Under the influence: sublethal exposure to an insecticide affects personality expression in a jumping spider

Raphaël Royauté, Christopher M Buddle, Charles Vincent

To cite this version:

Raphaël Royauté, Christopher M Buddle, Charles Vincent. Under the influence: sublethal exposure to an insecticide affects personality expression in a jumping spider. 2023. hal-03955445

HAL Id: hal-03955445
https://hal.inrae.fr/hal-03955445v1

Preprint submitted on 21 Mar 2023

Received Date : 27-Jul-2014
Accepted Date : 15-Jan-2015
Article type : Standard Paper
Editor : Dana Hawley
Section : Behavioural Ecology

Under the influence: sublethal exposure to an insecticide affects personality expression in a jumping spider

Raphaël Royauté* [a,b,c], Christopher M. Buddle [b], Charles Vincent [c]

[a] Current address: Department of Biological Sciences, North Dakota State University, Fargo, USA

[b] Department of Natural Resource Sciences, McGill University, Macdonald Campus, Ste-Anne-de-Bellevue, Canada

[c] Horticultural Research and Development Centre, Agriculture and Agri-Food Canada, Saint-Jean-sur-Richelieu, Canada

* Corresponding author: raphael.royaute@gmail.com

Summary

1. Consistent behavioural differences between individuals have far-reaching implications for ecology and evolution, including how populations cope with increasing anthropogenic changes, notably pesticides. Although sublethal doses of insecticides are known to alter behaviour, current studies on the relationship between toxicants and behaviour tend to ignore effects on individual variation.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/1365-2435.12413

2. Our objective was to determine whether sublethal exposure to an organophosphate insecticide could affect the consistency of individual behaviour and disrupt behavioural correlations, in a jumping spider occurring in agroecosystems. Adults of the jumping spider *Eris militaris* (Araneae: Salticidae) were scored by an open-field and a prey-capture assay, each conducted as pre- and post-insecticidal exposure to the organophosphate phosmet. Half of the individuals received no exposure to the insecticide to provide a control group. We then estimated the changes in repeatability, a measure of the extent of personality differences, and in behavioural correlations between control and insecticide-treated groups.
3. Although insecticide exposure had no discernable effects on the population's average behaviours, insecticide-exposed individuals showed an average of 23 % lower repeatability and the correlation between activity and prey capture was more strongly collapsed in females.
4. Our results provide clear evidence that exposure to sublethal doses of insecticides on an important arthropod predator in agroecosystems causes substantial alteration of personality differences even in absence of a population-wide shift in behaviour. This suggests insecticide effects are more complex than previously thought and indicates high variation in the way individuals coped with insecticidal exposure.
5. By altering the consistency of behavioural traits and their correlations, exposure to sublethal concentrations of insecticides can have subtle effects on behavioural expression, which may ultimately affect biocontrol performance in an important arthropod predator in agroecosystems. Our study calls for an increasing focus on individual behavioural variation when testing the effects of pesticides on non-targeted fauna.

Key-words: animal personality, anthropogenic disturbance, behavioural syndromes, consistent individual variation, contaminants, multivariate mixed models, pesticides

Introduction

The study of animal personalities has received much attention in recent years. This emerging field has shown that consistent behavioural differences among individuals are common in animals (Réale *et al.* 2007). These differences are sometimes correlated across different behavioural contexts, forming behavioural syndromes (Sih, Bell & Johnson 2004b; Sih, Bell & Johnson 2004a). Behavioural differences have important implications for ecological and evolutionary dynamics since they affect individual fitness and can have cascading effects on animal communities by shaping the magnitude and direction of species interactions (Sih *et al.* 2012; Wolf & Weissing 2012). In addition, behavioural differences provide a framework to study how individuals cope with increasing anthropogenic activities (Sih *et al.* 2010). Human-induced environmental changes challenge species to respond adaptively to those novel conditions. The effects of these changes may vary depending on the behavioural phenotype being considered. Some personality types perform better in human-disturbed environments (Martin & Réale 2008; Madden & Whiteside 2013), and the overall architecture of behavioural syndromes can differ between populations with varying degrees of anthropogenic pressures (Miranda *et al.* 2013; Royauté, Buddle & Vincent 2014). In addition, certain classes of anthropogenic changes (e.g., urban noise, pollutants) may amplify or attenuate behavioural and physiological variation (Killen *et al.* 2013).

Pesticides used in agriculture are important stressors for animals. These compounds are often wide-spectrum and are linked to declines in populations of seed dispersers (Krebs *et al.* 1999; Donald, Green & Heath 2001), pollinators (Brittain *et al.* 2010) and biocontrol agents (Geiger *et al.* 2010). Most pesticides degrade rapidly after spraying, and organisms are frequently exposed to pesticide residues, which cause changes in physiology and behaviour, rather than to doses causing direct mortality (reviewed in Desneux, Decourtye & Delpuech 2007).

While our knowledge of the effects of sublethal exposure to pesticides on behaviour has increased rapidly, individual differences remain poorly accounted for in ecotoxicological assays (Montiglio & Royauté 2014). Current practices tend to report shifts in average behaviour post-exposure rather than focusing on how behavioural expression of individuals may change through pre- and post-exposure phases. Stated another way, current studies ignore potential effects that may be due to personality differences. Most studies focus on unique traits rather than using the multidimensional approach favoured by behavioural syndrome studies.

Studying how personality differences and correlations among personality traits vary under exposure to pesticides addresses a significant gap in our knowledge on the consequences of pesticide exposure on non-targeted organisms. It is important to understand if differences in aggressive, bold or exploratory behaviours remain consistent when individuals are exposed to pesticide stress. These effects can be particularly relevant for predator species with regulating effects on the population dynamics of prey species. In an agroecological context, certain behavioural phenotypes may participate more actively in biocontrol (e.g., active and voracious individuals) and a decoupling of these differences through pesticide exposure may limit their contribution to pest control.

Spiders (Araneae) are an ideal taxon to study the interaction between behavioural variation and pesticide exposure. Spiders are abundant in many agroecosystems and provide important pest regulation services (Carter & Rypstra 1995). They are sensitive to pesticides (Pekar 2012) and their personality traits and behavioural syndromes are well documented

(Pruitt & Riechert 2012). We used the jumping spider *Eris militaris* (Araneae: Salticidae) as a model taxon in this research. This species is commonly found in apple orchards and is easily reared under laboratory conditions. Previous work indicated differences in syndrome structure when comparing insecticide-free and insecticide-exposed populations, most notably regarding the strength of an activity-voracity syndrome (Royauté, Buddle & Vincent 2014). Here, we expand on this work by testing how direct exposure to an insecticide can impact personality traits and their syndromes. We focus on traits related to activity and prey capture behaviours because these traits are frequently correlated in spiders with important consequences for individuals' survival and fitness (Pruitt, Riechert & Jones 2008; Pruitt & Krauel 2010).

Our objective was to test whether sublethal exposure to an organophosphate insecticide can alter personality, either by affecting the consistency of behavioural traits or by affecting the strength of correlation between traits. We tested several hypotheses by which sublethal exposure to insecticides is expected to alter the expression of personality traits and their correlations. First, insecticidal exposure may affect patterns of repeatability, a measure of the extent of personality differences (Fig. 1a). Such differences may occur because 1) insecticide-exposed individuals become more similar (collapse of behavioural differences hypothesis); 2) each individual becomes more variable after exposure (variability increase hypothesis); or 3) personality differences are amplified after insecticidal exposure (amplification of behavioural differences hypothesis). Second, insecticidal exposure may affect patterns of correlation between behavioural traits and either reduce (syndrome collapse hypothesis) or amplify (syndrome amplification hypothesis) the magnitude of behavioural correlations (Fig1b).

Materials and methods

SPIDER COLLECTION AND REARING

Juvenile *E. militaris* were collected from three populations in habitats where insecticide exposure is expected to be low. The first site was an apple orchard managed without insecticidal applications since its implementation 20 years ago (Agriculture and Agri-Food Canada experimental farm in Frelighsburg, QC, W 45.0462, N -72.8565). The other sites were shrubby areas located near the McGill Morgan Arboretum (Ste Anne de Bellevue, QC, W 45.440185, N -73.946893) and the Pin Rigide Ecological Reserve (Saint-Chrysostome, QC, W 45.111657, N -73.876557). Spiders were collected haphazardly by beating the foliage of trees and brought to the laboratory. We also included laboratory-reared specimens (F1) collected in the apple orchard site. Juveniles were reared to adults in cylindrical containers (760 mL Plastipak®) that included a plastic plant to mimic natural conditions (Carducci & Jakob 2000) and a small plastic straw retreat (L = 2.5 cm, ⌀ =1.2 cm). They were kept at 24 °C and 40 % humidity, under a 16L:8D photoperiod. Water was provided *ad lib* using dental cotton inserted in an Eppendorf tube. Spiders were fed weekly with a mixed diet of cabbage looper larvae (*Trichoplusia ni*), two species of adult fruit flies of different sizes (*Drosophila melanogaster* and *Drosophila hydei*) and juvenile domestic crickets (*Acheta domestica*).

BEHAVIOURAL TESTS

We tested 176 adult individuals for behavioural correlations (Arboretum population - females: n = 15, males: n = 7; apple orchard population - females: n = 42, males: n = 51; laboratory-reared population - females: n = 33, males: n = 14; Pin Rigide population -

females: n = 9, males: n = 5). We used a repeated measure design that allowed us to simultaneously compare effects of insecticides on differences in average behavioural values between treatments as well as investigating effects on behavioural variations at between and within-individual levels. We processed the spiders through the behavioural tests with up to 16 individuals at a time (minimum 8). In order to standardize satiety, we offered spiders one adult fruit fly (*D. hydei*) during the week preceding the tests and one adult *D. melanogaster* 12 h prior to the tests. We then recorded spiders' behaviour using open-field and prey capture tests performed before and after exposure to the insecticide (hereafter referred to as pre and post-exposure phases). We consistently conducted behavioural tests in the same order, with the open-field conducted from 8:30 to 11:00, and prey capture from 14:00 to 16:00. At the end of the first day of testing, we exposed spiders to a sublethal dose of the organophosphate phosmet for 24 h using the procedure described in Appendix S1 (see Supporting Information). Phosmet was selected as it is a widely-used, broad-spectrum insecticide which mode of toxicity, inhibition of acetylcholinesterase, activity, is well documented (Mineau 1991). The sublethal dose we used was in the range of field concentrations and was therefore ecologically relevant for our experiment (~1 ppm) (Pettis *et al.* 2013).

Half of the spiders were introduced in test tubes containing the sublethal dose and the other half were introduced in control tubes (control group: n = 81, insecticide-treated group: n = 95). After 24 h exposure, we reintroduced spiders in their containers, offered them one *D. melanogaster*. Because sublethal effects of insecticides are often short-lived and reversible (Desneux, Decourtye & Delpuech 2007), we repeated the behavioural tests immediately on the following day (inter-test interval: 48 h). In the insecticide-treated group, two individuals (out of 96) died, while in the control group, one individual (out of 81) died. We took mass and body-size measurements on 151 individuals. Spiders were weighed immediately after the prey capture test at pre- and post-exposure phases. Body mass (± 0.1 mg) was determined using a Sartorius TE214S scale. We used cephalothorax width (± 0.001 mm) as a proxy for body size and measured using a WILD MMS 225 digital length measuring set. Body-condition was estimated as a residual index (following Jakob, Marshall & Uetz 1996). All tests were videotaped using a Canon Vixia HF200 camera. To remove traces of conspecific cues, we cleaned test arenas with 70% ethanol and air-dried them for 120 s between trials. We used video playback with the software The Observer XT (Noldus Information Technology, Wageningen, The Netherlands) to acquire the parameters related to activity and prey capture.

Open-field test

We used a wooden open-field arena of 30 × 30 cm divided in 5 × 5 cm quadrats for the open-field test (Carducci & Jakob 2000). The arena was subdivided in three zones: a central zone (4 quadrats), an intermediate zone (12 quadrats) and an edge zone (20 quadrats). We let each spider rest for 120 s in a 5 cc syringe before we released it at the center of the arena. We started recording as soon as the spider entered one of the four central quadrats. During 300 s, we recorded the latency to exit the first quadrat (s), the total number of quadrats visited, the number of unique quadrats visited, the number of quadrats visited during the first minute of the test and the number of quadrats visited in each zone of the arena.

Prey capture test

We introduced each spider in a 9 cm Petri dish and left it to rest for 120 s. At the end of the resting period, we inserted an adult *D. hydei* into the Petri dish through a hole on its side using a buccal aspirator. Due to a technical problem during the rearing of *D. hydei*, we used the smaller prey species *D. melanogaster* for a subset of 42 individuals. As none of the observed behaviours differed significantly depending on fly species ($p > 0.3$), this variable was removed from subsequent analyses. Spiders were given a duration of 600 s to capture the prey. We recorded the latencies to detect (defined as the first orientation toward the prey) and capture the prey, as well as the average time performing visual and active tracking of the prey. We defined visual tracking as instances where the spider oriented its cephalothorax toward the prey and visually followed the prey at a distance. Active tracking was defined as a fast forward motion toward the prey, often concluded by a capture attempt. The test was stopped as soon as the spider captured the prey or when the 600 s duration was reached. The fly was removed from the spider by probing it with the tip of a small brush in order to keep satiety consistent between tests. We assigned a capture latency value of 600 s for spiders that failed to capture the prey. Proportion of capture success did not differ between treatments (Fisher exact test, $P= 0.25$).

STATISTICAL ANALYSES

We conducted all analyses with R, version 3.0.0 for Macintosh (R Core Team 2013). We used the Bayesian package MCMCglmm (Hadfield 2010) for mixed modelling analyses and lavaan (Rosseel 2012) for structural equations modeling analyses. Prior to all analyses, count data (e.g., number quadrats travelled, number of attacks on prey) were square-root transformed and continuous data (e.g., detection and capture latencies) were $\ln(x+1)$-transformed.

Exploratory analysis

We used the exploratory analysis described in Appendix S1 to identify the behavioural variables that were the best indicators of activity and prey capture. For activity, the variables selected as best indicators were the total activity (total number of quadrats explored), the surface explored (number of unique quadrats explored) and the edge activity (number of edge quadrats visited). For prey capture, those variables were the latency to detect and capture the prey as well as the amount of active and visual tracking performed by the spider (Appendix S1, Table S1, Fig. S1). We hereafter collectively refer to these seven variables as behavioural traits.

Effect of insecticidal exposure on average behaviour

In order to investigate the effects of insecticidal treatments on the expression of average behaviour, we performed Bayesian univariate linear mixed models on each behavioural trait selected through the previous exploratory analyses (Appendix S2a). Fixed effects included population, sex and the interaction between treatment and test phase. Individuals were included as random effects to account for repeated measures. Our hypothesis was that sublethal effects on behaviours would occur only in the post-exposure phase and would be detected through a significant treatment × test interaction. We reported the posterior mode for each fixed effect estimates along with their 95% credible intervals (CI).

Effect of insecticidal exposure on behavioural repeatability

Repeatability is commonly used as a measure of the extent of individual differences in behaviour, and is defined as the ratio of between-individual variance over the total phenotypic variance: $R = V_{BI} / (V_{BI} + V_{WI})$, where the phenotypic variance V_P is expressed as $V_P = V_{BI} + V_{WI}$ (V_{BI} : between-individual variance, V_{WI} : residual or within-individual variance). We used Bayesian univariate mixed models to compare behavioural repeatability between treatments and sex (Appendix S2a). Individuals were included as random effects. Significant fixed effects selected through the above analyses (i.e. population and sex) were included to avoid over confident estimates of repeatability (Nakagawa & Schielzeth 2010; Westneat *et al.* 2011). All behavioural variables were expressed as standard deviation units to facilitate convergence. We report the posterior mode and 95 % credible intervals for repeatability as well as the effect size for the difference in repeatability between treatments: ΔR (defined as $R_{\text{insecticide-treated}} - R_{\text{control}}$). To further test which component of the phenotypic variance most influenced differences in repeatability, we calculated ΔV_{BI} and ΔV_{WI} (defined as $V_{\text{insecticide-treated}} - V_{\text{control}}$). We based inference on overlap of the 95% CIs with zero.

Effect of insecticidal exposure on behavioural correlations

To test whether sublethal insecticide exposure would affect patterns of trait covariance at between and within-individual levels, we performed Bayesian multi-response mixed models on each treatment group separately (Appendix S2b). We assessed the magnitude of between (r_{BI}) and within-individual (r_{WI}) correlation based on the posterior mode of its estimate and used the 95% credible intervals and the percentage of estimates excluding zero as a measure of the precision of the estimates. We then performed Mantel's test on each "slice" of the posterior correlation matrices to test for overall differences in correlation structure between treatments. We also report the average difference in pairwise correlation between treatments, Δr, to provide a qualitative assessment of the magnitude of the difference in correlation between treatments. This value was calculated as the average of $r_{\text{insecticide-treated}} - r_{\text{control}}$. This is appropriate since correlation estimates for each treatment come from separate models. We based statistical inference on the following scale: $0 < |\Delta r| < 0.2$, no to low effect; $0.2 < |\Delta r| < 0.5$, medium effect; $|\Delta r| > 0.5$, strong effect (Nakagawa & Cuthill 2007).

Results

EFFECTS ON AVERAGE BEHAVIOURS

We found no evidence for an effect of phosmet on average behaviour between control and treated groups. Estimates of the treatment × test phase interaction were < 1 and showed substantial overlap of their CIs with zero (Table S3). Other sources of variation such as sex for activity traits and population for prey capture traits had significant effects and were included as fixed effects in all subsequent models to provide unbiased estimates of repeatability and behavioural correlations.

EFFECTS ON BEHAVIORAL VARIANCE

Repeatability of behavioural traits ranged from 0.07 to 0.68 with strong differences detected between treatments and sex (Fig 2, Table S4, S5). Trait repeatability decreased by an average of 23 % in the insecticide-exposed group, as indicated by the average difference in repeatability among treatments, ΔR (posterior mode [95 % CI], ΔR = -0.23 [-0.48; 0.07], negative values indicates lower repeatability in the insecticide-treated group). Males showed a pronounced decline in the repeatability of all activity traits in the insecticide-treated group (Fig 2, Table S5). In contrast, prey capture repeatability was primarily affected in females.

Males showed stronger evidence for a variability increase scenario than a collapse in behavioural difference. Between-individual variance decreased simultaneously with an increase in within-individual variance but the magnitude of the effect was stronger for the within-individual variance component (ΔV_{BI} = [-0.37; -0.41]; ΔV_{WI} = [0.45; 0.95]). Insecticide-treated females followed both the variability increase and behavioural difference amplification scenarios depending on the prey capture trait considered. Females exposed to the insecticide increased their within-individual variation in active tracking (ΔV_{BI} = 0.07 [-0.10; 0.58]; ΔV_{WI} = 2.46 [1.66; 3.21]), while increasing their between-individual variation in visual tracking (ΔV_{BI} = 0.27 [-0.02; 0.79]; ΔV_{WI} = 0.09 [-0.32; 0.39]) (Fig. 2).

EFFECTS ON BEHAVIORAL CORRELATIONS

We found strong sex-specific differences in the way behavioural correlations responded to insecticide exposure (Fig 4). Females exposed to the insecticide showed the most evidence of a collapse of the activity-prey capture syndrome (Fig. 1b) (Mantel test r [95% CI] indicating the overall correlation between behavioural correlation matrices of the control and insecticide-treated groups, females - between-individuals: r = 0.31 [-0.08; 0.77], within-individuals: r = 0.77 [0.57; 0.92]; males - between-individuals: r = 0.59 [0.12; 0.88], within-individuals: r = 0.78 [0.53; 0.93]).

The decrease in between-individual correlations in insecticide-exposed females was mostly the result of a weakening of correlations between activity and prey capture traits. Females that were more active on average tended to have higher capture latencies and spend a higher proportion of their time actively pursuing prey. Such tendencies were not noticed in the insecticide-treated group (total activity-capture latency: $r_{BI} = 0.33$ [-0.08; 0.63], $\Delta r_{BI} = -0.29$; total activity-active tracking: $r_{BI} = 0.40$ [-0.11; 0.62], $\Delta r_{BI} = -0.34$). Investigation of pair-wise difference in correlation estimates also indicated a sign inversion of certain within-individual correlations (Table S6) (total activity-active tracking: $r_{WI} = -0.28$ [-0.55; 0.05], $\Delta r_{WI} = 0.46$; edge activity-active tracking: $r_{WI} = -0.27$ [-0.50; 0.08], $\Delta r_{WI} = 0.43$). In the absence of insecticide exposure, females who increased their activity in between test phases lowered the amount of time spent actively pursuing prey, while insecticide-exposed females showed the reverse trend.

Males did not show evidence of a collapse of the activity-prey capture syndrome as a result of insecticide exposure. They did show, however, subtle changes in between-individual correlations among activity traits, suggesting insecticide exposure changed the way males explored their environment. Control individuals that traveled further into the arena also tended to explore a greater surface and this pattern weakened in insecticide-treated males (total activity-surface explored: $r_{BI} = 0.73$ [0.43; 0.84], $\Delta r_{BI} = -0.29$; total activity-edge activity: control, $r_{BI} = 0.73$ [0.41; 0.82], $\Delta r_{BI} = -0.27$). Contrary to females, we did not notice any notable difference in patterns of behavioural correlations at the within-individual level ($\Delta r_{WI} \leq 0.20$).

Discussion

Our objective was to test sublethal insecticidal exposure as a potential disrupter of personality expression in the jumping spider *E. militaris*. By exposing spiders to an ecologically relevant concentration of insecticide, we found that sublethal effects can occur at the individual level even in the absence of a population-wide shift in average behaviour. We detected substantial differences in repeatability and in the correlation between personality traits expressed among insecticide-exposed and non-exposed groups. We also found that sex had a strong influence on which specific traits or correlation was most likely to be altered, suggesting males and females differ in the way they cope with insecticide exposure.

SEX-SPECIFIC DIFFERENCES IN INDIVIDUAL RESPONSE TO INSECTICIDE EXPOSURE

Our study shows that sublethal exposure to an insecticide produces complex effects on the expression of personality traits and their correlations. Previous work conducted with *E. militaris*, showed evidence that populations from insecticide-free and insecticide-treated apple orchards differed in the overall architecture of their behavioural syndromes (Royauté, Buddle & Vincent 2014). In the present study, we were able to investigate these results further by directly manipulating individuals' insecticide exposure level. As a result, the repeatability of personality traits declined by an average of 23% in the insecticide-exposed group, and these differences were mostly mediated by an increase in within-individual variance, thus supporting the variability increase hypothesis.

Interestingly, while alterations of behavioural repeatability between treatments occurred for five out of the seven traits considered, males and females showed important differences in which type of trait was most affected and in which direction. Females showed strongest differences in the repeatability of prey capture behaviours between treatments, with insecticidal exposure either increasing an individual's variability (i.e., active tracking) or amplifying between-individual differences (i.e., visual tracking). Males, in contrast, showed an increased variability for all activity traits while prey capture traits were largely unaffected. These sex-specific differences were also maintained when investigating how behavioural correlations responded to insecticide exposure. We found more support for a collapse of the activity-prey capture syndrome in females than in males. Inactive females were quicker to capture prey in the absence of insecticide exposure, a tendency no longer expressed in the treated group. Males did not show evidence for such an activity-prey capture syndrome, even in the control group, but showed a decrease in correlation strength among all activity traits. Taken together, our results suggest that insecticide-exposed individuals showed a strong departure from their personality tendencies. In other words, an active spider becoming "under the influence" of insecticides may no longer behave as active as it would otherwise. These effects were expressed differently among sexes as insecticide exposure was more likely to cause changes in female hunting strategies while male exploration was altered irrespectively of prey capture.

Several mechanisms are likely to contribute to these patterns. Personality traits are underpinned by a variety of physiological mechanisms, including differences in metabolic rates, immune responses and wiring of neuroendocrine pathways (Sih, Bell & Johnson 2004a; Careau *et al.* 2008; Niemelä *et al.* 2012). Since organophosphates disrupt the activity of acethylcholinesterase, differences in acethylcholine receptor density among individuals would likely affect the sensitivity of individual to a given insecticide dose. Another possibility could be that some individuals experience reduced immune function as a result of insecticide exposure (Desneux, Decourtye & Delpuech 2007). This could result in changes in energy budgets and ultimately alter the energy allocation priorities of individuals.

The sublethal toxicity of organophosphate on beneficial arthropods has been found to affect many behaviours related to mobility and locomotion, with sexual differences reported in some cases (Tietjen & Cady 2007; Hanna & Hanna 2013). However, such studies tend to focus on shifts in average behavioural or physiological response. In contrast, our results suggest that sublethal effects may still be present even when population-wide behavioural shifts remain undetected. This result could have important repercussions on evolutionary and ecological processes, which we outline below.

EVOLUTIONARY AND ECOLOGICAL IMPLICATIONS

Personality traits are often adaptive and are related to individual differences in physiology, life-history trajectories and fitness (Koolhaas *et al.* 1999; Dingemanse & Réale 2005; Réale *et al.* 2010). By reducing the amount of personality differences expressed in certain traits, sublethal exposure to insecticides can cause insidious effects on an individual's fitness, even in absence of a population-wide response. For example, by altering exploration and foraging strategies, insecticidal exposure could affect an individual's capacity to capture prey or to disperse into suitable habitats. In many spider species, prey capture efficiency is part of a broader "aggression syndrome" involving aggressive tendencies against conspecific and antipredator tendencies (Riechert & Hedrick 1993; Johnson & Sih 2005; Johnson & Sih 2007; Pruitt, Riechert & Jones 2008, reviewed in Pruitt & Riechert 2012). Such "packages" of traits often share similar physiological underpinnings (Sih, Bell & Johnson 2004b). Any insecticide compound affecting behavioural differences in one trait is likely to profoundly affect all related traits. Ultimately, such alterations may result in changes in an individual's life-history strategy.

Individual-level effects could also precede population-wide effects, especially in the case of chronic exposure. This scenario is most likely if individuals differ in their sensitivity to the insecticide. More sensitive individuals would experience behavioural shifts sooner than the rest of the population and thus experience reduced fitness. Therefore, monitoring effects of insecticides on trait variance may provide a better estimate of early disruption of behavioural traits.

By uncoupling behaviours related to activity and prey capture in arthropod predators, insecticides exposure may cause cascading effects on prey-population dynamics. Personality differences are known to influence the strength of trophic cascades as certain individuals can contribute more strongly to the top-down control of prey species (Griffen, Toscano & Gatto 2012; Toscano & Griffen 2014). In agricultural landscapes frequently exposed to insecticides, effects on the overall architecture of personality differences may therefore reduce the environmental services provided by biocontrol agents.

TOWARDS AN ECOTOXICOLOGY OF INDIVIDUAL DIFFERENCES

Ecotoxicological studies focusing on effects of contaminants on behavioural differences remain scarce and have been mostly restricted to aquatic systems. For example, exposure to heavy metals can decrease the repeatability of critical swimming speed in fathead minnows (Kolok, Plaisance & Abdelghani 1998), and exposure to anxiolytic drugs resulted in the emergence of correlations between previously uncorrelated personality traits in perches (Brodin *et al.* 2013). Change in personality expression resulting from contaminant exposure is most likely a widespread phenomenon occurring in multiple types of ecosystems independently of contaminant class. Yet, these types of effects remain widely understudied in terrestrial systems (but see Morales *et al.* 2013 for a recent example).

Our study addresses a significant gap in our knowledge by revealing the complex ways by which multiple behavioural functions can be altered by insecticide exposure. We suggest that, whenever possible, longitudinal studies should be implemented to monitor the effects of insecticides over time at the individual level. While longitudinal studies have been included with great effect in the past, they typically treat repeated measures on individuals as a nuisance parameter (e.g., Gill & Raine 2014). We favour a more integrative approach where effects of contaminants on both average *and* (co)variance among behaviours can be fully explored. We believe such an approach will expand our understanding of how sublethal effects of insecticides and other types of contaminants operate and will generate more robust predictions for population persistence.

Finally, we investigated only one dose of a very specific compound. In field conditions, sublethal effects may be even more severe. For example, insecticidal exposure and accumulation may be mediated by personality differences. Individuals with higher activity may explore larger areas and be more frequently in contact with insecticide residues. An important way forward is to consider that personality differences may affect insecticide exposure and accumulation, and the received insecticide dose may in turn affect personality expression in a feedback loop (Montiglio & Royauté 2014). It is also important to consider the interactions between multiple insecticidal compounds, since different insecticides are used to control different types of pests. Such “cocktails” often act in synergy, having drastically different effects than exposure to each compound separately (Kortenkamp 2007). These types of interaction can be extended to study various classes of anthropogenic contaminants and model different paths of effects, allowing better predictions of the consequences of exposure to anthropogenic contaminants.

Our results point to several key conclusions, relevant to both basic and applied perspectives. First, assessing the effects of insecticides solely on differences in average behaviour between insecticide-exposed and control groups is somewhat limited, as it cannot account for effects at the individual level and thus risk underestimating the toxicity of these compounds. Second, using the multivariate approach favoured by behavioural syndromes studies can generate considerable more insights into the specific ways behaviours are altered by insecticide stress. Finally, our results can be applied in bioassay procedures by incorporating behavioural variations in dose-response ecotoxicological studies. Our research calls attention on a poorly studied source of behavioural variation: the presence of neurotoxic insecticides in the environment and shows that these compounds can significantly affect personality expression in an important arthropod predator.

Acknowledgements

We thank Monique Audette, Gay Grunner and Mary Pavlik for access to field sites. Martin Ballot, Laurianne Besnard, Caroline Charrier, Anne Danthony and Benoît Valle assisted in data collection and spider rearing. We also thank Mélanie Dammhahn, Ned Dochtermann, P.-O. Montiglio, members of Chris Buddle's laboratory and NDSU Biological Sciences graduate students for helpful comments on earlier versions of this paper. We thank Dr. Charles Fox, the associate editor and two anonymous reviewers for their thoughtful comments. Funding was provided in part by the National Science and Engineering Council of Canada (NSERC Discovery Grant to CMB), Agriculture and Agri-food Canada (CV) and McGill University (Margaret DuPorte Fellowship to RR).

Data Accessibility

Data deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.4s90s, (Royauté et al. 2015).

References

Brittain, C.A., Vighi, M., Bommarco, R., Settele, J. & Potts, S.G. (2010) Impacts of a pesticide on pollinator species richness at different spatial scales. *Basic and Applied Ecology,* **11,** 106-115.

Brodin, T., Fick, J., Jonsson, M. & Klaminder, J. (2013) Dilute concentrations of a psychiatric drug alter behavior of fish from natural populations. *Science,* **339,** 814-815.

Carducci, J.P. & Jakob, E.M. (2000) Rearing environment affects behaviour of jumping spiders. *Animal Behaviour,* **59,** 39-46.

Careau, V., Thomas, D., Humphries, M.M. & Réale, D. (2008) Energy metabolism and animal personality. *Oikos,* **117,** 641-653.

Carter, P.E. & Rypstra, A.L. (1995) Top-down effects in soybean agroecosystems: spider density affects herbivore damage. *Oikos,* **72,** 433-439.

Desneux, N., Decourtye, A. & Delpuech, J.M. (2007) The sublethal effects of pesticides on beneficial arthropods. *Annual Review of Entomology,* **52,** 81-106.

Dingemanse, N.J. & Réale, D. (2005) Natural selection and animal personality. *Behaviour,* **142,** 1159-1184.

Donald, P.F., Green, R.E. & Heath, M.F. (2001) Agricultural intensification and the collapse of Europe's farmland bird populations. *Proceedings of the Royal Society of London. Series B: Biological Sciences,* **268,** 25-29.

Geiger, F., Bengtsson, J., Berendse, F., Weisser, W.W., Emmerson, M., Morales, M.B., Ceryngier, P., Liira, J., Tscharntke, T., Winqvist, C., Eggers, S., Bommarco, R., Pärt, T., Bretagnolle, V., Plantegenest, M., Clement, L.W., Dennis, C., Palmer, C., Oñate, J.J., Guerrero, I., Hawro, V., Aavik, T., Thies, C., Flohre, A., Hänke, S., Fischer, C., Goedhart, P.W. & Inchausti, P. (2010) Persistent negative effects of pesticides on biodiversity and biological control potential on European farmland. *Basic and Applied Ecology,* **11,** 97-105.

Gill, R.J. & Raine, N.E. (2014) Chronic impairment of bumblebee natural foraging behaviour induced by sublethal pesticide exposure. *Functional Ecology,* **28,** 1459-1471.

Griffen, B.D., Toscano, B.J. & Gatto, J. (2012) The role of individual behavior type in mediating indirect interactions. *Ecology,* **93,** 1935-1943.

Hadfield, J.D. (2010) MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. *Journal of Statistical Software,* **33,** 1-22.

Hanna, C. & Hanna, C. (2013) The lethal and sublethal effects of three pesticides on the striped lynx spider (*Oxyopes salticus* Hentz). *Journal of Applied Entomology,* **137,** 68-76.

Jakob, E.M., Marshall, S.D. & Uetz, G.W. (1996) Estimating fitness: a comparison of body condition indices. *Oikos,* **77,** 61-67.

Johnson, J.C. & Sih, A. (2005) Precopulatory sexual cannibalism in fishing spiders (Dolomedes triton): A role for behavioral syndromes. *Behavioral Ecology and Sociobiology,* **58,** 390-396.

Johnson, J.C. & Sih, A. (2007) Fear, food, sex and parental care: a syndrome of boldness in the fishing spider, *Dolomedes triton*. *Animal Behaviour,* **74,** 1131-1138.

Killen, S.S., Marras, S., Metcalfe, N.B., McKenzie, D.J. & Domenici, P. (2013) Environmental stressors alter relationships between physiology and behaviour. *Trends in Ecology & Evolution,* **28,** 651-658.

Kolok, A.S., Plaisance, E.P.P. & Abdelghani, A. (1998) Individual variation in the swimming performance of fishes: an overlooked source of variation in toxicity studies. *Environmental Toxicology and Chemistry,* **17,** 282-285.

Koolhaas, J.M., Korte, S.M., De Boer, S.F., Van Der Vegt, B.J., Van Reenen, C.G., Hopster, H., De Jong, I.C., Ruis, M.A.W. & Blokhuis, H.J. (1999) Coping styles in animals: current status in behavior and stress-physiology. *Neuroscience & Biobehavioral Reviews,* **23,** 925-935.

Kortenkamp, A. (2007) Ten years of mixing cocktails: a review of combination effects of endocrine-disrupting chemicals. *Environmental Health Perspectives,* **115,** 98.

Krebs, J.R., Wilson, J.D., Bradbury, R.B. & Siriwardena, G.M. (1999) The second Silent Spring? *Nature,* **400,** 611-612.

Madden, J.R. & Whiteside, M.A. (2013) Selection on behavioural traits during 'unselective' harvesting means that shy pheasants better survive a hunting season. *Animal Behaviour*.

Martin, J.G.A. & Réale, D. (2008) Animal temperament and human disturbance: implications for the response of wildlife to tourism. *Behavioural Processes,* **77,** 66-72.

Mineau, P. (1991) *Cholinesterase-inhibiting insecticides : their impact on wildlife and the environment*. Elsevier, Amsterdam.

Miranda, A.C., Schielzeth, H., Sonntag, T. & Partecke, J. (2013) Urbanization and its effects on personality traits: a result of microevolution or phenotypic plasticity? *Global Change Biology,* **19,** 2634-2644.

Montiglio, P.O. & Royauté, R. (2014) Contaminants as a neglected source of behavioural variation. *Animal Behaviour,* **88,** 29-35.

Morales, J.A., Cardoso, D.G., Della Lucia, T.M.C. & Guedes, R.N.C. (2013) Weevil x Insecticide: Does 'Personality' Matter? *PLoS ONE,* **8,** e67283.

Nakagawa, S. & Cuthill, I.C. (2007) Effect size, confidence interval and statistical significance: a practical guide for biologists. *Biological Reviews,* **82,** 591-605.

Nakagawa, S. & Schielzeth, H. (2010) Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. *Biological Reviews,* **85,** 935-956.

Niemelä, P.T., Vainikka, A., Hedrick, A.V. & Kortet, R. (2012) Integrating behaviour with life history: boldness of the field cricket, *Gryllus integer*, during ontogeny. *Functional Ecology,* **26,** 450-456.

Pekar, S. (2012) Spiders (Araneae) in the pesticide world: an ecotoxicological review. *Pest Management Science,* **68,** 1438-1446.

Pettis J.S., Lichtenberg E.M., Andree M., Stitzinger J., Rose R., et al. (2013) Crop Pollination Exposes Honey Bees to Pesticides Which Alters Their Susceptibility to the Gut Pathogen *Nosema ceranae*. PLoS ONE 8(7): e70182.

Pruitt, J.N. & Krauel, J.J. (2010) The adaptive value of gluttony: predators mediate the life history trade-offs of satiation threshold. *Journal of Evolutionary Biology,* **23,** 2104-2111.

Pruitt, J.N. & Riechert, S.E. (2012) The ecological consequences of temperament in spiders. *Current Zoology,* **58,** 589-596.

Pruitt, J.N., Riechert, S.E. & Jones, T.C. (2008) Behavioural syndromes and their fitness consequences in a socially polymorphic spider, *Anelosimus studiosus*. *Animal Behaviour,* **76,** 871-879.

R Core Team. (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Réale, D., Garant, D., Humphries, M.M., Bergeron, P., Careau, V. & Montiglio, P.-O. (2010) Personality and the emergence of the pace-of-life syndrome concept at the population level. *Philosophical Transactions of the Royal Society B,* **365,** 4051-4063.

Réale, D., Reader, S.M., Sol, D., McDougall, P.T. & Dingemanse, N.J. (2007) Integrating animal temperament within ecology and evolution. *Biological Reviews,* **82,** 291-318.

Riechert, S.E. & Hedrick, A.V. (1993) A test for correlations among fitness-linked behavioural traits in the spider *Agelenopsis aperta* (Araneae, Agelenidae). *Animal Behaviour,* **46,** 669-675.

Rosseel, Y. (2012) lavaan: An R Package for Structural Equation Modeling. *Journal of Statistical Software,* **48,** 1-36.

Royauté, R., Buddle, C.M. & Vincent, C. (2014) Interpopulation variations in behavioral syndromes of a jumping spider from insecticide-treated and insecticide-free orchards. *Ethology,* **120,** 127-139.

Royauté R, Buddle C, Vincent C Data from: Under the influence: sublethal exposure to an insecticide affects personality expression in a jumping spider. Functional Ecology http://dx.doi.org/10.5061/dryad.4s90s

Sih, A., Bell, A. & Johnson, J.C. (2004a) Behavioral syndromes: An ecological and evolutionary overview. *Trends in Ecology and Evolution,* **19,** 372-378.

Sih, A., Bell, A. & Johnson, J.C. (2004b) Behavioral syndromes: an ecological and evolutionary overview. *Trends in Ecology & Evolution,* **19,** 372-378.

Sih, A., Cote, J., Evans, M., Fogarty, S. & Pruitt, J. (2012) Ecological implications of behavioural syndromes. *Ecology Letters,* **15,** 278-289.

Sih, A., Stamps, J., Yang, L.H., McElreath, R. & Ramenofsky, M. (2010) Behavior as a key component of integrative biology in a human-altered world. *Integrative and Comparative Biology,* **50,** 934-944.

Tietjen, W.J. & Cady, A.B. (2007) Sublethal exposure to a neurotoxic pesticide affects activity rhythms and patterns of four spider species. *Journal of Arachnology,* **35,** 396-406.

Toscano, B.J. & Griffen, B.D. (2014) Trait-mediated functional responses: predator behavioural type mediates prey consumption. *Journal of Animal Ecology*.

Westneat, D.F., Hatch, M.I., Wetzel, D.P. & Ensminger, A.L. (2011) Individual variation in parental care reaction norms: integration of personality and plasticity. *The American Naturalist,* **178,** 652-667.

Wolf, M. & Weissing, F.J. (2012) Animal personalities: consequences for ecology and evolution. *Trends in Ecology & Evolution,* **27,** 452-461.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

Appendix S1. Determination of a sublethal dose of phosmet.

Appendix S2. Model specifications for exploratory analyses of activity and prey capture behaviours.

Appendix S3. Model specifications for comparing repeatability and behavioral correlations across treatments.

Figure S1. Results of the exploratory analysis for activity and prey capture.

Table S1. Between and within-individual correlations for activity and prey capture variables.

Table S2. Correlation of behavioural traits with body condition and body-size.

Table S3. Effect of insecticidal exposure and other sources of variation on behavioural traits.

Table S4. Between (V_{BI}) and within-individual (V_{WI}) variance components compared across treatments.

Table S5. Repeatability of behavioural traits compared between sex and treatments.

Table S6. Between and within-individual correlations of behavioural traits compared across treatment groups.

Figure Legends

Fig. 1. Different scenarios explaining how sublethal insecticidal exposure may alter patterns of behavioural consistency **(a)** and covariance **(b)**. **(a)** All lines represent a single individual measured before and after exposure to an insecticide. A1 indicates a control case where behavioural repeatability (R) is unchanged in the absence of insecticidal exposure. A2 and A3 represent cases where insecticidal exposure causes repeatability to decrease either by shifting individuals toward the population mean (behavioural differences collapse) or by increasing the behavioural variability of each individual (variability increase). A4 describes a case where insecticidal exposure amplifies individual differences (behavioural differences amplification). **(b)** Ellipses indicate the strength of correlation between two behavioural traits in presence (grey ellipses) and absence of insecticidal exposure (white ellipses). Insecticidal exposure may either decrease (B1, syndrome collapse scenario) or amplify (B2, syndrome amplification scenario) behavioural correlations.

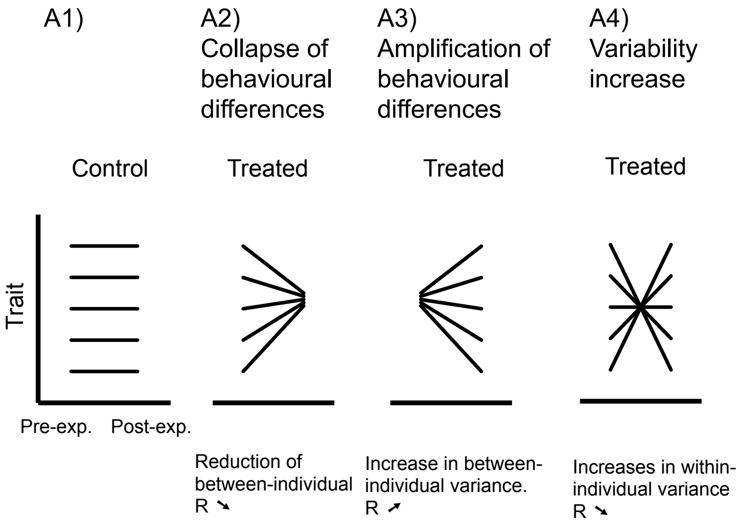
Fig. 2. Extent of personality differences (i.e. repeatability) compared across control and insecticide-treated individuals and sex (females: white circles, males: dark circles) for (a) activity and (b) prey capture traits. Values represent posterior modes ± 95 % credible interval.

Fig. 3. Difference in between **(a)** and within-individual variance components **(b)** compared across treatment groups and sex (females: white circles, males: dark circles). Values

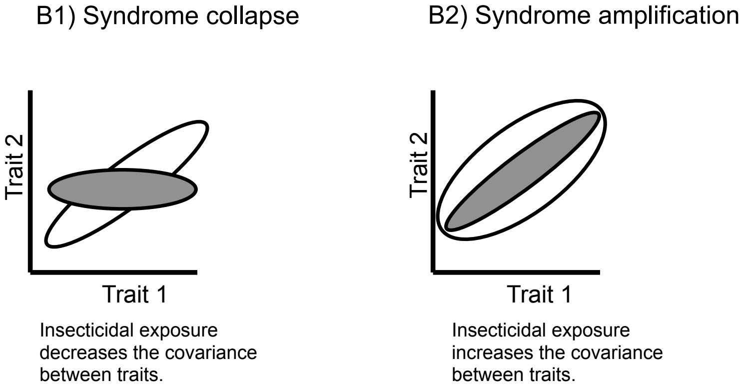
represent posterior modes ± 95 % credible interval and are expressed as deviation from the control group (i.e., positive values indicate an increase in variance in the insecticide-treated group relative to the control).

Fig. 4. Difference in behavioural correlations between control (white circles) and treated groups (black circles) for between **(a,b)** and within-individual levels **(c,d)**. Plots represent pairwise correlation with highest difference in correlation estimates between treatments (Δr) and sex (females: **a,c**, males: **b,d**). Values were simulated from the correlation matrices obtained through multivariate mixed models. Lines represent least square regressions for control (dotted lines) and insecticide-treated groups (solid lines).

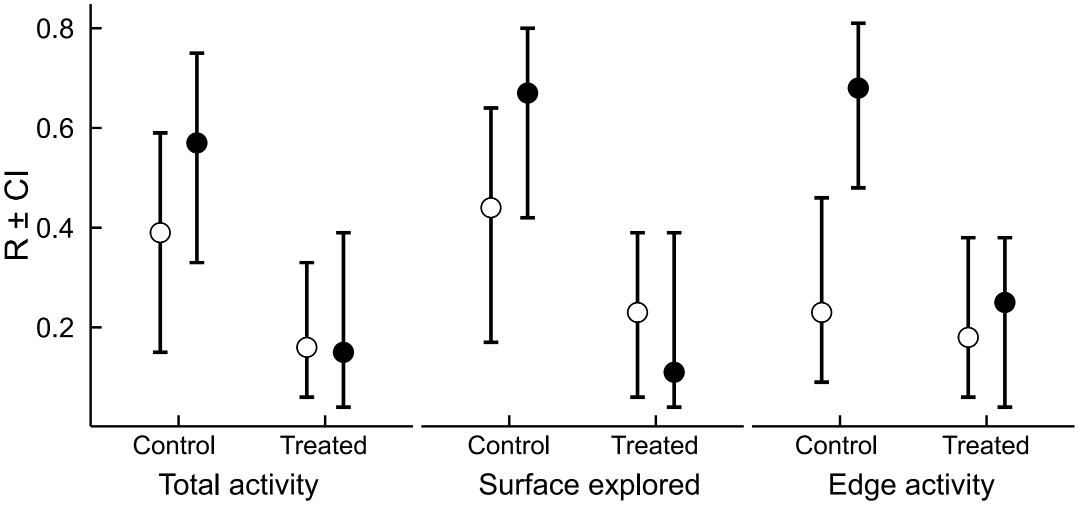
(a) Behavioural consistency



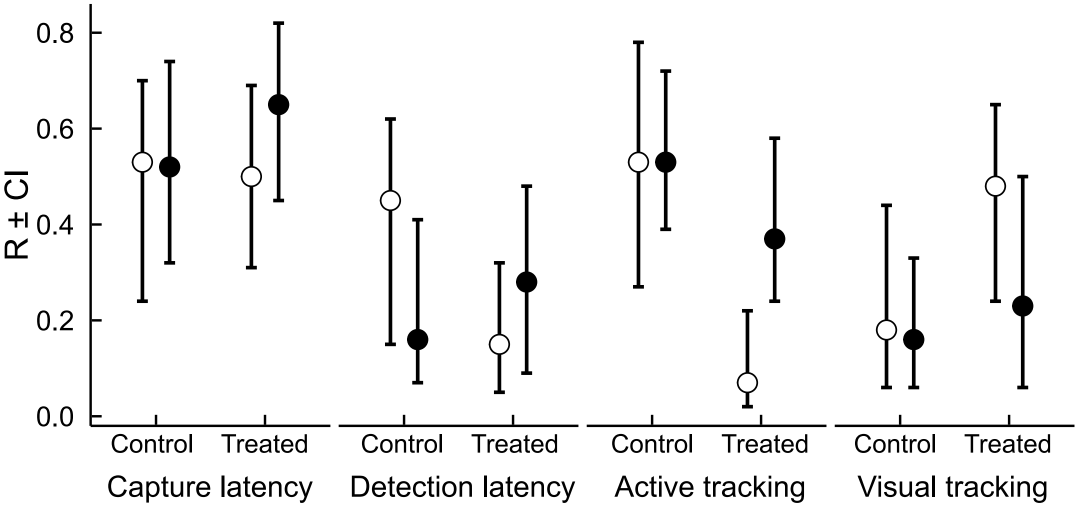
(b) Behavioural covariance



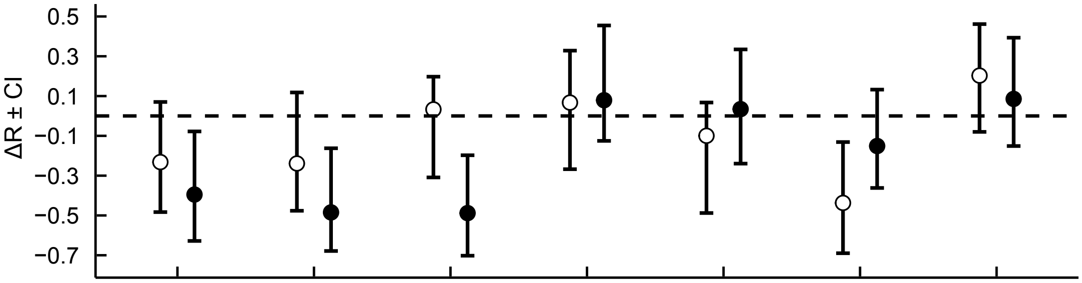
(a) Activity



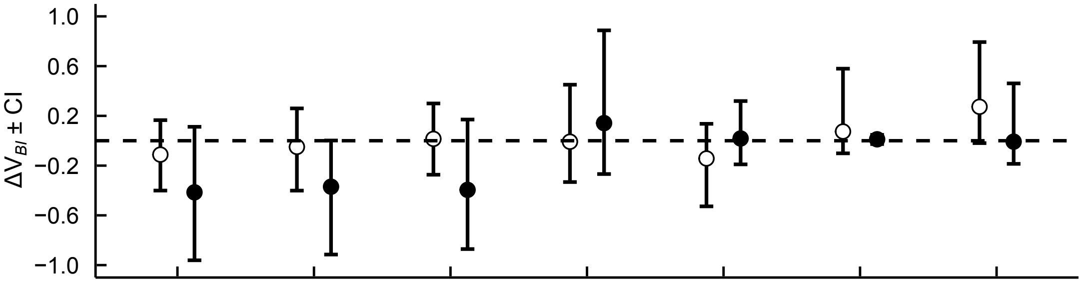
(b) Prey capture



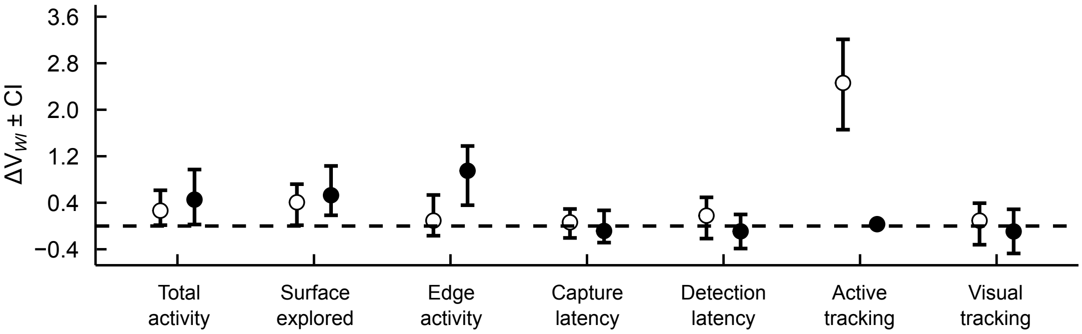
(a) Repeatability

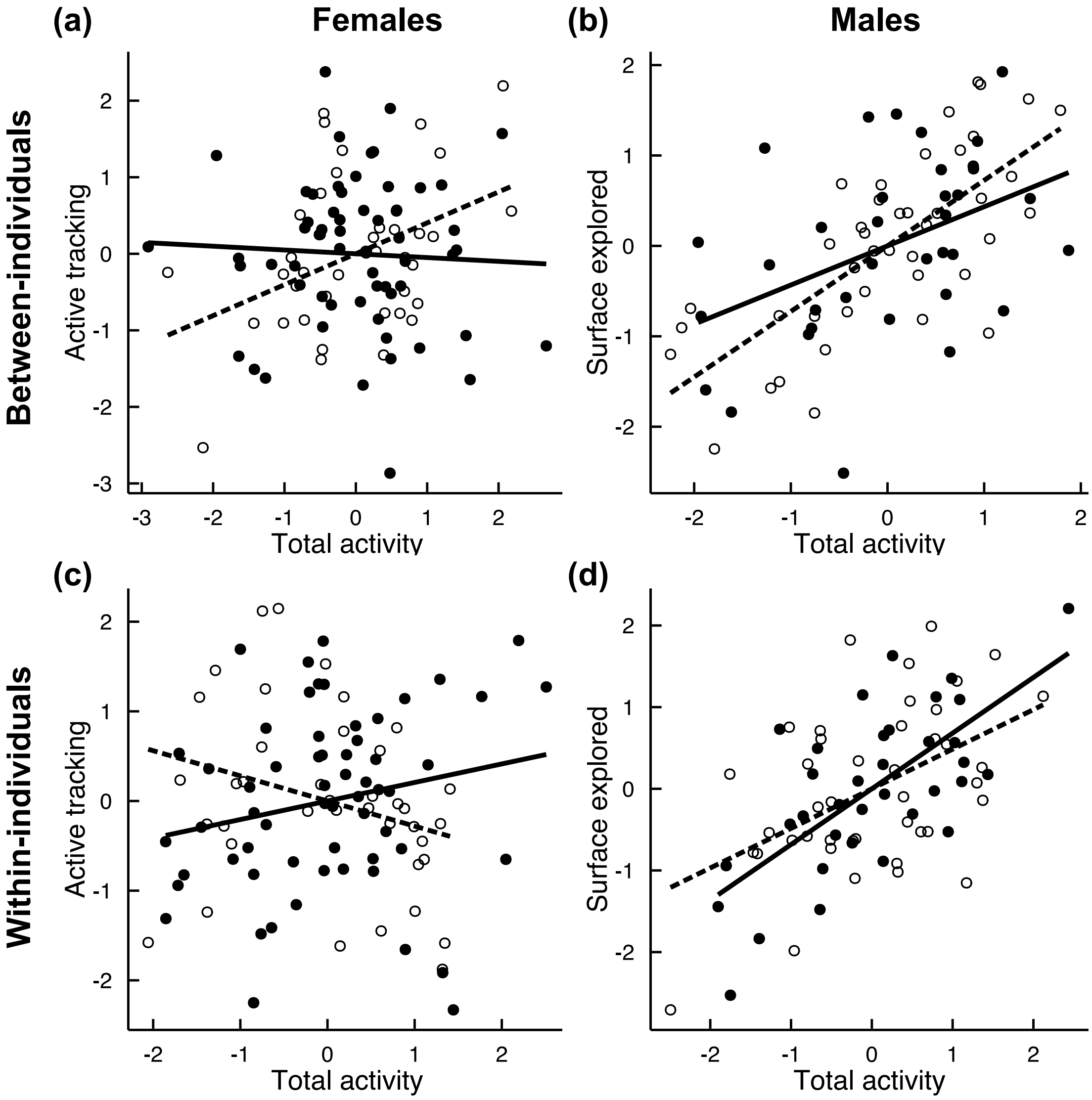


(b) Between-individual variance



(c) Within-individual variance




(a)
Females
(b)
Males
Between-individuals
Active tracking
Surface explored
Total activity
Total activity
(c)
(d)
Within-individuals
Active tracking
Surface explored
Total activity
Total activity
2
1
0
-1
-2
-3
-3
-2
-1
0
1
2