; Wolf, Van Doorn, & Weissing, 2011). Although predators and prey coevolve, the presence of different predators and a diversity of prey may dilute these effects in comparison to social interactions within species. Thus, we may expect social interactions to have more pronounced effects on behavioural plasticity. Much remains to be learned about the ways in which environmental cues shape between- and within-individual behavioural
variation. Here we investigate the effects of different environmental cues (social vs. non-social) on behavioural plasticity within the same experimental framework.

We use Australian field crickets (*Teleogryllus oceanicus*) to test the hypothesis that the environment, and changes therein, can affect behavioural expression (phenotypic plasticity), the repeatability of behaviours (a phenomenon often referred to as 'animal personality': Bell et al., 2009; Gosling, 2001) and correlations between multiple behavioural traits ('behavioural syndromes': Bell, 2007; Sih, Bell, Johnson, & Ziemba, 2004). We manipulated two aspects of the environment, the social environment via acoustic cues from conspecifics, and the physical environment via mechanical disturbance, and examined the effects of the presence and absence of these cues on male behaviour. In crickets, acoustic sexual signals have been found to affect aggression, dominance, female mate choice and alternative mating tactics (Bailey and Zuk 2008; Bailey et al. 2010; DiRienzo et al. 2012). Males from various cricket species have been found to be attracted to conspecific song, forming clusters in which individuals remain relatively stationary whilst broadcasting acoustic sexual signals (Campbell and Shipp 1979; Simmons 1988; Tinghitella et al. 2009). Based on these findings, we predicted that males would be more likely to engage in searching behaviour (emerge quickly from a shelter and be more explorative and active in search of conspecifics) in the absence compared to the presence of conspecific calls. Different levels of predation or parasitism risk can affect how cautiously individuals behave (Lewkiewicz and Zuk 2004; Hedrick and Kortet 2006). Therefore, we might expect the presence of a physical disturbance to render crickets less active and less bold compared to crickets that are not exposed to disturbances. However, we expect the effect of disturbances in the environment to be small compared to changes in the social environment due to the special role that the social environment is imputed to have in the evolution of animal behaviour (Bailey et al. 2017).

In a previous study (Rudin et al. 2017), we found that changes in dominance status eroded the repeatability of some behaviours, but that boldness (latency to emerge from a shelter) remained relatively stable. Additionally, changes in social status had a disruptive effect on the correlation between boldness and activity, but not on the correlation between boldness and exploration. Because of the links between social status and acoustic sexual signals (Simmons 1986; e.g. Brown et al. 2006; Callander et al. 2013), we predict that changes in such signals will similarly affect the repeatability of and correlations between behaviours. Previous studies have investigated environmental effects on the repeatability and expression of behaviours by exposing animals to different environments, measuring them repeatedly in the same environment. There is a distinct lack of studies that have investigated the effects of relatively short-term environmental changes on the repeatability and expression of and correlations between behaviours. Our experimental design allowed us to investigate the effects of such changes. Additionally, comparing individuals that experienced a switch in
environments to those that did not allow us to infer the presence or absence of between-individual variation in behavioural plasticity, or individual-by-environment interactions (IxEs) (Mathot et al. 2012; Dingemanse and Wolf 2013; Alonzo 2015; Stamps 2016). Although researchers have recently begun to focus on IxEs, much remains to be learned about them, especially in light of changes in the social environment (Bailey et al. 2017).

2.3 Methods

2.3.1 Study population
The animals used in this experiment came from a large outbred laboratory stock population (>1000 individuals) which is restocked annually with freshly collected individuals from Carnarvon (Western Australia). Animals were reared with ad libitum access to food and water and held at 26°C on a 12:12 h light:dark cycle. At the final larval instar, males (N = 208) were taken from the stock population and housed in individual clear plastic containers (7 × 7 × 5 cm). Individuals were checked daily and placed into experimental treatments the day following their final moult to adulthood.

2.3.2 Experiment 1: socio-sexual environment
In our first experiment, crickets were exposed to the presence and absence of acoustic sexual signals from conspecific males. Four groups of 26 crickets each (total N = 104) were assigned to four separate environmental chambers, two silent and two acoustic. The tegmen of all crickets were clipped to ensure they could not produce song. Within the acoustic chambers, five-minute recordings of ~30 sexually mature males housed with an equal number of females were played back continuously. These recordings included a mixture of calling, courtship and aggressive song. The playback devices were MP3 players (iPod nano 7th Gen and iPod classic 6th Gen) and speakers (Logitech Z200 Multimedia Speakers and Philips Speaker Dock SBD8000/79). The light:dark cycles of all chambers were set to 12:12 light:dark and all were held at 26°C. After one week of exposure to either the silent or acoustic environments, behavioural trials were conducted as described below. After behavioural trials, half of the crickets were returned to the same treatment they had been exposed to previously, while the other half switched treatment, either from the silent to the acoustic or from the acoustic to the silent treatment. Crickets were again exposed to these treatments for a week after which behavioural trials were repeated. This resulted in 4 groups of individuals at the end of the two trials: AA (acoustic environment for 1st week, acoustic environment 2nd week), AS (acoustic environment for 1st week, silent environment 2nd week), SS (silent environment for 1st week, silent environment 2nd week) and
SA (silent environment for 1st week, acoustic environment 2nd week) (Figure 1). Each of these groups consisted of 26 individuals.

2.3.3 Experiment 2: physical disturbance

In our second experiment, a different set of crickets (again silenced by clipping of the tegmen) was exposed to the presence or absence of disturbance stimuli. We modified a commercial ceiling extraction fan by removing the fan blades and cable-tying a weight (large steel nut) eccentrically to the spindle. When the fan was turned on, the eccentric weight caused the fan to shake. The modified fan was then cable-tied to the top of a transparent plastic box (41 × 30 × 25 cm), into which the crickets were later placed in their individual containers. Thus the plastic box was shaken when the fan was turned on. The fan was attached to an HPM Slim Digital Timer (D817SLIM) which was set to turn on for 3 minutes, 5 times a day, at random intervals (5 – 535 min) with the random interval repeating every other day. Intervals were randomised to reduce potential habituation to the cue. For the undisturbed environment, crickets, in their individual boxes, were placed into an identical large transparent plastic box that was not fitted with a shaker. Again each of these disturbed/undisturbed treatments were replicated twice across two environmental chambers held on a 12:12 light:dark cycle.

Figure 1. Diagram of experimental design for both experiments (N = 104 for each experiment). ‘Treatment’ was either exposure to male acoustic sexual signals or physical disturbance (speaker symbols used to illustrate the acoustic manipulation used in experiment 1). ‘Control stimuli’ was the absence of acoustic signals or physical disturbances. After one week of being exposed to either the treatment or the control environment, crickets underwent the behavioural trials. After the trials, half of the crickets spent another week in the same environment they were exposed to during week 1 and the other half were switched to the environment they had not previously experienced. After week 2 each cricket underwent another behavioural trial.

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and at 26°C. Of the 104 crickets used in this experiment, half were initially exposed to the disturbance treatment for a week \( (N = 52, 26 \text{ for each replicate chamber}) \) while the other half were left undisturbed \( (N = 52, 26 \text{ in each replicate chamber}) \). Behavioural trials were then conducted (see below). After behavioural trials, half of the crickets were returned to the same treatment that they had been exposed to previously, while the other half switched treatments (Figure 1). Crickets were again exposed to these treatments for a week after which behavioural trials were repeated. This yielded 4 groups of individuals: DD (disturbed environment for 1\(^{st}\) week, disturbed environment 2\(^{nd}\) week), DU (disturbed environment for 1\(^{st}\) week, undisturbed environment 2\(^{nd}\) week), UU (undisturbed environment for 1\(^{st}\) week, undisturbed environment 2\(^{nd}\) week) and UD (undisturbed environment for 1\(^{st}\) week, disturbed environment 2\(^{nd}\) week).

### 2.3.4 Behavioural trials

All behavioural trials were conducted in an environmental chamber at 26°C during the dark phase. To ensure that the crickets were not disturbed by the observer, trials were conducted under dim red illumination. The experimental setup (Figure 2) consisted of a plastic trough (31 cm deep, 38 \times 52 cm at top, 32 \times 46 cm at base) into which a shelter cut from PVC pipe (height: 8.5 cm; diameter: 8 cm) was placed. The shelter had an opening that was fitted with a movable door which could be opened from outside the trough by pulling a piece of string attached to the door. Fine sand was used to cover the base of the trough (approx. 2 cm deep).

Motion analysis software (EthoVision v8.5) was used to track the movement of individuals within the arena, resulting in objective, quantifiable measurements of their behaviour. Crickets were filmed with a video camera (Panasonic WV-CL930) installed 80 cm above the base of the arena. Within the software, we defined different areas: the area closest to the shelter (17 cm radius around the corner of the arena closest to the shelter) was defined as ‘near’. The area farthest from the shelter (17 cm radius around the corner opposite the shelter) was defined as ‘far’. The area between ‘near’ and ‘far’ was defined as ‘middle’. The specific behaviours quantified through EthoVision were as follows: the latency to emerge from the shelter (once the whole body of the cricket was outside the shelter); the total distance moved within the arena; the average velocity within the arena; the latency to ‘far’; the time spent moving (any movement \( >0.3 \text{ cm/s} \)); the total time spent within the arena as well as within the ‘near’, ‘middle’ and ‘far’ zones.

After 1 week of exposure to the environmental treatments, each cricket was placed inside the shelter with the door closed, and the shelter was tapped with a plastic rod for approximately 10 s to disturb the cricket. Thirty seconds later the door was carefully opened. This was defined as the starting point of the trial. Each cricket was given 10 min to emerge from the shelter. Those crickets that failed to
emerge from the shelter within 10 min resulted in the termination of the trial for those individuals. Once a cricket emerged, its movements were tracked by EthoVision for 10 min. We did not include the time individuals spent inside the shelter after exiting the shelter for the first time in the analysis because it is the inverse of the total time spent in the arena.

Within 1.5 h of completing their first behavioural trial, crickets were transferred to their subsequent environments (either reversed or kept the same) (Figure 1) where they remained for a further week. The second behavioural trial was then conducted as described above. After the second behavioural trial crickets were frozen, and later their size and weight measured.

2.3.5 Size and weight measurements
We measured pronotum width using Mitutoyo CD-6 ASX callipers (precision: 0.01 mm) and weight using a KERN PLS 510-3 scale (precision: 0.001 g). There was a high degree of correlation between the 2 measures across all crickets used for both experiments ($r_s = 0.71$, $N = 208$, $P < 0.001$). Therefore, pronotum width was used as an estimator of individual size for all statistical analyses because it is a
fixed measure of size that does not vary with recent food or water intake. Mean ± SE pronotum width was 6.36 ± 0.02 mm, mean ± SE weight was 0.556 g ± 0.006 g.

2.3.6 Statistical analyses

We grouped behavioural measures into different categories as follows: the latency to emerge from the shelter following a startle cue was defined as ‘boldness’ (sensu Carter et al. 2013). For all other behavioural measures we performed a single principal components analysis (PCA) for each experiment on the correlation matrix of data pooled from trials conducted after the first and second week. The axes of variation were Varimax rotated and scores on the axes with eigenvalues >1 were extracted. For both experiments, the number of components extracted was 2 (Table 1). The number of components to be extracted was confirmed by running a parallel analysis using the fa.parallel function (psych package in R). In both cases, average velocity, the total time spent in the arena, time spent ‘middle’, time spent ‘far’ and time spent moving loaded most strongly onto the first principal component (PC1). The latency to ‘far’, distance moved and time spent ‘near’ loaded most strongly onto the second principal component (PC2). Comparing the two sets of components using the factor.congruence function (psych package in R) resulted in the following coefficients: 0.852 for PC1 Acoustic – PC1 Disturbance; -0.854 for PC2 Acoustic – PC2 Disturbance. These coefficients suggest relatively low marginal similarity between both sets of components (Lorenzo-Seva and ten Berge 2006). Component scores extraction was regression-based. Henceforth, PC1 scores will be used as a measure of ‘activity’, while PC2 scores will be used as a measure of ‘exploration’. Since a high score of PC2 equates to more time ‘near’ and a lower proportion of time spent moving (Table 1), the score indicates how ‘unexplorative’ a cricket is. We therefore reverse signed PC2 to ease interpretation.

Both experiments had a sample size of 104 crickets and individuals underwent two trials each. This resulted in 208 trials for each experiment. In the socio-sexual experiment, crickets did not emerge from their shelter in 18 out of 208 trials (8.7%), 10 of them after week 1 and 8 after week 2. In the disturbance treatment, crickets did not emerge in 16 out of 208 trials (7.7%), 8 after week 1 and 8 after week 2. The crickets that did not emerge from their shelter were assigned the maximum time allowed for emergence, i.e. 600 seconds. If an individual failed to express a behaviour within ‘activity’ or ‘exploration’ (which was the case if they failed to emerge from the shelter or if they, for example, never moved to the ‘far’ area of the arena), they were considered missing values for those two behavioural axes. This occurred in 53 out of 208 (25.5%) trials (34 after week 1 and 19 after week 2) in the socio-sexual experiment treatment and in 31 out of 208 (14.9%) trials (15 after week 1 and 16 after week 2) in the disturbance experiment. In both experiments, sample sizes were therefore lower for activity and exploration (socio-sexual: 155 overall, 70 after week 1 and 85 after week 2;
Table 1. Loadings of the original behavioural variables on the principal axes of variation (PC1 and PC2) for the socio-sexual and the disturbance environment. All behaviours were measured twice for each individual (after week 1 and after week 2 of the experiment) and the principal components analysis was run on both measures for each individual.

<table>
<thead>
<tr>
<th></th>
<th>Socio-sexual environment</th>
<th>Disturbance environment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PC1</td>
<td>PC2</td>
</tr>
<tr>
<td>Eigenvalue</td>
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<td>2.22</td>
</tr>
<tr>
<td>Variance explained</td>
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<td>27.68</td>
</tr>
<tr>
<td>Distance moved</td>
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<td>0.756</td>
</tr>
<tr>
<td>Latency to ‘far’</td>
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<td>-0.829</td>
</tr>
<tr>
<td>Total time in arena</td>
<td>0.860</td>
<td>-0.411</td>
</tr>
<tr>
<td>Time ‘near’</td>
<td>0.033</td>
<td>-0.863</td>
</tr>
<tr>
<td>Time ‘middle’</td>
<td>0.829</td>
<td>0.030</td>
</tr>
<tr>
<td>Time ‘far’</td>
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<td>0.143</td>
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<tr>
<td>Velocity</td>
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</tr>
<tr>
<td>Time spent moving</td>
<td>0.584</td>
<td>0.117</td>
</tr>
</tbody>
</table>

disturbance: 177 overall, 89 after week 1 and 88 after week 2) than for boldness (both treatments: 208, 104 after week 1 and 104 after week 2). For some analyses we used differentials, which were calculated by subtracting the week 2 score from the week 1 score (latency to emerge for boldness and the principal component scores for activity and exploration). In those cases, sample sizes were further reduced for activity and exploration. This is because differentials could not be calculated if a cricket did not emerge and express all behaviours within ‘activity’ and ‘exploration’ at both week 1 and week 2. Consequently, the sample sizes for changes in activity and exploration between week 1 and week 2 were 62 for the socio-sexual experiment and 76 for the disturbance experiment.

To check whether there was a difference between individuals that spent week 1 exposed to the treatment stimulus versus those that were not, we ran a series of univariate general linear models. Within each, the fixed factor was the environment: Acoustic (A) and Silent (S) for experiment 1; Disturbed (D) and Undisturbed (U) for experiment 2. The dependent variables were the behavioural measures (latency to emerge for boldness, principal component scores for activity and exploration) and the covariate was pronotum width. To look for changes in behavioural expression in week 2, univariate general linear models were run for each behavioural trait (boldness and the principal component scores for activity and exploration) using the treatment profiles (AA, AS, SS, SA in experiment 1, or DD, DU, UU, UD in experiment 2) as the fixed factor. In each case, the dependent variables were the differentials (calculated as described above). Pronotum width was again used as the covariate. To determine whether individuals that switched environments after week 1 (AS and SA
or DU and UD) differed significantly from those that experienced the same environment for 2 consecutive weeks (AA and SS or DD and UU) we used Fisher’s least significant difference post-hoc comparisons. Equality of variance assumptions were met for all traits.

To test the repeatability of behaviours across the 2 behavioural trials (week 1 and week 2) we used the R (version 3.4.2) package rptR (Nakagawa and Schielzeth 2010; Stoffel et al. 2017) to calculate repeatabilities (or intraclass correlation coefficients) from variance components obtained from linear mixed models. By using individual identities as random effects, repeatabilities explain the proportion of total variance accounted for by differences between individuals. In other words, repeatability represents both the variability of behavioural traits across individuals as well as their relative consistency within individuals. Looking at the individual variance components from which repeatabilities were calculated gave us an indication as to whether changes in within- or between-individual variation drove changes in repeatability (though these differences could not be formally tested). Response variables were analysed using REML estimation and Gaussian fit because they were all approximately normally distributed. Repeatability estimates were calculated with 1000 bootstrappings and 1000 permutations. Besides calculating the repeatabilities of the response variable (boldness, activity and exploration) pooled across all treatment profiles, repeatabilities of the response variables were also calculated for each treatment profile (AA, AS, SA, SS and DD, DU, UD, UU) separately. To test whether repeatabilities differed significantly from each other we calculated 84% confidence intervals around the repeatability estimates. Repeatabilities were considered significantly different from each other when those confidence intervals did not overlap. Two 95% confidence intervals that do not overlap may not differ significantly at the 0.05 level and the 0.05 significance level can be visualised by the non-overlap criterion when adjusting the confidence levels to 1.39 times the standard error (or 84%), which is why we used 84% (and not 95%) confidence intervals (Goldstein and Healy 1995).

Spearman’s rank correlations ($r_s$) were used to check for the existence of between-individual behavioural correlations (Huntingford 1976; following examples and suggestions in Bell 2007). Because activity and exploration were represented by principal components scores (which erased any possible correlations), correlations between those two traits could not be assessed. Correlations were assessed separately for week 1 and week 2 measures. Week 1 measures were split into individuals that were exposed to the cue (male acoustic signals or disturbance) and those that were not. Week 2 measures were split into four groups for each experiment: those that were exposed to the cue for 2 weeks, those that were exposed to the cue in week 1 but not in week 2, those that were never exposed to the cue and those that were not exposed to the cue in week 1 but exposed to it in week 2. To
determine whether differences between correlation coefficients were significant we used Fisher’s r-to-z transformation (Myers and Sirois 2006).

2.4 Results

2.4.1 Experiment 1: socio-sexual environment

The effect of environment on behaviour

There were significant boldness, activity and exploration differences between individuals exposed to the male acoustic signals and individuals held in the silent environment during the first week. Crickets that were not exposed to male calls were significantly bolder (decreased latency to emerge; Figure 3a: $F_{2, 104} = 4.104; P = 0.019$), more active (Figure 3b: $F_{2, 77} = 7.171; P = 0.001$) and more explorative (Figure 3c: $F_{2, 77} = 7.356; P = 0.001$) than those that were exposed to calls. Size did not have a significant effect on any of the behaviours (boldness: $F_{1, 104} = 1.311, P = 0.255$; activity: $F_{1, 77} = 3.361, P = 0.071$; exploration: $F_{1, 77} = 0.413, P = 0.522$).

The effects of changing the environment

At week 2 there were significant changes in boldness, activity and exploration depending on whether crickets switched environments (Figure 4). General linear models were significant for all 3 traits (boldness: $F_{3, 103} = 3.687, P = 0.015$; activity: $F_{3, 63} = 5.685, P = 0.002$; exploration: $F_{3, 63} = 6.545, P = 0.001$). For boldness and activity, these changes were driven by significant differences between
individuals that did not change environments (AA and SS respectively) and individuals that did (AS and SA respectively) (Figure 4), as revealed by post-hoc analyses. Individuals that switched from acoustic to silent (AS) were bolder and more active than those that stayed in the acoustic environment (AA) while individuals that switched from silent to acoustic (SA) were less bold and active than individuals that stayed in the silent environment (SS). For exploration, only individuals that spent both weeks in the silent environment (SS) differed from individuals that switched from silent to being exposed to male acoustic signals (SA). Individuals that were switched from the silent to the acoustic environment were less explorative than those that remained in the silent environment. No difference was found between individuals that spent both weeks in the acoustic environment (AA) and those that switched from being exposed to male acoustic signals to the silent environment (AS) (Figure 4). Size had no significant effect on either behavioural trait (boldness: $F_{1, 103} = 0.433$, $P = 0.512$; activity: $F_{1, 63} = 2.196$, $P = 0.144$; exploration: $F_{1, 63} = 0.030$, $P = 0.863$).

**Figure 4.** Differences in behaviour at week 2 (behavioural score week 2 minus score at week 1) for boldness (latency to emerge), exploration and activity. Crickets were either exposed to the same treatment for 2 weeks (acoustic: AA; silent: SS) or experienced a change after the 1st week (AS or SA). The bottom and top of the box represent the first (Q1) and third quantiles (Q3) of the data, respectively (interquartile region, IQR). The horizontal line within the box represents the median. The whiskers end at the largest and smallest non-outliers. Outliers (1.5 x IQR above Q1 and below Q3) are represented by dots.
**Repeatabilities**

Figure 5 shows the repeatabilities of all personality traits for the different treatment profiles (AA, AS, SS and SA) and the overall repeatabilities for each trait (pooled across all treatment profiles). Within boldness and activity, the 84% confidence intervals around the repeatabilities of the individuals that changed from the silent to the acoustic environment (SA) did not overlap with those of the other treatment profiles (AA, AS and SS) and the repeatabilities were therefore significantly different (the repeatability of SA being lower than AA, AS and SS). For exploration, the 84% confidence intervals around the repeatabilities of both treatment profiles in which crickets spent the first week in the silent treatment (SS and SA) did not overlap with and was lower than the other two profiles (AA and AS). The within- and between-individual variance components from which repeatabilities were calculated are shown in Figure A1.

![Figure 5](image)

**Figure 5.** Repeatabilities for boldness, activity and exploration pooled across all treatment profiles and for the different subsets (AA and SS i.e. no change in environment; AS and SA i.e. change in environment). The 84% confidence intervals around repeatabilities are represented by the whiskers.

**Correlations among behaviours**

Correlations between boldness and activity and boldness and exploration for week 1 are compared in Table 2, and for week 2 in Table 3. After week 1, correlations between all behavioural traits (boldness — activity and boldness — exploration) were significant. After week 2, correlations between all traits were significant for both cases in which individuals did not experience a change in environments (AA and SS) but not for cases in which a change occurred (AS and SA). The one exception was the
correlation between boldness and activity, which was still significant for individuals that changed from the acoustic to the silent environment (AS). Only the correlations between boldness and exploration differed significantly between AA and AS individuals.
2.4.2 Experiment 2: disturbance

The effect of environment on behaviour

Significant differences in behaviour between individuals exposed to random bouts of disturbance and individuals that were undisturbed were found only for exploration, with individuals that were exposed to disturbance being less explorative than those that were exposed (Figure 6c: $F_{2, 89} = 5.073; P = 0.008$). No significant effects were found for boldness (latency to emerge; Figure 6a: $F_{2, 104} = 1.835; P = 0.165$) and activity (Figure 6b: $F_{2, 89} = 0.830; P = 0.439$). Pronotum width had no significant effect on any of the behaviours (boldness: $F_{1, 104} = 0.397, P = 0.530$; activity: $F_{1, 89} = 0.344, P = 0.559$; exploration: $F_{1, 77} = 0.669, P = 0.416$).

The effects of changing the environment

After week 2 there were significant changes in exploration depending on whether a switch in the environment occurred ($F_{3, 75} = 3.383, P = 0.014$) (Figure 7). The other general linear models were non-significant (boldness: $F_{3, 103} = 2.201, P = 0.074$; activity: $F_{3, 75} = 1.767, P = 0.145$). Individuals that did not experience a change midway through the experiment (DD and UU) did not alter their behaviour in week 2. There was a significant difference between individuals that spent both weeks in the undisturbed environment (UU) and those that switched from the undisturbed to the disturbed environment (UD) (Figure 7). Here, individuals became less explorative (the differential became negative) when switching from the undisturbed to the disturbed environment, whereas no change was observed for individuals that spent both weeks in the undisturbed environment. No such difference was observed between individuals that spent both weeks in the disturbed environment.

Figure 6. Behavioural differences between individuals that were exposed to randomly applied bouts of disturbance for 1 week and those that were not exposed using univariate general linear models. The bottom and top of the box represent the first (Q1) and third quantiles (Q3) of the data, respectively (interquartile region, IQR). The horizontal line within the box represents the median. The whiskers end at the largest and smallest non-outliers. Outliers (1.5 x IQR above Q1 and below Q3) are represented by dots. Asterisks indicate significance levels: **$P < 0.01$. 

Figure 7. Behavioural differences between individuals that were exposed to randomly applied bouts of disturbance for 1 week and those that were not exposed using univariate general linear models. The bottom and top of the box represent the first (Q1) and third quantiles (Q3) of the data, respectively (interquartile region, IQR). The horizontal line within the box represents the median. The whiskers end at the largest and smallest non-outliers. Outliers (1.5 x IQR above Q1 and below Q3) are represented by dots. Asterisks indicate significance levels: **$P < 0.01$. 


(DD) and those that switched from the disturbed to the undisturbed (DU). No such effects were observed for boldness and activity. Size had no significant effect on either behavioural trait (boldness: $F_{1, 103} = 1.663, P = 0.200$; activity: $F_{1, 75} = 1.376, P = 0.245$; exploration: $F_{1, 63} = 1.548, P = 0.218$).

**Figure 8** shows the repeatabilities for the three behavioural traits, both pooled across all treatment profiles (DD, DU, UU and UD) and within each profile separately. All of the 84% confidence intervals around the repeatabilities within each personality trait overlapped and could therefore not be considered significantly different from each other.

**Repeatabilities**

Figure 8 shows the repeatabilities for the three behavioural traits, both pooled across all treatment profiles (DD, DU, UU and UD) and within each profile separately. All of the 84% confidence intervals around the repeatabilities within each personality trait overlapped and could therefore not be considered significantly different from each other.

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**Figure 7.** Differences in behaviour at week 2 (behavioural score at week 2 minus score at week 1) for boldness (latency to emerge), exploration and activity. Crickets were either exposed to the same treatment for 2 weeks (disturbance: DD; undisturbed: UU) or experienced a change after the 1st week (DU or UD). The bottom and top of the box represent the first (Q1) and third quantiles (Q3) of the data, respectively (interquartile region, IQR). The horizontal line within the box represents the median. The whiskers end at the largest and smallest non-outliers. Outliers (1.5 x IQR above Q1 and below Q3) are represented by dots.
Correlations across behaviours

Tables 2 and 3 show the correlations between the three behavioural traits after week 1 and 2 respectively. Correlations between all behavioural traits were significant after week 1. All but one of the correlations (boldness – exploration for DU individuals) were also significant after week 2. Comparing DD to DU individuals and UU to UD individuals, none of the correlations were significantly different from each other.

2.5 Discussion

The social environment is expected to have a particularly strong effect on animal behaviour (Bailey et al. 2017). We found that when crickets were switched from a silent environment to an environment in which male acoustic sexual signals were present, they became less bold, active and explorative. When switched from an acoustic to a silent environment, individuals became bolder and more active. Changes in the social environment also affected the repeatability of and correlations between some behaviours. In contrast, changing between an undisturbed and disturbed environment had either no, or considerably weaker effects on behavioural plasticity, repeatability and correlations. These findings support the hypothesis that the social environment affects behaviour more markedly than the non-
social environment and highlight the importance of interacting phenotypes in understanding behavioural consistency and plasticity.

2.5.1 Variation in the social environment

Balenger & Zuk (2015) showed that *T. oceanicus* from a population in which a genetic mutation rendered approximately 90% of the males incapable of calling, exhibited increased locomotor behaviour when reared in silence. They argued that, in the absence of calling rivals, it would be beneficial for individuals to increase overall movement if such behavioural alterations increase mating opportunities. Furthermore, crickets have been found to cluster in areas in which other crickets are calling; searching behaviour is increased in the absence and decreased in the presence of other calling males (Campbell and Shipp 1979; e.g. Cade 1981b). When reared in the presence of male calling song, male *T. oceanicus* were less likely to employ satellite behaviour (settling near calling males to parasitize their song to gain access to females) as adults compared to crickets reared in silence (Bailey et al. 2010). While the population used for our experiment does not exhibit mutations rendering them silent and our crickets were not reared as juveniles in the absence of conspecific song, we can nonetheless draw parallels between these previous studies and our findings. Here, crickets held in a silent environment for the first week of their adult lives emerged more quickly from a shelter and displayed more active and explorative behaviour than those that spent the week in the presence of male acoustic sexual signals. Thus, the presence of male song may be an indicator for an individual that it is within a chorus, reducing its need to search for an aggregation of singing conspecifics. Conversely, males that did not experience song during the first week of their adult lives may increase their explorative behaviour and activity and more readily leave a shelter and search for a chorus. Females are attracted by male acoustic sexual signals (Ulagaraj and Walker 1973) and the choruses males form have been compared to ‘lek’ type mating systems (Alexander 1975). In such systems, females compare males and choose mates (e.g. Beehler and Foster 1988). Female *T. oceanicus* have been found to become more choosy when reared in an environment that mimicked an aggregation of calling males compared to females reared in a silent environment (Bailey and Zuk 2008). Thus, male behaviour may be explained as a response to female behaviour; staying put in a chorus (as simulated here through playback of male song) is expected to increase a male’s mating success. Within calling aggregations, males of multiple field cricket species space out relatively evenly. This is thought to be a result of maintaining an exclusive female-attraction zone with calls acting as aggressive signals (Campbell and Shipp 1979; Cade 1981b; Simmons 1988). Thus, acoustic spacing within aggregations would further decrease exploration, activity and boldness in crickets exposed to the acoustic sexual signals of rivals.
We found considerable behavioural plasticity in response to changes in the acoustic environment. Interestingly, both scenarios (changing from the acoustic to the silent or silent to acoustic environments) elicited statistically significant changes in the average level of all behaviours, with the exception of exploration in crickets that experienced a switch from the acoustic to the silent environment. The fact that crickets become less bold, explorative and active when they go from a silent environment to being exposed to male calls further supports the notion that individuals are more likely to stay put, rather than engage in searching behaviour, upon ‘joining’ an aggregation of singing males. This, again, is in line with studies that found that males increased searching behaviour in the absence of male acoustic sexual signals (Campbell and Shipp 1979; e.g. Cade 1981b).

We found significant repeatability in behaviours indicating consistent among-individual variation in behaviour, or personality. Repeatability can be high due to both between-individual differences and within-individual consistency of behaviours (Nakagawa and Schielzeth 2010). A meta-analysis found that overall, the repeatability of animal behaviours is around 0.37 (Bell et al. 2009). We found overall repeatabilities of 0.44, 0.39 and 0.42 for boldness, activity and exploration respectively, close to or slightly higher than the average behavioural repeatabilities reported for insects (0.36; Bell et al. 2009). A recent study on the field cricket Gryllus campestris found repeatabilities of 0.06, 0.21 and 0.12 for boldness, activity and exploration respectively in the wild (Fisher et al. 2015). Although there is a trend for repeatabilities to be higher in the wild than in the laboratory (Bell et al. 2009), the repeatabilities found here were noticeably higher than those found by Fisher et al. (2015). The repeatability of behaviours was relatively stable across most treatment profiles. However, the repeatabilities of boldness and activity decreased considerably for individuals that spent their first week of adult life in the silent environment before experiencing a switch to the acoustic environment. For exploration, repeatabilities were low when individuals stayed in the silent environment and when they were switched from silent to acoustic. This may suggest that boldness and activity exhibit between-individual variation in plasticity, or individual-by-environment interactions (IxE) (Mathot et al. 2012; Dingemanse and Wolf 2013; Alonzo 2015; Stamps 2016). Exploration, on the other hand, did not appear to exhibit IxE. Repeatabilities can be reduced by IxEs when the rank order of individuals changes in response to variation in the environment, making individuals more unpredictable in their behaviour (i.e. an increase in within-individual variation). If the reduction in repeatabilities is driven by decreases in between- rather than increases in within-individual variation, the presence of an IxE becomes less likely. We could not test for IxEs formally since more observations per individual would be needed to run the appropriate mixed effects models. However, our data suggest that variation in the social environment might affect between-individual variation in plasticity (IxE) because reductions in repeatability were driven by increases in within- and not decreases in between-individual variances.
(Figure A1). The fact that certain changes in the social environment can lead to changes in behavioural repeatability is similar to our previous finding that changes in social dominance can erode the repeatability of some behaviours (Rudin et al. 2017). Fighting success (Gryllus bimaculatus; Wedell and Tregenza 1999) and the tendency to adopt alternative mating tactics (Gryllus integer; Cade, 1981a) have both been found to exhibit significant additive genetic variance in crickets. Environmentally dependent genetic variation (GxE) in either of these traits in T. oceanicus could result in individual variation in plasticity in light of increased competition. Being switched from a silent to an acoustic environment may indicate increased competition and thus explain the disruption in the repeatability of behaviours we observed. Interestingly, changing from the acoustic to the silent environment did not result in the same disruption of repeatabilities, suggesting that all individuals reacted in a similar way when switched from a competitive to a non-competitive environment.

Even though individuals adjusted their behaviour in response to changes in their social environment, between-individual behavioural differences persisted when crickets spent their first week of adult life in the acoustic environment and were then switched to the silent environment. Some studies have indicated that the development of personalities may be delayed if sufficient information on the state of the environment cannot be acquired (Fischer et al. 2014; Fawcett and Frankenhuis 2015). Furthermore, the development of between-individual behavioural differences in a population may be driven by environmental challenges associated with the social environment to a considerable degree (e.g. Edenbrow and Croft 2013; Rittschof et al. 2014). Here, we found that the repeatability of all behaviours was lower when crickets spent week 1 in the silent and week 2 in the acoustic environment, and for exploration when both weeks were spent in the silent environment. This may suggest that being exposed to silence early during adulthood delays or even inhibits the establishment of certain repeatable, predictable behaviours. This would be especially true if male acoustic sexual signals are important for behavioural differentiation. Indeed, this is supported by our finding that the behaviours of crickets that spent their first week in the acoustic environment did not exhibit reduced repeatability, regardless of whether they experienced a switch. Furthermore, some recent studies found that the acoustic rearing environment affects behaviour later in life in both male and female crickets (Bailey and Zuk 2008; Bailey et al. 2010). Little is known about the effects of the social environment on behavioural repeatability at different ages. Our study cannot address whether or how long these effects would persist past the relatively short time-frame of two weeks adopted in our experiment. Longer-term studies would be needed to fully explore this question. However, our results indicate that the presence or absence of sexual signals early in adult life may affect the degree of behavioural repeatability later on, at least for some behaviours.
Correlations between all behaviours were strong when individuals did not experience a switch in their environment; the only correlation that was strong when there was a change in the environment was that between boldness and activity for individuals experiencing a switch from acoustic to silent. IxEs are thought to cause changes in correlations between traits (Brommer and Class 2017), so it is surprising that the only correlation that changed significantly was one for which none of the traits exhibited an IxE; the correlation between boldness and exploration was higher among individuals that stayed in the acoustic environment than those that were switched from acoustic to silent. Strong genetic correlations have been thought to constrain the evolvability of behaviours (Dochtermann and Dingemans 2013). Although this study was conducted at the phenotypic level, it has been argued that phenotypic correlations can serve as a proxy for genetic correlations (Dochtermann 2011). The fact that some aspects of the social environment (e.g. dominance status, social environment) can weaken otherwise strong behavioural correlations leads us to caution against neglecting such social interactions when estimating the evolvability of different behaviours.

2.5.2 Variation in physical disturbance

The ability to adjust behaviour in response to risks, such as predation, in the environment should be beneficial from an adaptive perspective (Dall, Houston, & McNamara, 2004; Lima & Dill, 1990; Wolf & Weissing, 2012). In general, experiencing predator cues can be expected to lead to more cautious behaviour (Smith and Blumstein 2008). Our expectation that the presence of a disturbance might affect cricket behaviour was only partly met. Here, we found that crickets were less explorative after exposure to disturbances for a week, and this is in line with the expectation of predator-avoidance behaviour when faced with predator cues. Similarly, crickets that were switched from being exposed to disturbance to an undisturbed environment became less explorative (but not vice versa). Although we lacked the sample size for formal statistical analyses, the behavioural response (especially boldness and activity) to the disturbance stimuli appeared to be quite weak and less pronounced than the response to the presence of male acoustic sexual signals. It is possible that the stimuli presented to the crickets in this study did not elicit strong responses because it was not perceived as a predation threat, although crickets were visibly startled when being disturbed by the shaker. The levels of boldness and activity we observed may show low plasticity (with exploration being somewhat more plastic) to maximise survival within the population from which these crickets were derived. Plasticity in response to social cues may be less costly (wrong decision = reduced reproductive success) than plasticity in response to potential threats (wrong decision = death). Alternatively, or in addition, the relatively weak plasticity of exploration and the absence of plasticity in boldness and activity may have been due to habituation, resulting from one week of exposure to shaking without negative
consequences for the crickets. A study on Christmas tree worms found such an effect, with behavioural plasticity only being detected over short time scales (1 day) but not longer time scales (4 days) (Pezner et al. 2017). Thus, the treatment period in our experiment may have been too long to detect behavioural plasticity in relation to disturbance.

We found no significant differences between the repeatabilities of each behaviour (boldness, activity and exploration) within each subset (DD, DU, UU, and UD). Thus, disturbance stimuli had less pronounced effects than acoustic sexual signals on repeatabilities. In response to changes in the environmental stimuli presented here, between-individual behavioural differences and behavioural correlations appear to be relatively stable, regardless of the direction of such changes. This was to be expected since we found that the effects of disturbance on the levels of behavioural expression were negligible (no effects on boldness or activity and only a small effect on exploration). Again, this may be attributable to habituation and/or point to the fact that all individuals react similarly to the disturbance cue. Our findings suggest that there is no between-individual variation in behavioural plasticity (IxE) in response to changes in physical disturbance. Likewise, Mathot and colleagues (2011), investigated escape flight duration in red knots (Calidris canutus islandica), finding no between-individual variation in behavioural plasticity within flocks. They attributed this finding to the fact that escape flight durations exhibit positive frequency-dependent payoffs, which in turn constrain individual variation in plasticity.

2.5.3 The social environment and behavioural plasticity

We found considerable behavioural plasticity in response to the social environment but not to a non-social environmental disturbance. These findings address gaps in our understanding of social and non-social effects on behaviour since there appears to be a distinct lack of studies comparing such effects within a single framework (Bailey et al. 2017). The evidence found here and in our previous study (Rudin et al. 2017) shows that social cues (dominance status, acoustic sexual signals) can have appreciable effects on behaviour and that changes in these social cues can affect the repeatability of behaviours and between-individual behavioural correlations. The fact that such changes can potentially disrupt personalities and correlations between different behaviours is intriguing and has, in our opinion, been somewhat neglected in the literature.

Behaviour is thought to be the result of an individual’s intrinsic properties and external abiotic and biotic effects. Traditionally, genetic and environmental influences have been considered separate. However, a distinctive property of social interactions is that they are simultaneously environmental and genetic. Thus, environmental effects can be heritable if the environment is social and heritable differences contribute to these effects. Bailey and Zuk (2012) found that female T. oceanicus from
different populations show different levels of choosiness depending on whether male acoustic signals were present or not, indicating the presence of indirect genetic effects. We note that we could not explicitly test for indirect genetic effects in our study, since it was conducted at the level of the phenotype, and we only had measurements from the receivers of the social cue and not its broadcasters. Future studies could build on ours by measuring behaviours in response to different calling males rather than to recordings of song (e.g. Santostefano et al. 2016). We previously demonstrated that the outcome of agonistic social interactions affected an individual’s behavioural phenotype, namely the expression of boldness, activity and exploration (Rudin et al. 2017). Here, we show that the social environment has strong effects on behavioural plasticity, but the non-social environment does not. This provides further support for the notion that responses to the social, rather than the non-social environment, are likely to be important drivers of rapid coevolutionary dynamics (Drown and Wade 2014). This is also supported by our finding that there is individual variation in plasticity in response to a change from the silent to the acoustic environment. Since the behaviours considered here are likely to affect access to food or mates, carry-over fitness effects are a likely consequence for interacting individuals, resulting in differential selection. The non-social environment (physical disturbance) did not appear to be as important as the social environment in shaping behavioural plasticity. Future work should use a quantitative-genetic framework to determine the extent to which changes in the social environment have the potential to affect evolutionary changes in behavioural plasticity and personality.
Figure A1. Between- (light grey) and within-individual (dark grey) variances of the three behavioural traits (a, d: boldness; b, e: activity; c, f: exploration) for each treatment profile within both the acoustic sexual signal experiment (AA, AS, SS, SA) and the mechanical disturbance experiment (DD, DU, UU, UD).
Chapter Three

Social cues alter quantitative genetic variation and covariation in animal personality traits
3.1 Abstract

The social environment is expected to have substantial effects on behaviour, and as a consequence its heritability and evolvability. We investigated these effects by exposing Australian field crickets (*Teleogryllus oceanicus*) to either silence or recordings of male acoustic sexual signals. We used a combined pedigree and full-sib/half-sib breeding design to estimate the repeatability, heritability, and evolvability of behaviours related to boldness, exploration, and activity. All behaviours measured were significantly repeatable in both social environments. Additionally, most behaviours showed significant heritabilities in the two environments. We found no difference in repeatabilities between the silent and the acoustic environment but did find significant differences in the heritabilities and evolvabilities between these environments. There was a high degree of similarity between the phenotypic covariance matrices across the two environments, while the genotypic covariance matrices were highly dissimilar. Reflecting this, we found significant genotype-by-environment interactions for most of the behaviours. Lastly, we found that the repeatable aspect of behaviour (‘personality’) was significantly heritable for most behaviours, but that these heritabilities were higher in the acoustic than in the silent environment. We conclude that the social environment can have a significant impact on the heritability and evolvability of behaviour, and argue that evolutionary inferences from phenotypic studies should be made with caution.
3.2 Introduction

Behaviour, by definition, gives animals the ability to respond to environmental stimuli relatively quickly (Levitis et al. 2009). As a consequence, all aspects of the environment can have marked impacts on the behavioural phenotype of an individual. Both abiotic factors, such as temperature (e.g. Brodie and Russell 1999) or light (e.g. Herberstein and Fleisch 2003), and biotic factors, such as predation threats (Bell and Sih 2007; Briffa et al. 2008) or food availability (e.g. Heithaus and Dill 2002), have been found to affect the way in which behaviours are expressed. In a recent review, Bailey et al. (2017) pointed out that among the biotic aspects of the environment, social interactions may play a particularly important role in shaping the way behaviours are expressed and evolve. Examples include the effects of rival male pheromones on ejaculate expenditure in bank voles (delBarco-Trillo and Ferkin 2004) and a study showing that guppies reared at high densities are less successful foragers than those reared at low densities (Chapman et al. 2008).

Within evolutionary ecology, evolutionary inferences are often drawn from phenotypic observations; an often unstated assumption termed the ‘phenotypic gambit’ (Grafen 1984). The phenotypic gambit therefore depends on the degree to which underlying genotypes (additive genetic variation) inform observed phenotypic variation. Whether the gambit holds for behaviours is often unclear, but this can be assessed whenever heritabilities and repeatabilities are estimated within a single framework (Dochtermann et al. 2014). If evolutionary trajectories are affected by the social environment (McGlothlin et al. 2010; Drown and Wade 2014) we would expect heritabilities to differ between environments, questioning the validity of the phenotypic gambit. A way of testing the phenotypic gambit is to compare phenotypic and genetic correlations and (co)variances of different behavioural traits among different social environments. The higher the similarity between phenotypic and genetic correlations/covariances, the more appropriate it would be to apply the gambit. The same applies to assessing the validity of ‘Cheverud’s conjecture’ (Roff 1995), which suggests that phenotypic correlations may be useful proxies of underlying genetic correlations (Roff 1995, 1997).

Here, we used the Australian field cricket (Teleogryllus oceanicus) to investigate the effect of the social environment on genetic variation and covariation in the expression of behaviours commonly quantified in animal personality studies; boldness, exploration, and activity (Carter et al. 2013; Rudin et al. 2017). These personality traits have been found to have fitness consequences (e.g. Wolf et al. 2007; Smith and Blumstein 2008; Cote et al. 2010; Niemelä et al. 2012) and can therefore be expected to be important in the evolution of behaviour. In a previous study, we found that in T. oceanicus, dominance status, and changes therein, can have marked impacts on the expression and repeatability of and correlations among boldness, exploration, and activity (Rudin et al. 2017). Additionally, changes in the social environment, namely male acoustic sexual signals, appear to have a greater impact on
the expression and repeatability of these behaviours than an abiotic startle stimulus (Rudin et al. 2018). Acoustic sexual signals affect traits such as female mate choice, alternative mating tactics, dominance, and aggression in crickets (Bailey et al. 2010; Bailey and Zuk 2012; DiRienzo et al. 2012). Furthermore, various cricket species are attracted by conspecific song and have been found to form clusters from which they broadcast acoustic sexual signals whilst remaining relatively stationary (Campbell and Shipp 1979; Simmons 1988; Tinghitella et al. 2009). These findings support the notion that behaviour, and the evolutionary rates thereof, might be particularly prone to variation in the social environment (Bailey et al. 2017), compared to other aspects of the environment such as predation threats. We employed a pedigreed full-sib/half-sib breeding design over two generations to assess the heritability and evolvability of different behaviours across two environments, one in which crickets were exposed to silence and one in which they were exposed to male acoustic sexual signals. This also allowed us to determine the degree to which the social environment affects genetic variation in the expression and repeatability (often equated with 'animal personality': Gosling 2001; Bell et al. 2009) of behaviours. From our previous phenotypic studies (Rudin et al. 2018) we expected crickets exposed to male acoustic sexual signals to be bolder and more explorative and active in an open area, but that the repeatabilities across these environments should be similar (Rudin et al. 2018). Furthermore, by estimating phenotypic and genetic covariances and correlations we were able to draw inferences about the degree to which behaviours are free to evolve independently and whether the phenotypic gambit or Cheverud’s conjecture apply. Lastly, using an approach suggested by Dochtermann et al. (2014), we used our quantitative genetic and repeatability data to assess the relative contributions of genetic and phenotypic variation to the repeatable aspects of behavioural variation (personality). Thus, we ask whether a social cue can affect the evolvability and heritability of behaviour, and so influence evolutionary trajectories. Besides some theoretical studies (Dochtermann et al. 2014), few empirical studies have reported quantitative genetic variation in personality traits (Petelle et al. 2015; Edwards et al. 2017).

3.3 Methods

3.3.1 Study population and breeding design

The animals used in this experiment were sourced from an outbred stock population (>1000 individuals) which is supplemented once a year with individuals collected from Carnarvon (Western Australia). All animals were housed in a controlled temperature room (26°C) on a 12:12 h light:dark cycle and fed and watered ad libitum. Water was supplied through moist pads of cotton wool, which also served as oviposition substrates for the females.
We combined a full-sib/half-sib design within a pedigree framework. To produce the first generation 17 F0 males, freshly collected from the field (Carnarvon, Western Australia, December 2015), were each mated to two or three virgin stock population dams. Male offspring from these matings (F1) were the first batch of experimental animals. The second generation (F2) of experimental animals was produced by mating one (F1) son from each of 18 field-inseminated females with one to three of the F1 females. To minimize environmental effects of rearing, all male offspring from the same dam were split into two to four groups and each group housed in a different 15-liter plastic container. Within each container, we provided cat chow (Purina Friskies Meaty Grills) and water ad libitum and egg cartons for shelter. To avoid any confounding container effects, all offspring from the same mother were combined at 5 to 7 weeks post-hatching and then randomly assigned into two to four groups of ~ 30 individuals and each of these groups housed in a 15 litre container (except for one family which had <30 individuals and was housed in a single container). We again provided egg cartons and food and water ad libitum. A total of 35 males sired offspring with one to three dams resulting in 96 dam families across the two generations.

3.3.2 Environmental treatments

At the penultimate instar, male offspring were removed from their communal containers and placed into individual plastic containers (7 × 7 × 5 cm) with free access to water and food. These containers were checked several times daily for eclosion (final molt to adulthood). Once adult, the tegmen of all crickets were clipped so that they would be unable to produce song. Roughly half of each dam’s offspring (totalling N = 607 males), in their individual boxes, were then immediately placed into two environmental chambers where they were exposed to male acoustic sexual signals while the other half (N = 598) were placed into two ‘silent’ chambers where no male calls were played. All chambers were kept at 26°C with a 12:12 h light:dark cycle. Male acoustic sexual signals were presented through continuous playback of five-minute recordings of ~ 30 sexually mature males housed with an equal number of females. The recordings consisted of courtship, calling and aggressive song. Calls were broadcast using speakers (Logitech Z200 Multimedia Speakers and Philips Speaker Dock SBD8000/79) and MP3 players (iPod nano 7th Gen and iPod classic 6th Gen). After one week of exposure to either treatment, behavioural trials were conducted as described below. All individuals were then returned to their respective environmental chambers (treatments). After a further week of exposure to the treatments, behavioural trials were repeated a second time for each individual. Thus, all experimental animals experienced experimental treatments for a total of 2 weeks. Our manipulation of the social environment by exposing males to the acoustic calls of rivals or maintaining them in a silent environment is a biologically relevant social manipulation as populations of these crickets occur on
the islands of Hawaii where a mutation has rendered males unable to produce song (Zuk et al. 2006; Pascoal et al. 2014; Simmons et al. 2014), such that natural populations of these crickets experience either acoustic or silent environments.

3.3.3 Behavioural trials

Behavioural trials were conducted in a temperature controlled room (26°C) during the crickets’ dark cycle to ensure maximal activity levels. Trials were conducted under dimmed red light to minimize disturbance by the observer. The experimental setup (Figure 1) consisted of a plastic trough (31 cm deep, 38 × 52 cm at top, 32 × 46 cm at base), the base of which was covered with about 2 cm of fine sand, and a shelter cut from PVC pipe (height: 8.5 cm; diameter: 8 cm). To minimize disturbance, the shelter was equipped with a moveable door which could be opened from outside by pulling a piece of string attached to it. A video camera (Panasonic WV-CL930) was mounted 80 cm above the base of the arena to record the crickets’ movements.

After the first week of exposure to either the acoustic or the silent treatment, the crickets were placed into a sound-proof container and carefully transported to the room in which the trials were conducted. Then, each cricket was placed into the shelter, the door closed, and the shelter tapped with a plastic rod for approximately 10 s. The cricket was then given 30 s to settle, then the door was opened. This was considered the starting point of the trial and each cricket was given 10 min to emerge from the shelter. If a cricket failed to emerge from the shelter within 10 min the trial was considered terminated for that individual.

Once a cricket left the shelter it was given 10 min to roam within the open arena and its movement was tracked using motion analysis software (EthoVision v8.5) to quantify its behaviour (following methods used in Rudin et al. 2017). The software allowed us to define different areas within the arena (Figure 1): ‘near’ was defined as the area nearest the shelter (17 cm radius from the corner of the arena) and ‘far’ as the area excluding ‘near’. The following behaviours were quantified with EthoVision: the latency to emerge from the shelter (the time taken for before the whole body of an individual was outside the shelter); the average movement velocity; total distance moved; time spent moving >0.3 cm/s; total time spent in the arena and time spent ‘near’ and ‘far’. It should be noted that total time spent in the arena may not add up to 10 min since crickets were able to return to the shelter after emergence.

Crickets were returned to their respective environmental chamber within 2 h of completing their behavioural trial. A second set of behavioural trials was conducted in an identical manner one week later, following which crickets were frozen. Body weight and size measurements were recorded at a later date. A total of 1205 crickets were used in this study (598 in the silent and 607 in the acoustic
treatment), resulting in 2410 trials (1196 in the silent and 1214 in the acoustic treatment). Crickets that failed to emerge in either the first, second, or both trials were excluded from all statistical analyses, resulting in the following final sample sizes: 566 in the silent and 527 in the acoustic treatment.

3.3.4 Weight and size measurements

The weight of each cricket was measured using a KERN PLS 510-3 balance (precision: 0.001 g). Pronotum width was measured with Mitutoyo CD-6 ASX calipers (precision: 0.01 mm). Mean ± SE weight was 0.552 ± 0.005 g, mean ± SE pronotum width was 6.24 ± 0.01 mm. Weight and pronotum width were highly correlated (r₁₂₀₅ = 0.74, P < 0.001). For this reason, and because pronotum width does not vary with recent water or food intake, we used pronotum width as a proxy for individual size in all statistical analyses.

Figure 1. Diagram of the arena in which behavioural trials were conducted.
3.3.5 Statistical analyses

Due to the nature of our breeding design, and to allow for the inclusion of fixed effects and the analysis of unbalanced datasets, all quantitative genetic analyses were performed using restricted maximum-likelihood models (i.e. 'animal model'; Kruuk 2004) in ASREML 4.1 (Gilmour et al. 2015). We fitted animal models for repeated measures data that allowed for the decomposition of individual variance into additive genetic and permanent environmental variance, and the estimation of repeatabilities (τ).

To estimate additive genetic (co)variance components we used the additive genetic relatedness matrix (Kruuk 2004) to determine the variance-covariance matrix to be associated with the models. We found that size did not explain a significant proportion of the variation in any of the traits. Size was initially included as a fixed factor, however the statistical conclusions were unaffected by its removal and to simplify the reporting size was not included as a fixed factor in the analyses presented here.

\[ V_A \] (additive genetic variance), \( V_R \) (residual variance) and \( V_{PE} \) (variance due to permanent environmental effects, i.e. the repeated measures) were calculated from univariate animal models. Narrow-sense heritabilities (\( h^2 \)) were calculated as \( V_A/(V_A + V_R + V_{PE}) \), while repeatabilities (\( \tau \)) were calculated as \( (V_A + V_{PE})/(V_A + V_R + V_{PE}) \) (Dochtermann et al. 2014). We used REML likelihood ratio tests (using 1 degree of freedom) (Meyer 1992; Gilmour et al. 2015) to determine the significance of the additive genetic variance components and the repeatabilities. For comparison between the two environments (acoustic and silent), we first estimated repeatabilities and heritabilities separately for each environment. Paired \( t \)-tests were then used to check for differences in quantitative genetic parameters between environments (the heritability of total time in the arena could not be estimated for the acoustic environment since it exhibited additive genetic variance close to zero). Additionally, we conducted paired \( t \)-tests for differences in the individual variance components (\( V_A \), \( V_R \), and \( V_{PE} \)) between environments to assess which of them drove the between-environmental differences in repeatabilities and heritabilities. All data were then pooled and ‘environment’ included as a fixed factor to test whether trait means differed significantly between environments using Wald F statistics (Gilmour 2015). To obtain measures of evolvability we standardized the additive genetic variation by the trait mean, giving us the coefficients of additive genetic variance (\( CV_A \)) (Houle 1992; Garcia-Gonzalez et al. 2012):

\[ CV_A = \frac{\sqrt{V_A}}{X} \]

Additionally, additive genetic variance standardized by the squared trait mean (\( I_A \)) was obtained (Houle 1992; Garcia-Gonzalez et al. 2012):

\[ I_A = \frac{V_A}{X^2} \]
These two mean-standardized additive genetic variances were also calculated for the residual variances (CV\textsubscript{R} and l\textsubscript{RI}) and the permanent environment variances (CV\textsubscript{PE} and l\textsubscript{PPE}) and were calculated using the same formulae but replacing V\textsubscript{A} with V\textsubscript{PE} and l\textsubscript{PE} respectively.

Genotype-by-environment effects for the different traits were tested by calculating cross-environment genetic correlations for each trait. Thus, we included an interaction term (environment by individual identity; the individual identity term provides information on each individual’s relatedness to all other individuals in the pedigree) as an additional random factor in the models. The data sets for both environments were pooled and a univariate model fitted with environment as a fixed effect. The significance of the interaction term (significance indicating a genotype by environment effect) was tested with likelihood ratio tests (with 2 degrees of freedom because of the additional interaction term; Andrews et al. 2017) comparing the complete models to models in which the interaction term was excluded.

Genotypic and phenotypic correlations were calculated for each environment separately. Genotypic correlations (r\textsubscript{A}) were estimated by dividing the additive genetic covariances between different traits (e.g. additive genetic covariance between trait X and trait Y: Cov\textsubscript{AXY}) by the square root product of the additive genetic variances of the individual traits (V\textsubscript{AX} and V\textsubscript{AY}):

\[
r_{A} = \frac{Cov_{AXY}}{\sqrt{V_{AX} \times V_{AY}}}
\]

Phenotypic correlations (r\textsubscript{P}) were calculated by dividing the sum of all covariances between different traits (residual, additive genetic and repeated measures) by the square root product of the sum of all variances of the individual traits:

\[
r_{P} = \frac{Cov_{AXY} + Cov_{AXY} + Cov_{PEXY}}{\sqrt{(V_{AX} + V_{AX} + V_{PEX}) \times (V_{AX} + V_{AX} + V_{PEY})}} = \frac{Cov_{APY}}{\sqrt{V_{AX} \times V_{AX}}}
\]

This resulted in two pairs of variance-covariance matrices (silent and acoustic additive genetic correlations; silent and acoustic phenotypic correlations). To test whether the two covariance matrices within each pair were similar or dissimilar we ran Mantel tests (Mantel 1967; Roff et al. 2012). Likewise, we tested similarity between the two acoustic covariance matrices and the two silent covariance matrices. The Mantel test is non-parametric and calculates the correlation (and its significance) between two matrices through permutations of the rows and columns of one of the input matrices. The test statistic is the Pearson product-moment correlation. Note that pairs in which at least one matrix component had a missing value were ignored. We used the Excel add-in XLSTAT v19.5 to run Mantel tests.
To estimate the contribution of additive genetic variation to the variation in between-individual behavioral differences (i.e. ‘personality’ differences) we calculated the proportion of personality variation (permanent environmental variation: $V_{PE}$) that can be attributed to additive genetic variation with $V_A/(V_A + V_{PE})$. This equation gave us among-individual variation ($V_A + V_{PE} = V_{ind}$) and its heritability ($h^2_{ind}$) and can be considered the heritability of personality (Dochtermann et al. 2014). Essentially, this is the same as dividing the heritability of each trait by its repeatability (Dochtermann et al. 2014). We considered $h^2_{ind}$ estimates significant when their 95% confidence intervals did not overlap zero. These calculations were done for the silent and the acoustic environments separately and the $h^2_{ind}$ then compared with a paired t-test.

3.4 Results

3.4.1 Data summary

For all behavioural measures analyzed in this study, there were significant differences between individuals exposed to the silent environment and individuals exposed to male acoustic sexual signals (Table 1). Distance moved, average velocity, time spent moving, time spent ‘far’ and total time spent in the arena were all greater for crickets held in silence than those exposed to acoustic sexual signals. Latency to emerge and time spent ‘near’ were greater for crickets experiencing acoustic sexual signals than those experiencing silence (Table 1). In the silent environment, crickets failed to emerge in 65 out of 1196 trials (5.43%) while crickets failed to emerge in 159 out of 1214 trials (13.09%) in the acoustic environment (the difference between these two groups was significant: $\chi^2 = 33.783, P < 0.001$). Crickets (including those that did not emerge) never ventured into ‘far’ during 88 of 1196 trials (7.36%) in the silent environment and during 233 of 1214 trials (19.19%) in the acoustic environment (the difference between these two groups was significant: $\chi^2 = 73.089, P < 0.001$). Thus, the sample size for time spent ‘far’ was lower than that of the other measures (Table 1).

3.4.2 Repeatabilities and heritabilities

Across both environments, the repeatabilities of all behavioural measures were significant (Table 2). In the silent environment, repeatabilities ranged from 0.336 to 0.585; in the acoustic environment, they ranged from 0.381 to 0.621 (Table 2). There was no significant difference between the repeatabilities in the silent (mean ± SE: 0.42 ± 0.032) and the acoustic environment (0.48 ± 0.033) (paired t6 = -1.924, $P = 0.103$).

Narrow sense heritabilities for the behavioural measures were relatively low (though all were significant with the exception of time spent ‘near’) in the silent environment, with only average velocity above 0.2 (Table 2). In the acoustic environment all measures except total time in the arena
(which could not be estimated) showed significant heritabilities and of those that could be estimated only time spent ‘far’ and time spent moving were below 0.2 (Table 2). Heritabilities differed significantly between environments (paired $t_5 = 6.629, P < 0.001$), with those in the acoustic ($0.227 \pm 0.016$) being higher than those in the silent environment ($0.114 \pm 0.023$) (Table 2). Furthermore, the coefficients of additive genetic variance, $CVA$, were significantly higher in the acoustic ($0.028 \pm 0.006$) than in the silent environment ($0.013 \pm 0.004$) (paired $t_5 = 5.815, P = 0.002$). Accordingly, IAs were significantly higher in the acoustic ($0.165 \pm 0.016$) than in the silent environment ($0.110 \pm 0.018$) ($t_5 = 5.992, P = 0.002$). Mean ± SE pronotum width was $6.24 \pm 0.010$ and its heritability was $0.392 \pm 0.075$ ($h^2 \pm SE$), with $VA (SE) = 0.049 (0.012)$, $VR (SE) = 0.077 (0.008)$, $CVA = 0.036$, $CVR = 0.044$, $IA = 0.001$ and $IR = 0.002$. We found no significant differences in individual variance components ($VA$, $VR$, and $VPE$) between environments ($VA$: paired $t_5 = -1.533, P = 0.186$; $VR$: paired $t_6 = 1.169, P = 0.287$; $VPE$: paired $t_6 = -0.813, P = 0.447$).

3.4.3 Genotype by environment interactions

We found significant genotype-by-environment interactions for all but one behavioural trait: latency to emerge (Table 3). Interaction coefficients (SE) ranged from $0.061 (0.028)$ to $0.215 (0.067)$.

3.4.4 Comparing trait correlations

In the silent environment, phenotypic correlations ($r_P$), calculated from repeated measures animal models, were significant in 18 of 20 cases for which phenotypic correlations could be estimated (Table 4). Of the significant correlations, 16 were positive and 2 were negative (Table 4). In the acoustic environment, phenotypic correlations were significant in 16 of 20 cases and of the significant correlations, 12 were positive and 4 negative.

Of the 20 genetic correlations ($r_A$) that were estimable in the silent environment, 4 were significant and positive (Table 5). In the acoustic environment, we were able to estimate 13 genetic correlations of which 12 were significant and positive (Table 5). Some correlations were inestimable because either one or both of the traits had very low additive genetic variances.

Comparing the two phenotypic covariance matrices using the Mantel test revealed that they were highly correlated (i.e. high degree of similarity) ($r = 0.863, P < 0.001$). In contrast, the genetic covariance matrices were not significantly correlated and therefore highly dissimilar ($r = 0.243, P = 0.482$). The acoustic phenotypic and the acoustic genetic covariance matrices were strongly correlated ($r = 0.989, P < 0.001$) while there was no correlation between the silent phenotypic and the silent genetic covariance matrices ($r = 0.225, P = 0.340$).
3.4.5 Heritability of personality

Within the silent environment, the heritabilities of the repeatable components ($h^2_{ind}$) of all but one trait (time spent ‘near’) were significant, ranging from 0.16 to 0.51 (mean ± SE: 0.27 ± 0.04) (Table 6). In the acoustic environment, all of the estimable traits (total time in the arena could not be estimated) showed significant $h^2_{ind}$, ranging from 0.40 to 0.58 (mean ± SE: 0.48 ± 0.03) (Table 6). There was a significant difference in $h^2_{ind}$ between environments (mean difference ± SE = 0.215 ± 0.045), paired $t_5 = -4.772$, $P = 0.005$). There was a significant difference between repeatabilities and $h^2_{ind}$ in the silent environment (0.149 ± 0.057, $t_6 = 2.594$, $P = 0.041$) but not in the acoustic environment (0.009 ± 0.059, $t_5 = -0.151$, $P = 0.886$).
Table 1. Summary statistics and effects of the environment (silent or acoustic) from univariate animal models of each trait. The table shows sample sizes and means (with SE) for each trait. Additionally, the effect of the environment on the trait means and its significance is given (Wald $F$ statistic). Because there were two measurements for each trait (week 1 and week 2), this table shows sample size, mean and SE averaged across the two measurements. Summary statistics for the two measurements separately can be found in the supplementary material (Supplementary Tables 1 and 2).

<table>
<thead>
<tr>
<th>Trait</th>
<th>$N$ silent</th>
<th>$N$ acoustic</th>
<th>$N$ combined</th>
<th>mean (SE) silent</th>
<th>mean (SE) acoustic</th>
<th>mean (SE) combined</th>
<th>Environment $F$</th>
<th>Environment $P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latency to emerge (s)</td>
<td>566</td>
<td>527</td>
<td>1093</td>
<td>144.92 (3.15)</td>
<td>195.33 (3.93)</td>
<td>169.25 (2.62)</td>
<td>154.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Distance (cm)</td>
<td>566</td>
<td>527</td>
<td>1093</td>
<td>168.45 (2.05)</td>
<td>143.55 (1.66)</td>
<td>94.50 (1.27)</td>
<td>128.29</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Velocity (cm/s)</td>
<td>566</td>
<td>527</td>
<td>1093</td>
<td>1.23 (0.01)</td>
<td>1.12 (0.01)</td>
<td>1.18 (0.01)</td>
<td>62.91</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time spent moving (s)</td>
<td>566</td>
<td>527</td>
<td>1093</td>
<td>127.60 (1.26)</td>
<td>96.22 (1.50)</td>
<td>112.46 (1.08)</td>
<td>378.97</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time spent ‘near’ (s)</td>
<td>566</td>
<td>527</td>
<td>1093</td>
<td>92.55 (1.20)</td>
<td>117.19 (1.74)</td>
<td>104.44 (1.11)</td>
<td>213.98</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time spent ‘far’ (s)</td>
<td>554</td>
<td>490</td>
<td>1044</td>
<td>130.45 (2.16)</td>
<td>91.86 (1.73)</td>
<td>112.32 (1.53)</td>
<td>289.31</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total time in arena (s)</td>
<td>566</td>
<td>527</td>
<td>1093</td>
<td>220.34 (2.36)</td>
<td>202.59 (2.28)</td>
<td>211.78 (1.67)</td>
<td>43.81</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Table 2. Quantitative genetic parameters and repeatabilities (τ) estimated from repeated measures animal models for seven behavioural traits in male *T. oceanicus* exposed to a silent environment and exposed to acoustic sexual signals. All traits were measured twice (after one week and after two weeks). The table shows the different variance components (permanent environmental \(V_{PE}\), additive genetic \(V_A\) and residual \(V_R\)) and their standard errors for all behavioural traits. Repeatabilities (τ) and heritabilities (\(h^2\)) with standard errors are bolded where significant. Different mean-standardised measures of genetic variation (frequently used as measures of evolutionary potential or evolvability) are also given (\(CV_{PE}\), \(CV_A\), \(CV_R\), \(I_{PE}\), \(I_A\), and \(I_R\)).

### Silent

<table>
<thead>
<tr>
<th>Trait</th>
<th>(V_{PE}) (SE)</th>
<th>(V_A) (SE)</th>
<th>(V_R) (SE)</th>
<th>τ (SE)</th>
<th>(h^2) (SE)</th>
<th>(CV_{PE})</th>
<th>(CV_A)</th>
<th>(CV_R)</th>
<th>(I_{PE})</th>
<th>(I_A)</th>
<th>(I_R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latency to emerge</td>
<td>2618.73 (340.98)</td>
<td>660.69 (320.72)</td>
<td>2325.41 (140.25)</td>
<td>0.585 (0.028)</td>
<td>0.118 (0.055)</td>
<td>0.353</td>
<td>0.177</td>
<td>0.333</td>
<td>0.125</td>
<td>0.031</td>
<td>0.111</td>
</tr>
<tr>
<td>Distance</td>
<td>767.81 (129.26)</td>
<td>215.47 (105.11)</td>
<td>1405.90 (85.52)</td>
<td>0.412 (0.036)</td>
<td>0.090 (0.043)</td>
<td>0.111</td>
<td>0.115</td>
<td>0.186</td>
<td>0.012</td>
<td>0.013</td>
<td>0.035</td>
</tr>
<tr>
<td>Velocity</td>
<td>0.02 (0.01)</td>
<td>0.02 (0.01)</td>
<td>0.05 (0.00)</td>
<td>0.426 (0.036)</td>
<td>0.221 (0.064)</td>
<td>0.111</td>
<td>0.115</td>
<td>0.186</td>
<td>0.012</td>
<td>0.013</td>
<td>0.035</td>
</tr>
<tr>
<td>Time spent moving</td>
<td>203.10 (46.80)</td>
<td>92.59 (40.43)</td>
<td>587.01 (35.51)</td>
<td>0.336 (0.039)</td>
<td>0.105 (0.044)</td>
<td>0.112</td>
<td>0.075</td>
<td>0.190</td>
<td>0.012</td>
<td>0.006</td>
<td>0.036</td>
</tr>
<tr>
<td>Time spent ‘near’</td>
<td>234.07 (41.36)</td>
<td>43.57 (26.73)</td>
<td>541.07 (32.77)</td>
<td>0.339 (0.038)</td>
<td>0.053 (0.032)</td>
<td>0.165</td>
<td>0.071</td>
<td>0.251</td>
<td>0.027</td>
<td>0.005</td>
<td>0.063</td>
</tr>
<tr>
<td>Time spent ‘far’</td>
<td>865.94 (142.89)</td>
<td>255.22 (120.39)</td>
<td>1462.69 (90.12)</td>
<td>0.462 (0.036)</td>
<td>0.099 (0.045)</td>
<td>0.226</td>
<td>0.122</td>
<td>0.293</td>
<td>0.051</td>
<td>0.015</td>
<td>0.086</td>
</tr>
<tr>
<td>Total time in arena</td>
<td>633.20 (167.07)</td>
<td>348.16 (150.07)</td>
<td>2180.29 (131.11)</td>
<td>0.382 (0.039)</td>
<td>0.111 (0.046)</td>
<td>0.114</td>
<td>0.085</td>
<td>0.212</td>
<td>0.013</td>
<td>0.007</td>
<td>0.045</td>
</tr>
</tbody>
</table>

### Acoustic

<table>
<thead>
<tr>
<th>Trait</th>
<th>(V_{PE}) (SE)</th>
<th>(V_A) (SE)</th>
<th>(V_R) (SE)</th>
<th>τ (SE)</th>
<th>(h^2) (SE)</th>
<th>(CV_{PE})</th>
<th>(CV_A)</th>
<th>(CV_R)</th>
<th>(I_{PE})</th>
<th>(I_A)</th>
<th>(I_R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latency to emerge</td>
<td>3357.14 (541.47)</td>
<td>1986.69 (651.37)</td>
<td>2706.03 (173.80)</td>
<td>0.621 (0.027)</td>
<td>0.247 (0.074)</td>
<td>0.297</td>
<td>0.228</td>
<td>0.266</td>
<td>0.088</td>
<td>0.052</td>
<td>0.071</td>
</tr>
<tr>
<td>Distance</td>
<td>436.77 (94.54)</td>
<td>354.75 (113.34)</td>
<td>669.64 (43.43)</td>
<td>0.542 (0.034)</td>
<td>0.243 (0.071)</td>
<td>0.146</td>
<td>0.131</td>
<td>0.180</td>
<td>0.021</td>
<td>0.017</td>
<td>0.032</td>
</tr>
<tr>
<td>Velocity</td>
<td>0.02 (0.01)</td>
<td>0.03 (0.01)</td>
<td>0.04 (0.00)</td>
<td>0.487 (0.029)</td>
<td>0.287 (0.074)</td>
<td>0.131</td>
<td>0.137</td>
<td>0.172</td>
<td>0.017</td>
<td>0.019</td>
<td>0.030</td>
</tr>
<tr>
<td>Time spent moving</td>
<td>340.91 (69.86)</td>
<td>220.84 (74.61)</td>
<td>614.18 (39.47)</td>
<td>0.426 (0.036)</td>
<td>0.188 (0.059)</td>
<td>0.192</td>
<td>0.154</td>
<td>0.258</td>
<td>0.037</td>
<td>0.024</td>
<td>0.066</td>
</tr>
<tr>
<td>Time spent ‘near’</td>
<td>524.77 (99.58)</td>
<td>343.00 (114.33)</td>
<td>707.84 (45.43)</td>
<td>0.393 (0.040)</td>
<td>0.218 (0.069)</td>
<td>0.195</td>
<td>0.158</td>
<td>0.227</td>
<td>0.038</td>
<td>0.025</td>
<td>0.052</td>
</tr>
<tr>
<td>Time spent ‘far’</td>
<td>478.40 (93.80)</td>
<td>263.25 (97.86)</td>
<td>722.09 (49.73)</td>
<td>0.381 (0.032)</td>
<td>0.180 (0.063)</td>
<td>0.238</td>
<td>0.177</td>
<td>0.293</td>
<td>0.057</td>
<td>0.031</td>
<td>0.086</td>
</tr>
<tr>
<td>Total time in arena</td>
<td>1053.16 (129.86)</td>
<td>NA</td>
<td>1680.75 (107.26)</td>
<td>0.424 (0.039)</td>
<td>NA</td>
<td>0.160</td>
<td>NA</td>
<td>0.202</td>
<td>0.026</td>
<td>NA</td>
<td>0.041</td>
</tr>
</tbody>
</table>
Table 3. The table shows interaction (environment by genotype) coefficients and the variance components of the interaction for the different behavioural traits. Bolded coefficients indicate significant interactions, tested by comparing the log likelihoods (df = 2) of models where the interaction term was excluded and models where it was present. Significant coefficients indicate genotype by environment interactions.

<table>
<thead>
<tr>
<th>Trait</th>
<th>$V_{\text{env.ID}}$ (SE)</th>
<th>env.ID (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latency to emerge</td>
<td>501.34 (332.01)</td>
<td>0.074 (0.049)</td>
</tr>
<tr>
<td>Distance</td>
<td>275.62 (75.93)</td>
<td><strong>0.142 (0.037)</strong></td>
</tr>
<tr>
<td>Velocity</td>
<td>0.02 (0.01)</td>
<td><strong>0.215 (0.067)</strong></td>
</tr>
<tr>
<td>Time spent moving</td>
<td>141.08 (54.47)</td>
<td><strong>0.138 (0.052)</strong></td>
</tr>
<tr>
<td>Time spent ‘near’</td>
<td>159.02 (64.91)</td>
<td><strong>0.134 (0.055)</strong></td>
</tr>
<tr>
<td>Time spent ‘far’</td>
<td>181.70 (96.65)</td>
<td><strong>0.089 (0.047)</strong></td>
</tr>
<tr>
<td>Total time in arena</td>
<td>179.63 (82.40)</td>
<td><strong>0.061 (0.028)</strong></td>
</tr>
</tbody>
</table>
Table 4. Phenotypic models with trait-by-permanent-environmental-effects as random effects. Tables show the variance-covariance-correlation matrices for each environment (silent or acoustic), with the phenotypic variances on the diagonal (italics), the phenotypic correlations above the diagonal and the phenotypic covariances below the diagonal. Standard errors (SE) for each value are shown in brackets. Significance is indicated by bolded font. Blank cells indicate that the covariance could not be estimated for the trait combination (due to small variance components).

### Silent

<table>
<thead>
<tr>
<th>Trait</th>
<th>Latency to emerge</th>
<th>Distance</th>
<th>Velocity</th>
<th>Time spent moving</th>
<th>Time spent ‘near’</th>
<th>Time spent ‘far’</th>
<th>Total time in arena</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latency to emerge</td>
<td>5616.33 (282.56)</td>
<td>-0.44 (0.03)</td>
<td>-0.29 (0.03)</td>
<td>-0.27 (0.04)</td>
<td>-0.40 (0.03)</td>
<td>-0.31 (0.03)</td>
<td>-0.05 (0.04)</td>
</tr>
<tr>
<td>Distance</td>
<td>-1619.10 (136.09)</td>
<td>2388.46 (110.59)</td>
<td>0.48 (0.03)</td>
<td>0.33 (0.05)</td>
<td>-0.30 (0.03)</td>
<td>0.38 (0.03)</td>
<td>0.30 (0.03)</td>
</tr>
<tr>
<td>Velocity</td>
<td>-6.63 (0.84)</td>
<td>7.02 (0.54)</td>
<td>0.09 (0.01)</td>
<td>0.27 (0.03)</td>
<td>-0.14 (0.03)</td>
<td>0.24 (0.04)</td>
<td>0.15 (0.06)</td>
</tr>
<tr>
<td>Time spent moving</td>
<td>-602.64 (78.26)</td>
<td>490.56 (114.17)</td>
<td>2.38 (0.31)</td>
<td>883.36 (40.24)</td>
<td>-0.17 (0.03)</td>
<td>0.28 (0.03)</td>
<td>0.16 (0.03)</td>
</tr>
<tr>
<td>Time spent ‘near’</td>
<td>-953.03 (78.67)</td>
<td>-416.82 (47.35)</td>
<td>-1.27 (0.27)</td>
<td>-146.27 (27.45)</td>
<td>822.39 (36.75)</td>
<td>-0.19 (0.03)</td>
<td>-0.34 (0.03)</td>
</tr>
<tr>
<td>Time spent ‘far’</td>
<td>-1172.00 (138.87)</td>
<td>953.30 (101.27)</td>
<td>3.78 (0.59)</td>
<td>417.12 (51.71)</td>
<td>-278.13 (48.45)</td>
<td>2586.22 (122.21)</td>
<td></td>
</tr>
<tr>
<td>Total time in arena</td>
<td>-201.54 (143.49)</td>
<td>823.28 (93.01)</td>
<td>2.58 (0.87)</td>
<td>271.33 (54.60)</td>
<td>-553.67 (53.77)</td>
<td></td>
<td>3163.12 (143.48)</td>
</tr>
</tbody>
</table>

### Acoustic

<table>
<thead>
<tr>
<th>Trait</th>
<th>Latency to emerge</th>
<th>Distance</th>
<th>Velocity</th>
<th>Time spent moving</th>
<th>Time spent ‘near’</th>
<th>Time spent ‘far’</th>
<th>Total time in arena</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latency to emerge</td>
<td>8012.19 (445.67)</td>
<td>-0.47 (0.03)</td>
<td>-0.34 (0.03)</td>
<td>-0.38 (0.03)</td>
<td>-0.59 (0.02)</td>
<td>-0.27 (0.04)</td>
<td>-0.11 (0.05)</td>
</tr>
<tr>
<td>Distance</td>
<td>-1602.60 (149.89)</td>
<td>1455.81 (78.02)</td>
<td>0.46 (0.03)</td>
<td>0.45 (0.03)</td>
<td>-0.34 (0.03)</td>
<td>0.33 (0.03)</td>
<td>0.02 (0.03)</td>
</tr>
<tr>
<td>Velocity</td>
<td>-8.65 (1.10)</td>
<td>5.00 (0.48)</td>
<td>0.08 (0.01)</td>
<td>0.31 (0.03)</td>
<td>-0.23 (0.04)</td>
<td>0.28 (0.04)</td>
<td>0.00 (0.03)</td>
</tr>
<tr>
<td>Time spent moving</td>
<td>-1174.40 (127.96)</td>
<td>588.75 (54.42)</td>
<td>3.07 (0.39)</td>
<td>1173.84 (59.38)</td>
<td>-0.26 (0.03)</td>
<td>0.25 (0.03)</td>
<td>0.01 (0.04)</td>
</tr>
<tr>
<td>Time spent ‘near’</td>
<td>-2078.40 (166.28)</td>
<td>-505.42 (62.32)</td>
<td>-2.62 (0.46)</td>
<td>-353.56 (52.93)</td>
<td>1571.76 (82.96)</td>
<td>0.26 (0.04)</td>
<td>-0.57 (0.02)</td>
</tr>
<tr>
<td>Time spent ‘far’</td>
<td>-938.60 (149.79)</td>
<td>481.82 (51.07)</td>
<td>3.09 (0.45)</td>
<td>329.90 (45.09)</td>
<td>-338.24 (61.88)</td>
<td>1456.58 (76.25)</td>
<td></td>
</tr>
<tr>
<td>Total time in arena</td>
<td>-504.78 (197.64)</td>
<td>42.90 (68.02)</td>
<td>0.03 (0.51)</td>
<td>14.05 (60.17)</td>
<td>-1181.20 (79.60)</td>
<td></td>
<td>2744.43 (127.92)</td>
</tr>
</tbody>
</table>
Table 5. Tables show the variance-covariance-correlation matrices derived from multivariate animal models for each environment (silent or acoustic), with the genetic variances on the diagonal (italics), the genetic correlations above the diagonal and the genetic covariances below the diagonal. Standard errors (SE) for each value are shown in brackets. Significance is indicated by bolded font. Blank cells indicate that the covariance could not be estimated for the trait combination (due to small variance components).

<table>
<thead>
<tr>
<th>Silent</th>
<th>Latency to emerge</th>
<th>Distance</th>
<th>Velocity</th>
<th>Time spent moving</th>
<th>Time spent ‘near’</th>
<th>Time spent ‘far’</th>
<th>Total time in arena</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trait</td>
<td>660.69 (320.72)</td>
<td>-0.20 (0.42)</td>
<td>-0.68 (0.20)</td>
<td>-0.50 (0.26)</td>
<td>-0.23 (0.39)</td>
<td>-0.69 (0.22)</td>
<td>-0.66 (0.25)</td>
</tr>
<tr>
<td>Latency to emerge</td>
<td>67.55 (127.46)</td>
<td>215.48 (105.11)</td>
<td>-0.14 (0.31)</td>
<td>0.15 (0.31)</td>
<td>0.62 (0.50)</td>
<td>-0.21 (0.38)</td>
<td>0.09 (0.32)</td>
</tr>
<tr>
<td>Distance</td>
<td>2.37 (1.10)</td>
<td>-0.26 (0.55)</td>
<td>0.02 (0.01)</td>
<td>-0.05 (0.27)</td>
<td>0.25 (0.33)</td>
<td>0.41 (0.24)</td>
<td><strong>0.48 (0.23)</strong></td>
</tr>
<tr>
<td>Velocity</td>
<td>125.47 (87.13)</td>
<td>20.80 (48.35)</td>
<td>-0.07 (0.37)</td>
<td>92.59 (40.43)</td>
<td>0.27 (0.42)</td>
<td>-0.19 (0.33)</td>
<td>-0.17 (0.30)</td>
</tr>
<tr>
<td>Time spent moving</td>
<td>-46.54 (71.60)</td>
<td>-55.25 (36.59)</td>
<td>-0.24 (0.30)</td>
<td>-16.15 (23.08)</td>
<td>43.57 (26.73)</td>
<td>-0.05 (0.38)</td>
<td>-0.46 (0.30)</td>
</tr>
<tr>
<td>Time spent ‘near’</td>
<td><strong>286.07 (156.32)</strong></td>
<td>-51.95 (82.46)</td>
<td>0.94 (0.67)</td>
<td>-30.53 (50.05)</td>
<td>5.78 (41.27)</td>
<td>255.22 (120.39)</td>
<td></td>
</tr>
<tr>
<td>Time spent ‘far’</td>
<td>345.18 (170.04)</td>
<td>26.21 (90.35)</td>
<td><strong>1.24 (0.72)</strong></td>
<td>-31.73 (55.67)</td>
<td>58.03 (48.46)</td>
<td><strong>348.16 (150.07)</strong></td>
<td></td>
</tr>
</tbody>
</table>

Total time in arena

<table>
<thead>
<tr>
<th>Acoustic</th>
<th>Latency to emerge</th>
<th>Distance</th>
<th>Velocity</th>
<th>Time spent moving</th>
<th>Time spent ‘near’</th>
<th>Time spent ‘far’</th>
<th>Total time in arena</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trait</td>
<td>1986.69 (651.37)</td>
<td>-0.85 (0.10)</td>
<td>-0.72 (0.14)</td>
<td>-0.79 (0.12)</td>
<td>0.81 (0.09)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latency to emerge</td>
<td>704.43 (214.11)</td>
<td>354.75 (113.34)</td>
<td>0.68 (0.13)</td>
<td>0.67 (0.15)</td>
<td>-0.74 (0.13)</td>
<td>0.80 (0.14)</td>
<td></td>
</tr>
<tr>
<td>Distance</td>
<td>4.71 (1.64)</td>
<td>1.94 (0.71)</td>
<td>0.03 (0.01)</td>
<td><strong>0.38 (0.19)</strong></td>
<td>-0.36 (0.20)</td>
<td>0.65 (0.17)</td>
<td></td>
</tr>
<tr>
<td>Velocity</td>
<td>523.54 (174.35)</td>
<td>182.67 (73.36)</td>
<td>0.87 (0.54)</td>
<td><strong>220.84 (74.61)</strong></td>
<td>-0.72 (0.14)</td>
<td>0.77 (0.12)</td>
<td></td>
</tr>
<tr>
<td>Time spent moving</td>
<td>712.96 (223.75)</td>
<td>269.73 (87.86)</td>
<td>0.95 (0.65)</td>
<td><strong>211.11 (71.56)</strong></td>
<td><strong>343.00 (114.33)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time spent ‘near’</td>
<td>242.13 (80.44)</td>
<td>1.56 (0.62)</td>
<td><strong>202.90 (68.78)</strong></td>
<td><strong>263.25 (97.86)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time spent ‘far’</td>
<td>242.13 (80.44)</td>
<td>1.56 (0.62)</td>
<td><strong>202.90 (68.78)</strong></td>
<td><strong>263.25 (97.86)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 6. The table shows the contribution of additive genetic variation to variation in between-individual behavioural differences, i.e. the heritability of among-individual variation ($h^2_{\text{ind}}$) for each behavioural trait, within the two environments. The variance of among-individual variation for each trait is also given ($V_{\text{ind}}$). Standard errors are given in brackets (SE).

Silent

<table>
<thead>
<tr>
<th>Trait</th>
<th>$V_{\text{ind}}$ (SE)</th>
<th>$h^2_{\text{ind}}$ (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latency to emerge</td>
<td>3279.40 (280.54)</td>
<td>0.202 (0.093)</td>
</tr>
<tr>
<td>Distance</td>
<td>983.29 (112.63)</td>
<td>0.219 (0.102)</td>
</tr>
<tr>
<td>Velocity</td>
<td>0.04 (0.00)</td>
<td>0.506 (0.137)</td>
</tr>
<tr>
<td>Time spent moving</td>
<td>296.39 (40.82)</td>
<td>0.314 (0.129)</td>
</tr>
<tr>
<td>Time spent ‘near’</td>
<td>277.64 (37.16)</td>
<td>0.157 (0.094)</td>
</tr>
<tr>
<td>Time spent ‘far’</td>
<td>1121.20 (123.68)</td>
<td>0.217 (0.102)</td>
</tr>
<tr>
<td>Total time in arena</td>
<td>980.42 (144.44)</td>
<td>0.288 (0.123)</td>
</tr>
</tbody>
</table>

Acoustic

<table>
<thead>
<tr>
<th>Trait</th>
<th>$V_{\text{ind}}$ (SE)</th>
<th>$h^2_{\text{ind}}$ (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latency to emerge</td>
<td>5341.00 (457.01)</td>
<td>0.397 (0.107)</td>
</tr>
<tr>
<td>Distance</td>
<td>791.00 (80.99)</td>
<td>0.449 (0.123)</td>
</tr>
<tr>
<td>Velocity</td>
<td>0.04 (0.00)</td>
<td>0.576 (0.125)</td>
</tr>
<tr>
<td>Time spent moving</td>
<td>560.80 (61.27)</td>
<td>0.433 (0.119)</td>
</tr>
<tr>
<td>Time spent ‘near’</td>
<td>867.21 (84.94)</td>
<td>0.547 (0.116)</td>
</tr>
<tr>
<td>Time spent ‘far’</td>
<td>741.59 (80.12)</td>
<td>0.466 (0.119)</td>
</tr>
<tr>
<td>Total time in arena</td>
<td>1038.90 (129.41)</td>
<td>NA</td>
</tr>
</tbody>
</table>
3.5 Discussion

Animal behaviour and its evolutionary trajectories are predicted to be strongly affected by the social environment (McGlothlin et al. 2010; Bailey et al. 2017). Here, we found that variation in the social environment (the presence or absence of male acoustic sexual signals) had substantial effects on estimates of behavioural heritability and evolvability in the Australian field cricket (*Teleogryllus oceanicus*). While the repeatability of behaviours across environments was similar, their heritabilities and two mean-standardized measures of evolvability were significantly higher in the acoustic than in the silent environment. Within both environments, we found significant genotype-by-environment interactions for most behaviours measured. Phenotypic covariance (correlations) matrices did not differ between environments, yet genetic covariance (correlations) matrices did. Within both environments, there was significant heritability of the repeatable aspect (‘personality’) of most behaviours, but these heritabilities were higher in the acoustic than in the silent environment.

3.5.1 Effects of the social environment on behavioural expression

Behavioural plasticity allows individuals to respond to environmental stimuli, including social cues. In the presence of calling males, searching behaviour in crickets is expected to decrease as they form clusters in areas in which other males are calling (Campbell and Shipp 1979; e.g. Cade 1981). Such clusters are comparable to ‘lek’ type mating systems (Alexander 1975) in which females, attracted by male acoustic sexual signals (Ulagaraj and Walker 1973), compare males to choose mates (Beehler and Foster 1988). Indeed, male *T. oceanicus* reared in silent conditions have been found to be more likely to move about, seeking other calling males, compared to males reared in the presence of male acoustic signals (Bailey et al. 2010). Male behaviour may thus be a response to female behaviour. We found crickets displayed behaviours that indicate greater boldness, exploration, and activity (sensu Carter et al. 2013) when kept in a silent environment compared to crickets held in the presence of acoustic sexual signals. This indicates that there is a considerable amount of plasticity in behavioural responses to male acoustic sexual signals, a finding in line with previous studies (e.g. Cade 1981; Bailey et al. 2010; DiRienzo et al. 2012; Rudin et al. 2018).

Repeatability reflects both within-individual consistency and between-individual variation (Nakagawa and Schielzeth 2010). On average, the repeatability of animal behaviour has been found to be around 0.37 (Bell et al. 2009), and we found repeatabilities consistent with the average of 0.36 reported for insects (Bell et al. 2009). While repeatabilities in the wild tend to be higher than in laboratory settings (Bell et al. 2009), behavioural repeatabilities in another field cricket (*Gryllus campestris*) were found to range from 0.06 to 0.21 (Fisher et al. 2015), somewhat lower than those found here. Repeatabilities were comparable across environments, suggesting that between-individual differences are relatively
stable regardless of variation in the social environment (acoustic sexual signals). This finding replicates those of our previous study in which we found behavioural repeatabilities to be relatively stable across acoustic and silent environments (Rudin et al. 2018). Conversely, our finding contradicts some other studies which found that, for example, in sticklebacks (Gasterosteus aculeatus) the repeatability of boldness was affected by social conditions (Jolles et al. 2016) and in marmosets (Callithrix jacchus) explorative tendencies were less repeatable when individuals were tested alone compared to individuals tested in the presence of conspecifics (Koski and Burkart 2015).

3.5.2 Effects of the social environment on evolvability

The behaviours measured here relate to commonly used personality traits (behaviours that exhibit repeatable between-individual differences) such as boldness and exploration. The heritabilities we report are consistent with the range of heritability estimates for similar traits in nonhuman animals (reviewed by van Oers et al. 2005). One might expect these behaviours to have substantial fitness consequences since they may be involved in mate finding, foraging or predator avoidance. Indeed, Santostefano et al. (2017) found that in Mediterranean field crickets (Gryllus bimaculatus), exploratory tendencies were predictive of shorter lifespans, even in the absence of predators. Concerns have been raised about the applicability of heritability estimates obtained in laboratory settings to those that can be expected under field conditions. However, a meta-analysis of field and laboratory-derived heritabilities of life-history, morphological and behavioural traits found no difference in magnitude and significance between heritabilities estimated in natural and laboratory settings (Weigensberg and Roff 1996).

We found significant additive genetic variance for most behaviours when measured in both acoustic and silent environments. However, the elevated heritabilities and evolvabilities found in the acoustic environment indicate that the social environment has the potential to substantially alter the evolutionary trajectories of behaviours. Interestingly, none of the individual variance components used to calculate heritabilities (VA, VR, and VPE) differed significantly between environments. Thus, no single component can be considered the primary driver of the difference in heritabilities between environments. Phenotypic evolution is predicted to be affected by ‘interacting phenotypes’, which occur whenever the phenotypes of conspecifics affect each other (Moore et al. 1998, 2009; Wolf et al. 1999; Bleakley et al. 2010). Phenotypic expression of behaviours may depend on an individual’s own genes, but also the genes of the individual(s) it interacts with; such effects are also known as ‘indirect genetic effects’ (IGEs) (Moore et al. 1998, 2009; McGlothlin et al. 2010). If this socially mediated aspect of the environment is heritable, there will be coevolutionary dynamics between the actions and responses of the interacting individuals that can lead to a runaway process of adaptive
coevolution (Drown and Wade 2014). Thus indirect genetic effects mediated through social interactions may be important drivers of behavioural variation and evolution (Montiglio et al. 2013; Bailey et al. 2017). Indeed, whenever social interactions (e.g. aggression, signalling, sexual conflict or agonistic encounters) are involved, trait expression and evolutionary trajectories can be altered through IGEs (Wolf et al. 1998; Moore et al. 2009; Bleakley et al. 2010; McGlothlin et al. 2010). Santostefano et al. (2016) found that in male field crickets (G. campestris), activity, exploration, and aggressiveness of individuals were positively correlated with the aggressiveness they elicited in conspecifics. Since IGEs represent an environment that is heritable, this source of variance contributes to the evolutionary response to selection and evolutionary dynamics are therefore altered compared to that expected from traditional quantitative genetic models (Moore et al. 2009). We have previously shown that variation in the social environment (using acoustic sexual signals) has a greater effect on the expression of behaviour, than variation in a non-social environmental stimulus (mechanical shaking) (Rudin et al. 2018). Although we did not directly test for the presence of IGEs in this study, we show that besides behavioural expression, the social environment also has the potential to considerably affect the evolvability of behaviours. This is further confirmed by our finding of genotype-by-environment interactions (GxE) for most behaviours, indicating that genes related to these behaviours are expressed differently depending on the social environment and that there is a heritable basis to the plasticity in the behaviour (Via and Lande 1985; Kruuk et al. 2008).

3.5.3 Effects of the social environment on behavioural covariances
Most of the behaviours measured in this study showed significant phenotypic correlations with each other, and the absence or presence of male acoustic sexual signals had no effect on the sign or magnitude of these phenotypic correlations. We had expected correlations between behaviours based on our previous study (Rudin et al. 2017). Our finding implies the existence of ‘behavioural types’ (Bell, 2007) since, for example, crickets that covered more distance during a trial also emerged more quickly from a shelter or moved at higher velocities. Thus, individuals appear to be on a spectrum between active, explorative types and inactive, slow types. Combined with the significant repeatabilities found for all behaviours, this finding is consistent with what has generally been termed ‘animal personality’ (Gosling and John 1999).

The ‘phenotypic gambit’ (Grafen 1984) and ‘Cheverud’s conjecture’ (Cheverud 1988; Roff 1995) hypothesize that phenotypic behavioural patterns reliably reflect underlying genetic patterns. Elsewhere, phenotypic correlations between behaviours have been found to be generally informative of their genetic correlations (e.g. Dochtermann 2011; Brommer and Klun 2012). The fact that we found strong phenotypic correlations that were similar across environments might therefore predict
strong genetic correlations underlying these traits and that these genetic correlations are similar across environments. This prediction held true when crickets were exposed to male acoustic sexual signals since we found a high degree of similarity between the phenotypic covariance matrix and the genetic covariance matrix. However, in the absence of acoustic signals, hardly any of the behaviours correlated with each other genetically, and the phenotypic and genetic covariance matrices were consequently highly dissimilar. This means that under different social environments, phenotypic measurements may not be consistent with genetic patterns and neither the phenotypic gambit nor Cheverud’s conjecture hold. Thus, caution should be applied in drawing evolutionary inferences from phenotypic patterns, since one cannot know which aspects of the environment (perhaps particularly the social environment), in which animals are held or their behaviour measured, generate or ameliorate the covariances between phenotypic and genetic effects. The estimation of genetic correlations can be difficult as it requires large samples sizes and information about the relatedness among individuals (Lynch and Walsh 1998), yet it is crucial for our understanding of the ways in which distributions of phenotypes in a population can be changed by selection (Lande and Arnold 1983; Cheverud 1988).

Evolutionary trajectories are largely shaped by genetic correlations because such correlations can result in traits that are constrained in their direction of change (Blows and Hoffmann 2005; Roff and Fairbairn 2007). When genetic correlations exist, selection on a particular behavioural trait may have effects on other behaviours. While correlations between life-history or morphological traits have been found to limit potential evolutionary responses by 13—18% in a meta-analysis of additive genetic variance-covariance matrices, behavioural correlations seem to be more limiting, constraining responses by an average of 33% (Dochterman and Dingemanse 2013). Our results imply that when acoustic sexual signals are present, genetic correlations are strong and most behaviours measured here are predicted to evolve non-independently of each other, either in the same (positive correlations) or opposite directions (negative correlations). This may imply that these behaviours are constrained to some degree. Intriguingly, when acoustic sexual signals are absent, genetic correlations mostly disappear, predicting that behaviours will evolve independently of each other. Our results suggest that phenotypic correlations are likely stable, regardless of the level of acoustic sexual signals. Genetic correlations, on the other hand, may oscillate between low and high values depending on the level of acoustic sexual signals present.

3.5.4 The heritability of personality

A number of studies have investigated the heritable basis of behaviours that have been found to be repeatable (i.e. personality traits) (Stirling et al. 2002; Drent et al. 2003; Bell 2005; Sinn et al. 2006).
We measured the behaviours for each individual twice, allowing us to estimate not only the heritability and repeatability of those behaviours, but specifically whether there is a genetic basis for the repeatable differences among individuals; an aspect of behaviour about which little is known (Dochtermann et al. 2014). In other words, we are able to investigate whether personality (Dingemanse et al. 2010; Nakagawa and Schielzeth 2010; Dochtermann et al. 2014) per se, and not just the behaviour, is heritable. Very few studies have reported the repeatability of personality traits (e.g. Petelle et al. 2015; Edwards et al. 2017), although this could be calculated whenever estimates of VA and VPE are available. One of the reasons why the distinction between the heritability of personality and heritability of behaviour in general should be made is that it allows us to assess how additive genetic and non-genetic factors might influence the evolution of personality.

We found that there was a substantial genetic component underlying personality traits in the acoustic environment, with the average heritability of personality being 0.48. This again confirms that the phenotypic gambit could have been applied when acoustic signals were present, since phenotypic estimates were informative of underlying additive genetic variation. However, in the silent environment, the average heritability of personality was much lower at 0.27. This lends further support to our conclusion that the phenotypic gambit may not apply in the absence of acoustic sexual signals. Thus, drawing evolutionary inferences from phenotypic measurements, even when measured repeatedly, may be problematic when the influence of the (social) environment on behaviour is unknown. Neither of the individual variance components used to calculate the heritability of personality (VA and VPE) differed significantly between environments. Therefore, none of these components can be considered the primary driver of the difference in the heritability of personality between environments.

Since additive genetic components explain 48.4% and 27.2% of personality variation in the acoustic and the silent environment respectively, we can ask which other factors might explain the remaining 51.6 and 72.8%. The portion of personality variation that is not due to additive genetic effects must necessarily be due to variation in non-additive genetic effects and permanent environmental effects (Dochtermann et al. 2014). Non-additive genetic effects are often thought to be negligible (Lynch and Walsh 1998; Dochtermann et al. 2014). However, there is some literature suggesting that non-additive genetic effects should not be overlooked (Meffert et al. 2002; van Oers et al. 2005). Thus, it is not clear how much of the personality variation can be attributed to non-additive and permanent environmental effects. Our results suggest that permanent environmental effects and non-additive genetic effects explain approximately half of the variation in personality in the acoustic environment and close to three quarters in the silent environment. Disentangling how much of the personality variation can be attributed to the different permanent environmental effects (e.g. epigenetic effects,
maternal and paternal effects or long-term environmental effects on phenotypes) and non-additive effects (e.g. genetic dominance) is something we were unable to address in this study due to the nature of our breeding design. Some of these gaps could be addressed with different breeding designs, such as the North Carolina II design.

3.5.5 Conclusions
This study contributes to our understanding of the quantitative genetics of behaviour in light of variation in the socio-sexual environment. We have demonstrated that consideration of the social environment is critical when estimating the heritability and evolvability of behaviour. Evolutionary patterns may not be accurately predicted from phenotypic measurements alone. While variation in the social environment appears to have little effect on behavioural repeatabilities and phenotypic correlations, it does lead to differences in the heritability and evolvability of several behavioural traits.
General discussion

The three data chapters presented in this thesis document the effects of different social cues (dominance status and male acoustic sexual signals) on the behaviour of male Australian field crickets (*Teleogryllus oceanicus*). By experimentally manipulating these social cues I was able to elucidate whether and how they affect the expression and repeatability of, and correlations among behavioural traits such boldness, exploration and activity. Comparing the effects of male acoustic sexual signals to a non-social environmental cue (mechanical shaking intended to simulate a predator threat) provided further insight into the presumably special role of the social environment in the evolution and expression of behaviour. Lastly, I explored the effects of the social environment not only on phenotypic patterns of behaviour, but also genetic patterns.

In Chapter One I showed that dominance status affects the expression of behaviours, with dominant individuals being bolder, more explorative and active than subordinate individuals. Additionally, changes in dominance status resulted in changes in behaviour. More specifically, subordinate individuals that became dominant became bolder, more explorative and active, while dominant individuals that became subordinate reduced their boldness, exploration and activity. Furthermore, changes in dominance status disrupted the repeatability of and correlations between certain behaviours. Therefore, it appears to be important to consider dominance status (and possibly other manifestations of internal state) when investigating animal personalities or behavioural correlations as there is a danger of not estimating repeatabilities or correlations accurately without doing so. These results suggested that applying the ‘phenotypic gambit’ (i.e. estimating heritability from repeatability) could be problematic as it would be impossible to know which short-term environmental effects, especially in the social environment, may impact personality.

For Chapter Two I investigated whether social environmental cues (in this case male acoustic sexual signals) indeed play an important role in shaping behavioural expression by comparing them to non-social cues (physical disturbance via mechanical shaking). I found that the effects of variation in physical disturbance were less pronounced than those of variation in the social environment. Changes in physical disturbance hardly affected behavioural plasticity, repeatability or the correlations among behaviours, yet changes in the social environment had striking effects on these parameters. This study suggested that there is between-individual variation in plasticity (i.e. an individual-by-environment effect, IxE) in response to variation in the social environment, an effect not observed in responses to
changes in the physical environment. The findings of this experiment further support the findings presented in the first chapter, namely that changes in the social environment can potentially have disruptive effects in the repeatability of and correlations among behaviours, revealing gaps in the literature in this area of research.

In Chapter Three I presented the findings of an extensive quantitative genetics experiment in which I asked how the social environment (male acoustic sexual signals) affects the repeatability, heritability and evolvability of behaviour. I found similar repeatabilities across the silent and the acoustic environment. If the ‘phenotypic gambit’ (that phenotypic patterns are indicative of underlying genetic patterns) were applied at this stage, one would expect heritabilities and evolvabilities to be similar across the two environments as well. However, I found that heritabilities and evolvabilities significantly differed between environments. Additionally, while there was no difference between the phenotypic covariances in the two environments, the genetic covariance differed significantly. Consequently, genotype-by-environment interactions (different genotypes respond to environmental variation differently) are to be expected, and I was able to show that such interactions indeed exist for most behaviours. Lastly and intriguingly, I found that there was a considerable genetic component underlying personality variation in the acoustic environment, yet the heritability of personality was much lower in the silent environment. These findings combined lead me to advise against applying the phenotypic gambit since we cannot know which aspects of the social environment (or indeed other aspects of the environment) impact the evolutionary trajectory of behaviour.

The experiments presented in this thesis open up exciting opportunities for future research. The degree to which our results are more broadly applicable could be determined by conducting similar studies on different species. It would be particularly interesting to investigate differences between species that have different social structures. Similarly, investigating different social cues, both in crickets and in other species, would broaden our understanding of social environmental effects on behaviour. It has been shown, for example, that chemical signals such as cuticular hydrocarbons affect cricket behaviour (Thomas and Simmons 2009b) and that different pheromones influence behaviour in bank voles (delBarco-Trillo and Ferkin 2004) or honeybees (Le Conte et al. 2001). My studies were also limited in that I only investigated the effects of the social environment on male crickets. Investigating the effects of variation in the social environment on female behavioural expression and quantitative genetic parameters would improve our understanding of such effects. Female mate choice has been shown to be affected by male acoustic sexual signals (e.g. Bailey and Zuk 2008, 2012) and it would be interesting to see whether behavioural repeatability or heritability/evolvability of
female choice is similarly affected by changes in such signals. Another way in which my experiments could be expanded upon would be to include different kinds of behavioural contexts. We could ask, for example, how variation in the social environment affects the expression, heritability and evolvability of mating, courtship or contest behaviours.

Another angle which I was unable to explore in my experiments is the investigation of how selection acts upon the behavioural traits I measured, and how the social environment affects the selection process. The literature investigating the ways in which selection acts on personality traits has been growing in the past 15-20 years (for reviews see Dingemanse and Réale 2005; Smith and Blumstein 2008). Despite this, gaps remain in our understanding of such processes. For example, the fitness of a personality trait may depend on the context/environment in which it is expressed, yet not much work has been conducted on the fitness of behavioural traits in light of variation in the social environment. Using a meta-analytical approach, Smith & Blumstein (2008) found that in general, bolder individuals have reduced life-spans but exhibit increased reproductive success. Conversely, more shy individuals appear to have longer life-spans at the cost of reduced reproductive success. It would be interesting to test how such observations would be affected by changes in the social environment (e.g. the presence or absence of acoustic sexual signals in field crickets). One could even add additional layers of complexity by examining the interplay of predation threats and the social environment simultaneously when investigating the fitness consequences of different personality traits, and testing these effects on both males and females. This would take experiments such as the ones presented in this thesis from a more mechanistic (what are the genetic and environmental components of personality traits?) to a functional (how does the interaction between phenotypes and their environment affect fitness?) level.

Lastly, I was not able to explicitly test for the presence of indirect genetic effects (IGEs) in my experiments since I only had measurements from the receivers of acoustic sexual signals and not from its broadcasters. Both of these need to be known to directly test for IGEs since IGEs describe how the genes expressed in one individual affect the phenotype of another. Field crickets would be ideal animals to study such effects since they quite readily engage in calling behaviour, even under laboratory conditions. One could, for example, measure different behaviours in response to calls from different males whilst having knowledge about the relatedness between all individuals involved. This would allow us to test how calls from different males (genotypes) affect the expression of behaviour in other males (phenotypes), and therefore determine the importance of IGEs in these interactions.
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