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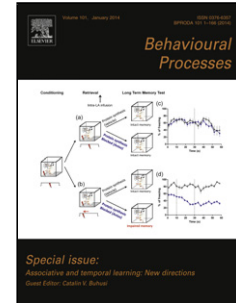
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Exploratory behaviour is not related to associative learning ability in the carabid beetle *Nebria brevicollis*

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Highlights

- Exploration in a novel environment is highly repeatable in *Nebria brevicollis*
- *N. brevicollis* show associative learning
- No beetle reversed learned associations from the initial associative learning task
- No correlation between learning performance and exploratory behaviour

Recently, it has been hypothesised that as learning performance and animal personality vary along a common axis of fast and slow types, natural selection may act on both in parallel leading to a correlation between learning and personality traits. We examined the relationship between risk-taking and exploratory behaviour and associative learning ability in carabid beetle *Nebria brevicollis* females by quantifying the number of trials individuals required to reach criterion during an associative learning task ('learning performance'). The associative learning task required the females to associate odour and direction with refugia from light and heat in a T-maze. Further, we assessed learning performance in a reversal task by quantifying the number of correct trials when the reinforcement was switched to previously unrewarding stimuli. We found that *N. brevicollis* females can associate conditional stimuli with a reward. No female was able to reverse the learned association within the number of trials given, however individuals differed in the number of correct trials in the reversal phase. Contrary to previous predictions neither exploratory behaviour, which was repeatable, nor risk-taking were correlated with learning performance. Our results suggest that the relationship between learning and personality may not take a common form across species.

Keywords: Cognition; exploration; ground beetle; invertebrate; novel environment; personality

1. Introduction

Cognition refers to the mechanisms by which animals acquire, store, process and use information. This includes processes such as learning, decision making, perception, and memory storage (Shettleworth, 2010). Associative learning is a particularly important

cognitive process and is defined as the ability to form a neural representation of an association between a stimulus and an environmental state (Dudai, 1989). Associative learning confers fitness advantages, for instance, by allowing animals to better seek stimuli that have previously been associated with positive fitness effects and avoid stimuli that have previously been associated with negative fitness effects (e.g. Dukas and Duan, 2000). A growing body of work is reporting consistent inter-individual differences in learning ability across a wide range of species (reviewed in Cauchoix et al., 2018). For instance, Ashton et al. (2018) found high individual consistency in Australian magpies (*Cracticus tibicen dorsalis*) which underwent a series of cognitive tasks quantifying inhibitive control, associative learning, reversal learning and spatial memory. Chittka et al. (2003) also reported consistent individual differences in speed and accuracy in buff-tailed bumblebees (*Bombus terrestris*) during a colour learning experiment in which individuals were tasked with discriminating between rewarding and non-rewarding virtual flowers. Elucidating the factors which drive and maintain individual differences in learning is essential for understanding the wider evolution of cognitive traits, as this variation is the prerequisite for natural selection. Factors that are being explored as drivers of individual differences in cognition include genotype, physical environment, social environment and animal personality (e.g. Kawecki, 2010; Croston et al., 2015; Dalesman, 2018; Pike et al., 2018; Sauce et al., 2018).

Animal personality refers to individual differences in behaviour which are stable across time. Individuals can vary in traits such as boldness, aggressiveness, sociability and exploratory tendency (Sih et al., 2004). Sih and Del Giudice (2012) hypothesised that as both associative learning and personality traits influence the way in which animals interact with the environment, natural selection may shape both in parallel and thus lead to a functional link. The authors postulate that personality traits vary along a risk-reward axis, where higher expression of traits increase an individual's likelihood of reward but involve greater risk

(such as predation). Similarly, associative learning may also vary along a speed-accuracy axis where individuals with ‘fast’ cognitive styles may acquire information from their environment more rapidly, however with less accuracy than individuals with ‘slow’ cognitive styles. The authors argue that as the risk-reward trade-off of personality traits and the fast-slow trade-offs of associative learning are both fundamentally fast-slow trade-offs, selection should shape both in parallel. Thus, according to Sih and Del Giudice (2012) individuals with proactive personality profiles (bold, more aggressive, more exploratory etc.) are predicted to acquire information more rapidly but at the cost of accuracy (i.e. making more mistakes), while reactive individuals (shy, less aggressive and less exploratory etc.) are predicted to gather and process information more slowly but with greater accuracy (i.e. making fewer mistakes). A growing number of studies have provided empirical support for this prediction. For instance, bold, active and aggressive caviies (*Cavia aperea*) are faster learners during an associative learning task (Guenther et al., 2014). However, during reversal learning tasks, where a previously non-rewarding stimulus is switched with a previously rewarding stimulus, less aggressive individuals required fewer trials to solve the task (Guenther et al., 2014). This suggests that less active individuals may have been more attentive to changes in the meaning of information. Alternatively, a better performance in reversal tasks may indicate quicker loss of learned associations as an adaptation to changing environments (Tello-Ramos et al., 2019). Further relationships between the expression of personality traits and reversal learning ability in accordance with Sih and Del Giudice (2012) have also been reported in other species such as Darwin’s finches (*Camarhynchus parvulus* and *Cactospiza pallida*, Tebbich et al., 2012) and black-capped chickadees (*Poecile atricapillus*, Guillette et al., 2011).

The relationship between personality traits and associative learning is a young field of research (Griffin et al., 2015), and as such the development of a comprehensive framework linking learning to personality has been problematic. Despite the theoretical work by Sih and

Del Giudice (2012) having initial empirical support, a recent meta-analysis found the strength and direction of the relationship to be highly variable (Dougherty and Guillette, 2018). It is thus possible that factors such as habitat, breeding systems or sexual dimorphism may exert different selection pressures on personality traits and/or learning and thus influence the way they covary. For instance, male and female great tits (*Parus major*) have opposite relationships between exploration and performance in reversal learning tasks (Titulaer et al., 2012), and alternative mating strategies have also been speculated to drive a bimodal distribution between learning ability and boldness in the eastern water skink (*Eulamprus quoyii*, Carazo et al., 2014). However, Dougherty and Guillette (2018) were unable to assess factors which may influence the relationship due to a small sample size (25 studies) across a narrow set of 19 study species. Further, most studied species were vertebrates, with only a single study looking at invertebrates (Udino et al., 2016). This appears to be symptomatic of the wider animal personality literature with a disproportionately small number of studies investigating animal personality in invertebrates (Kralj-Fišer and Schuett, 2014). Developing a comprehensive understanding of how learning and animal personality are related requires the broadening of empirical evidence across a wider range of species. This will allow a comparative approach to be employed to better understand the various factors which may influence their relationship.

Here, we used the carabid beetle *Nebria brevicollis* (Fabricius, 1792) as a model organism to assess the relationship between variation in personality traits and associative learning ability. *N. brevicollis* are eurytopic; they are night-active (Williams, 1959), reside in the variable microhabitat of forest litter layer (Greenslade, 1964), and have a highly variable diet consisting of Collembola, Diptera, earthworms, mites and spiders (Penney, 1966). Thus, as ecological factors such as prey type, prey location or shelter location may vary frequently, learning ability should provide an advantage during foraging and thus may be favoured by

natural selection. Though studies of learning in Coleopterans are scarce, learning ability has been reported in grain beetles (*Tenebrio molitor*, Alloway, 1969, 1970, 1972; Alloway and Routtenberg, 1967) and the carabid beetle *Pterostichus melanarius* (Plotkin, 1979).

Personality differences have also been reported in coleopterans, such as in the mustard leaf beetle *Phaedon cochleariae* (boldness, exploration and activity, Tremmel and Müller, 2013), and in the red flour beetle *Tribolium castaneum* (movement activity, Wexler et al., 2017).

Further, personality measures of exploration in response to novel environments have previously been found to be repeatable in *N. brevicollis* (Schuett et al., 2018). *N. brevicollis* therefore appears to be a suitable species to extend the study of the relationship between animal personality and learning into invertebrates.

In this study we addressed two main questions. Firstly, can *N. brevicollis* successfully learn an associative learning task, and reverse this learned association? Second, is there a relationship between performance in an associative learning task and personality measures of exploratory behaviour or risk-taking behaviour? In accordance with the prediction of Sih and Del Giudice (2012), we expected more exploratory or risk-taking individuals to require fewer trials to reach learning criterion. Upon changing the conditioning stimuli to previously unrewarding stimuli, we also expected more exploratory or risk-taking individuals to require more trials to reach criterion during this reversal phase, due to lower sensitivity to changes in environmental cues (Sih and Del Giudice, 2012). Alternatively, more exploratory individuals may generally have more opportunities to notice environmental change, hence may be able to reverse learned associations more quickly. The associative learning task was carried out in a T-maze in which one arm contained a reward (a soil pile to escape from warm and bright light produced by a lamp) with arm direction and odour acting as conditioning stimuli. Exploratory behaviour was measured in a novel environment ('open field test', Réale et al.,

2007; Schuett et al., 2018) and risk-taking behaviour was quantified as occurrence of the death feigning response ('thanatosis'; Schuett et al., 2018).

2. Materials and Methods

2.1 Test subject collection and maintenance

N. brevicollis were collected via pitfall traps in wooded areas on the University of Sussex campus (United Kingdom, latitude: 50.8679°N, longitude: -0.0877°W), between 22nd of October and 13th of December 2018. *N. brevicollis* is a ground beetle 11-14mm in length (Luff, 2007). Beetle collection followed the methods developed by Schuett et al. (2018). Nine traps were used with ca. 10m between each. The traps consisted of 10cm tall plastic cups sunk into the ground with their rims level with the soil surface and covered with wire mesh. A shorter, 5cm tall plastic cup with small holes on the bottom was placed inside the larger cup such that the top of each cup was at equal height, creating two compartments. Each cup had a 10cm diameter. This segregated catch according to size with smaller animals falling through the holes into the lower compartment. Vinegar soaked tissue was placed in both compartments which has previously been found to be attractive to *N. brevicollis*. Traps were emptied weekly.

Between collection and the start of experimental trials *N. brevicollis* individuals were stored in plastic containers (18x13x6cm) of 25-30 individuals. Each container was approximately half filled with moist soil, which was sprayed with water weekly and renewed with fresh soil monthly. The beetles were fed weekly *ad libitum* with crushed dead mealworms. Containers were stored in an incubator with a 9:15h light:dark cycle, set at a temperature of 8.8°C during the day and 3.6°C at night.

Only females were used in the study ($n=21$), as sex has previously been found to influence the way in which personality and cognition covary (Dougherty and Guillette, 2018). Females can be distinguished from males by their narrower front tarsi (Forsythe, 2000). From the 28th February 2019 onwards test beetles were stored individually in 11x8x4cm plastic containers approximately half-filled with soil which was sprayed with water every 3-4 days. Individuals were fed weekly with dead mealworms *ad libitum*. The containers were stored in the same room as the personality and learning trials to ensure the test subjects were acclimatised to the conditions of the test room. The temperature of the test room was recorded using a data logger (Voltcraft DL-210TH, Conrad Electronic, Hirschau, Germany).

2.2 Quantifying personality traits

All females were tested for their exploratory behaviour and risk-taking behaviour twice before the learning trials began. All study beetles were tested for exploratory and risk-taking behaviour on 3rd of March 2019, with a second round of tests three days later. Individuals were tested twice for each trait in order to quantify the repeatability of each and determine whether exploratory behaviour or risk-taking behaviour could be defined as a personality trait. Personality tests were conducted between 11.00-18.00, with the temperature of the test room ranging from 21.1°C to 22.1°C (mean=21.7°C, SD=0.3°C). Methods used for testing exploratory and risk-taking behaviour followed methods used by Schuett et al. (2018).

Measuring exploratory behaviour. Individuals were tested for activity in response to a novel environment ('open field test'; Réale et al., 2007). The novel environment consisted of an open white plastic box (35.5x25x10cm) divided into 28 squares. The individual was placed into a specific square at the centre of the box. The number of squares the beetle entered within 90s of being placed on the starting centre-square was then recorded. The

number of squares visited included revisits to the same square. Exploratory behaviour was quantified as the mean number of squares the individual visited in both tests.

Measuring risk-taking behaviour. Directly after the exploration test individuals were tested for their risk-taking behaviour by observing whether individuals exhibited thanatosis.

Thanatosis is a death-feigning response previously used to quantify risk-taking behaviour in *N. brevicollis* (Schuett et al., 2018), and has been found to be a component of behavioural syndromes in other species of beetle (*Tribolium castaneum*, Nakayama and Miyatake, 2009; *Callosobruchus chinensis*, Nakayama and Miyatake, 2010). This behaviour is believed to be a reliable proxy for risk-taking as work has demonstrated that flower beetles (*T. castaneum*) selected to exhibit greater frequency and duration of thanatosis had lower mortality rate due to predation (Nakayama and Miyatake, 2009). To start the test, the beetle was flipped onto its back using insect forceps and it was observed whether the individual showed thanatosis. We defined thanatosis to occur, if the beetle stopped movement completely. The beetle did not show thanatosis if it turned back onto its front or if it continued to move for 90 seconds after being flipped.

2.3 Test arena for associative learning task

The associative learning task was developed based on methods previously used to study learning and memory in grain beetles (*T. molitor*, Alloway, 1969, 1970, 1972; Alloway and Routtenberg, 1967). The task involved a T-maze setup in which a single arm contained a 5cm³ pile of soil in which the beetles could bury themselves (Figure 1). *N. brevicollis* are night-active (Williams, 1959) and noticeably negatively phototactic, burying themselves whenever uncovered (personal observation). This observation is in line with previous studies showing that night-active carabids are generally photophobic (Thiele, 1977). Two 20W

incandescent lamps (Handex International, Kowloon, Hong Kong) were mounted 15cm above the test arena. Therefore, when undergoing learning trials each beetle was exposed to comparable light and heat conditions. Escape from the light and heat constituted the reinforcement for choosing the soil-containing (=correct) arm. Each arm either had banana or strawberry odour, and the odour produced by the flavouring constituted the conditioning stimulus (hereafter CS). 2cm strips of tissue paper containing 0.5ml of food flavouring (Preema, Luton, United Kingdom) were placed above the entrance and reward area of each arm (Figure 1). Further, the location of the soil (left or right arm) also functioned as a CS. Thus, there were four possible combinations of CS an individual could be assigned to associate with the reward (left-strawberry, right-strawberry, left-banana, right-banana).

A combination of two CS was used to ensure beetles had sufficient stimuli to associate with the reinforcement. Few studies have been conducted assessing sensory perception in *N. brevicollis*. Kielty et al. (1996) found *N. brevicollis* use olfactory cues for prey and habitat detection. Further, work with grain beetles (*T. molitor*) found that individuals could associate arm direction with reward in a T-maze associative learning task (Alloway, 1969, 1970, 1972; Alloway and Routtenberg, 1967). Therefore, odour cues and direction were pertinent to our study species and were assumed to be suitable CS.

A transparent plastic box (11.5x15x7cm) served as the testing arena containing a T-maze constructed from Lego® (Figure 1). The test individual was placed at the starting area at the end of a 2x11cm corridor, the individual was then able to move to the opposite end where it could make a choice between two arms. The entrances to the arms were 1cm wide. The box was open-topped and had masking tape around the exterior to prevent the test subjects from being influenced by outside terrain.

To control for the potential odour of soil, in the non-rewarding arm 5cm³ of soil was placed inside a fabric mesh parcel and secured on the side wall of the test arena. The beetle could therefore not access the soil in the non-rewarding arm. In the reward arm mesh containing no soil was secured to the wall in the same way as the control arm to control for a potential effect the mesh might have.

2.4 Experimental procedure

Learning trials were conducted between 4th March and 12th April 2019 between 9.00-18.00. Temperature recorded immediately next to the trial arena ranged from 18.1°C to 23.9°C (mean=22.0°C, SD=0.7°C). Within the arena itself the beetles were exposed to comparable conditions due to being directly under the lamps. *N. brevicollis* are reproductively active following diapause, which occurs from mid-July to mid-August (Penney, 1969). Thus, the females used in our study were not reproductively active at the time of testing for exploratory behaviour or during the associative learning task. Four females were tested each week. Each individual was assigned conditioning stimuli pseudo-randomly, so each of the four individuals had one of the four potential CS combinations. Before the start of their first learning trial each individual was tested for potential biases by allowing each individual five unreinforced runs through the T-maze containing the odour-paper, recording the arm choice each time. If an individual chose an arm four or more times during these runs the individual was assigned conditioning stimuli against this bias. Seven individuals exhibited a bias.

During the learning trials the test beetle was placed at the starting area of the T-maze using insect forceps. Once in the maze the first arm entrance the head of the beetle entered was recorded, which was defined as either a correct choice (reward-containing arm) or incorrect (see Figure 1). Latency to move and latency to make choice were also quantified.

Trials lasted 5 minutes, during which the individual could move freely throughout the maze. Latency (in s) from first moving after being placed in the test arena to making an arm choice was used as a proxy for motivation, which has previously been suggested to influence learning performance (Liedtke and Schneider, 2014; Griffin et al., 2015).

Individuals were tested 10 times per day for three days for a maximum of 30 trials per individual. Inter-trial intervals lasted approximately 30 minutes within a day. Between trials the arena and maze were cleaned using 90% ethanol and the used soil was discarded and replaced with fresh soil. An individual was considered to have successfully learned, i.e. it reached criterion, if it made correct arm choices either during seven consecutive trials or nine times out of ten consecutive trials (i.e. $p < 0.05$, binomial test). Once an individual had successfully learned it received no more trials and began reversal learning trials the following day. The number of trials required to reach criterion was used to quantify learning performance in the initial association task. 11 Individuals did not reach learning criterion. Beetles which did not learn within the 30 trials were assigned scores of 30.

Only individuals that reached criterion during the initial learning task underwent reversal learning trials. Ten individuals reached criterion and proceeded to this stage. For reversal learning trials the same protocol as the initial associative learning phase was used. However, the CS was switched to the previously unrewarding CS combination (i.e. if an individual had learned to choose the right arm and banana odour, the left arm and strawberry odour would now be reinforced with the reward). One block of 10 trials was conducted for the reversal learning trials. Due to being limited to 10 reversal trials, we used two measurements of learning performance in the reversal task: (i) reaching the success criterion as defined above and (ii) the raw number of correct choices. The second measurement provided us with an estimation of the individual's accuracy in the reversal learning task even

when it did not reach the success criterion. Accordingly, individuals which made more correct choices were regarded as being more accurate in the reversal task.

2.5 Statistical analyses

All statistical analyses were conducted in R (version 3.5.1, R Development Core Team, 2017). Sample size was $n=21$ female beetles.

The time of personality test did not influence the number of squares visited in the novel environment (linear mixed effect model with beetle ID as random term: $\chi^2=2.34$, $df=1$, $p=0.13$) or likelihood of an individual exhibiting thanatosis (generalised linear mixed effect model, GLMM, with binomial error structure and beetle ID as random term: $\chi^2=0.50$, $df=1$, $p=0.48$). Hence, the time of day was not considered in further analyses. Similarly, individuals that had displayed a turning bias during unreinforced runs (i.e. before the learning trials started; binary variable) did not make more correct choices during the reversal phase when the previously favoured side was now rewarded (generalised linear model, GLM, with poisson error structure: $\chi^2=0.49$, $df=1$, $p=0.49$).

To test for consistent personality differences repeatability of the number of squares visited during the open field test was estimated with linear mixed effect models using the R package rptR (version 0.9.22, Schielzeth et al., 2017) with 1000 bootstrap steps and beetle ID as a random term. The repeatability (R) gives the amount of variance explained by among-individual variance divided by total variance of exploratory behaviour. Whether the repeatability of the number of squares visited during the open field test was significant was inferred if zero was not included within the 95% confidence intervals (CI). Model assumptions were confirmed visually using diagnostic plots. We did not assess repeatability of the occurrence of thanatosis as this behaviour was rarely shown (see below).

To test whether exploratory behaviour and thanatosis were associated, we conducted a GLMM with binomial error and beetle ID as random term. The number of squares visited in a novel environment was fit as explanatory variable; the occurrence of thanatosis was the dependent binary variable. In a previous study (Schuett et al., 2018), more exploratory *N. brevicollis* individuals were less likely to show thanatosis.

We conducted logistic regression analysis to assess whether the mean number of squares visited in a novel environment predicted whether an individual learned within the 30 trials (i.e. reached criterion; binary variable). The mean number of squares visited by an individual over the two novel environment trials was included as explanatory variable.

We tested for a relationship between exploratory behaviour and (reversal) learning performance with GLMs (for error structures see results). The mean number of squares visited by an individual over the two novel environments was set as the explanatory variable. The (i) number of trials to criterion in the learning phase (ii) number of correct arm choices during the reversal phase and (iii) mean arm choice latency in the initial learning phase (rounded to closest integer) were included as the dependent variables in separate models.

As only 5 individuals (out of n=21) exhibited thanatosis behaviour (in 7 out of 42 trials) only one GLM (quasipoisson error distribution) was conducted to assess whether demonstration of thanatosis behaviour (binary, independent variable) was related to learning performance (trials to criterion). If the beetle had shown thanatosis in at least one of the two behavioural trials it was classified as having demonstrated thanatosis behaviour.

To further assess whether there was a trade-off between the performance in the associative learning task (trials to criterion) and reversal phase (number of correct arm choices), we conducted a GLM (poisson error distribution) with the number of trials to

criterion as the explanatory variable and the number of correct reversal trials as the dependent variable.

Likelihood ratio tests provided p values to determine significance or lack thereof for explanatory variables.

3. Results

Measures of exploration (the number of squares visited during the open field test) showed high repeatability over time (mean=42.2, SD=34.2, R=0.66, 95% CI=[0.33, 0.85], $n_{ID}=21$, $n_{trials}=42$) but did not predict the probability of the beetles to exhibit thanatosis behaviour (GLMM: $\chi^2=0.01$, $df=1$, $p=0.92$).

Ten of the 21 tested female *N. brevicollis* reached criterion during their respective 30 associative trials. Those beetles who did learn exhibited variation in the number of trials required to learn, ranging from 10 to 29 trials (mean=17.3, SD=7.4). During the reversal learning phase no beetle successfully reached criterion, which was likely due to the limitation of a maximum of 10 reversal learning trials. However, the beetles exhibited variation in the number of correct trials, ranging from 1 to 7 (mean=4.4, SD=1.8) correct choices out of 10 reversal trials. We used this score as measurement for individuals' accuracy in the reversal task and used it for further analysis (see below).

The mean number of squares visited during the open field test did not significantly predict whether a beetle reached criterion within the 30 associative learning trials (logistic regression: $\chi^2=0.06$, $df=1$, $p=0.80$). Further, the mean number of squares visited did not predict the number of trials required to reach criterion (GLM with quasipoisson error structure: $F_{1,19}=0.04$, $p=0.85$; Figure 2a). For the individuals who progressed to the reversal learning stage ($n=10$) exploration did not predict the number of correct choices in the reversal

learning phase either (GLM with poisson error structure: $\chi^2=0.02$, $df=1$, $p=0.89$; Figure 2b). Further, exploratory behaviour did not predict the motivation of the beetles to make an arm choice during the initial learning phase (GLM with quasipoisson error structure: $F_{1,19}=0.06$, $p=0.80$).

There was no relationship between whether an individual exhibited thanatosis and associative learning performance (GLM with quasipoisson error structure: $F_{1,62}=0.32$, $p=0.58$). There was also no relationship between learning performance in the initial stage and the number of correct choices in the reversal stage (GLM with poisson error structure: $\chi^2=0.43$, $df=1$, $p=0.51$).

4. Discussion

Ten individuals reached learning criterion of seven consecutive correct choices or nine out of ten consecutive trials. It is possible that the remaining eleven individuals who did not learn may have also reached learning criterion if the maximum number of trials per individual had been extended beyond 30 trials. No beetles reached criterion during the reversal learning stage, and there was no relationship between learning performance in the initial learning phase and number of correct choices in the reversal learning phase. However, making inferences about the presence or lack of reversal learning ability is problematic due to the limitation of ten reversal trials. Nevertheless, we have demonstrated that *N. brevicollis* exhibits associative learning ability. Further, the number of squares visited during the open field test showed high individual repeatability over time, consistent with previous work showing that exploratory behaviour in *N. brevicollis* is a personality trait (Schuett et al., 2018). Repeatability of number of squares visited was higher than the average repeatability of behavioural traits found in a meta-analysis across 98 different species (Bell et al., 2009,

average repeatability estimate $R=0.37$). Our results also suggest that individuals that varied in exploratory tendency and risk-taking did not significantly differ in learning performance during either the initial associative learning task or the reversal learning task.

Our learning assay used escape from light and heat produced by lamps as a reinforcement for choosing the correct arm, indicating that the beetles were able to associate the location of soil with either location and/or odour. This is consistent with previous work assessing associative learning using a similar assay in the grain beetle *T. molitor* (Alloway, 1969, 1970, 1972; Alloway and Routtenberg, 1967), where individuals were found to be capable of associating location with refugia. *N. brevicollis* inhabits the leaf litter of woodland floors (Penney, 1966), and has a broad diet consisting of Collembola, Diptera, mites, earthworms and spiders, the relative proportions of which change throughout the year (Penney, 1966; Sunderland, 1975). The location of refugia and prey type will therefore change frequently, and rigid or stereotyped behaviours may be inappropriate for coping with environmental challenges. Thus, it is likely that *N. brevicollis* benefit from having the ability to learn cue-cue or cue-response associations for predator avoidance, refuge point location or food location (Morand-Ferron, 2017). An interesting line of further research would be to assess whether this associative learning ability is present across different learning assays, for example while using different positive reinforcements such as food or during aversive conditioning.

Despite finding that *N. brevicollis* is capable of associative learning, we found no relationship between exploratory behaviour and learning performance, even though both were highly variable between individuals. It could be that our sample size was too small to detect such a relationship. Nevertheless, several other studies found a significant relationship between exploratory behaviour and learning performance with a similar sample size, suggesting that in our study species this relationship is at least much weaker than in other

studied organisms (e.g. mammals: Guenther et al., 2014, birds: Guillette et al., 2009). Our finding is contrary to the prediction of Sih and Del Giudice (2012), who predicted that individuals with ‘fast’ personality types would form learned associations faster (i.e. exhibit ‘fast’ cognitive styles). Despite initial empirical support for this hypothesis (e.g. Guillette et al., 2011; Guenther et al., 2014), our results are not surprising given the emerging view that the relationship between animal personality and learning can be highly variable both within (Dalesman, 2018) and between species (Dougherty and Guillette, 2018). Even the single study investigating personality and learning in invertebrates (in the carpenter ant *Camponotus aethiops*) found learning performance was only predicted by a single personality dimension (exploration), however not by other personality traits such as sociability or aggression (Udino et al., 2017). This result also falls short of providing support for the prediction of Sih and Del Giudice (2012). Griffin et al. (2015) highlighted that it is necessary to demonstrate that multiple personality axes predict individual differences in cognitive abilities, as the hypothesis is predicated on the assumption that personality traits and learning are all underpinned by a common ‘fast-slow’ trade-off. Our finding that individual differences in exploratory behaviour and risk-taking are not related to individual differences in learning performance, in addition to the growing body of work demonstrating the highly variable nature of the personality-cognition relationship (Dougherty and Guillette, 2018), suggests that a more nuanced approach is required, considering personality and learning within the specific context of each study species.

Contrary to the prediction made by Sih and Del Giudice (2012), who postulate that the relationship between learning and personality will take a common form across species, several studies have highlighted the way in which the specific ecologies of each species and their associated selection pressures may cause differences in the way personality and learning covary. For example, Guillette et al. (2015) found no correlation between exploratory

behaviour and learning speed in black-capped chickadees (*Poecile atricapillus*). However, other studies using the same learning assays and personality tests found faster explorers were faster learners (Guillette et al., 2009). Further, Guillette et al. (2011) found slower explorers outperformed fast explorers in reversal learning trials. Guillette et al. (2015) speculated that such variation in the exploration-learning relationship may be due to the cohorts of each study being collected from different locations in different years. Thus, their respective populations may be subject to varying selection pressures which may drive the observed differences the relationship between personality and cognition. Indeed, it has been demonstrated that *N. brevicollis* individuals collected from urban areas have greater exploratory behaviour than individuals collected from less urbanised areas (Schuett et al., 2018). This may be due to individuals with greater exploratory behaviour benefiting from increased foraging success in urban areas where litter layers are thinner than in semi-natural habitats, thus selecting for more exploratory personality types (Schuett et al., 2018). It is possible that the selection pressures which give rise to this inter-population difference in exploratory behaviour may also generate differences in the way personality traits and learning ability covary at the intra-population level (compare Liedtke and Fromhage, 2019). Indeed, studies with wild-caught individuals have demonstrated that environments with conditions which vary between seasons (Dingemanse et al., 2004) or between sampling locations (Bell et al., 2013) can give rise to suites of correlated traits which differ between populations exposed to different selection pressures. We may therefore detect correlations between exploratory behaviour, or other personality traits, and learning in other *N. brevicollis* populations. Further, we only investigated exploratory behaviour and learning in females. However, sex-specific trait differences such as breeding schedule, reproductive roles, reproductive investment and resource availability may generate sexually dimorphic trait covariance patterns (Hämäläinen et al., 2018). Thus, the specific traits which covary and the

strength of covariance may differ between sexes due to differences in selection pressures arising from reproductive roles (Hämäläinen et al., 2018). Investigating the relationship between personality and learning in populations from different environments and comparing the relationship between males and females may be a valuable avenue of research to elucidate the ways in which ecological factors may shape relationships between personality traits and learning performance.

5. Conclusion

Our findings warrant further investigation. We showed large variation in the associative learning ability of female *N. brevicollis*. Nevertheless, we detected no relationship between learning performance and exploration or risk-taking, suggesting that generalisations about fast-slow trade-offs and relationships between personality and learning should be made cautiously with careful attention paid to the ecology and life history of each population studied. It is possible that relationships occur between specific personality and cognitive traits, rather than across all personality and cognitive axes, as had been reported by Udino et al. (2016). It is also necessary to quantify performance on a wider range of learning assays, for example by altering stimuli (e.g. light, food), response (e.g. inhibition, consumption), or outcome (e.g. escape, food, punishment), features which are important when considering cognition-personality relationships (Griffin et al., 2015). This study provides a solid foundation for further work assessing personality and cognition in *N. brevicollis*, which will aid the development of a comprehensive framework aiming to understand personality-cognition relationships and the underlying mediating factors.

Author contributions:

C.H. developed the experimental design, collected and analysed the data, and wrote the manuscript. J.L. developed the experimental design, reviewed and edited the manuscript. C.D. developed the experimental design, reviewed and edited the manuscript. W.S. supervised, developed the experimental design, analysed data, reviewed and edited the manuscript.

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Figure legends

Fig. 1 Schematic drawing of test arena. A transparent plastic box (11.5x15x7cm) served as the test arena with the walls of the T-maze (black area) constructed from Lego®. Different shades of odour paper (white and grey boxes, respectively) represent the two odours placed above the entrance and reward area of each arm. The dashed line represents the arm entrance, the point at which the beetle is defined as making a choice once their head has passed it. At the start of the learning trial the beetles were placed at the starting area (as indicated by the dashed line)

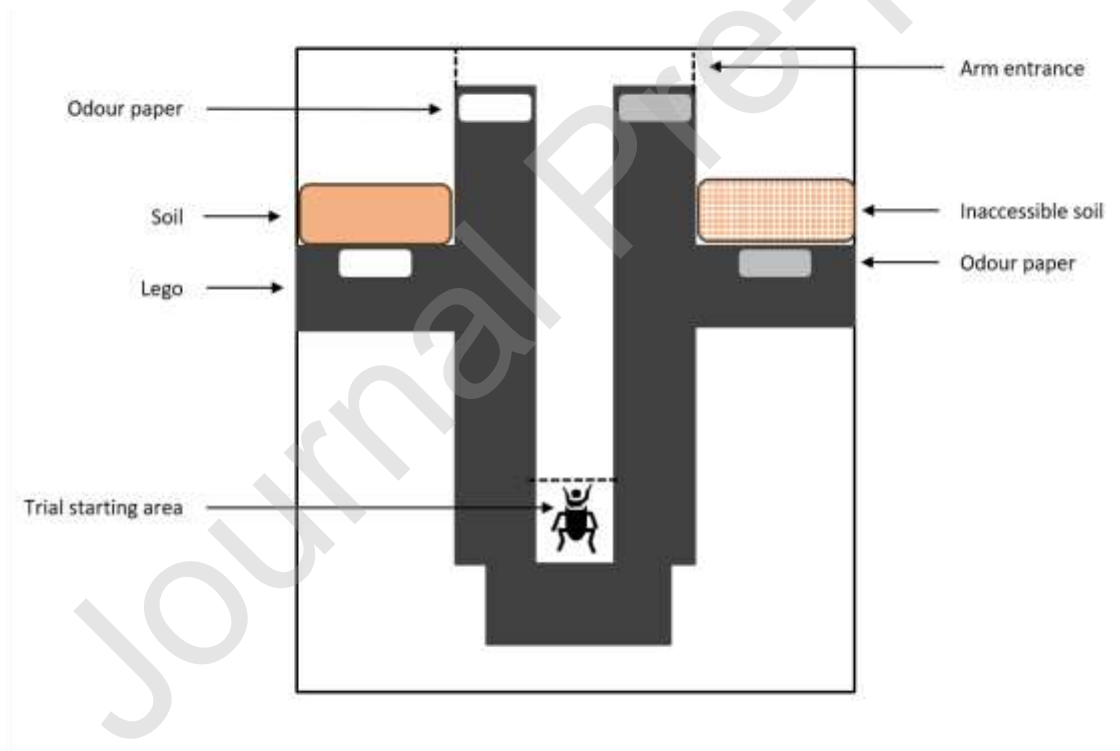


Fig. 2 Relationship between mean number of squares visited during the open field test and (a) number of trials to reach criterion in the initial learning trial (n=21 individuals) and (b) number of correct trials in the reversal learning phase (n=10 individuals). Larger points indicate two individuals had the same measurements in (a)

