Locomotor performance and behaviour: Covariance at the among-individual and residual level, and the impact of motivation

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I would like to dedicate this thesis to my two grandfathers, were they here today, I'd like to think they'd be happy to see all the amazing science I was able to write, and how much I learned. Vous me manquez.

Acknowledgments

Well, here we are, or I guess, here I am! What a journey this was, I don't think I could have foreseen what it would take to get here, sure I had an idea, but I did not realise what the road would be like. I have many, many people to thank, people who helped me, who supported me, who guided me, people I looked up to (some I still do, crazy, I know), my peers, my colleagues, my family, my friends…

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Abstract:

One of the main objectives of evolutionary biology is to understand the reasons behind the maintenance of individual differences in a multitude of traits that influence fitness such as locomotor performance and behaviour. Because locomotor performance sets an “envelope” within which behaviour is expressed, it is likely that a multitude of co-adaptations exists between these two suites of traits. In recent years, a growing number of studies have identified associations of different strength and directions between performance and behaviour. Two main hypotheses have received support, on one hand locomotor performance could be “co-specialized” with behaviour in a manner that behaviour reduces predation risk, such that shyer, less active, less explorative animals should be the best sprinters and the most endurant. On the other hand, locomotor performance could “compensate” for behaviours that lead to increased predation risk, in a way that bolder, more active and explorative animals should be able to sprint faster and for longer. In my thesis I provide a review of published studies that successfully identify associations between locomotor performance and behaviour and classify each association as supporting the co-specialization or compensation hypothesis respectively. I further elaborate on the importance of using repeated measurements and (co)variance partitioning when studying correlations between labile traits. I also discuss one of the main challenges that comes with studying locomotor performance, namely the importance of the variation in motivation, both methodologically, by using different performance tests, but also physiologically, by using blood corticosterone measurements as indicators of such variation.

Résumé :

L'un des principaux objectifs de la biologie évolutive est de comprendre les raisons à l'origine de la maintenance des différences individuelles chez une multitude de traits qui influencent la fitness, par exemple en performance locomotrice ou en comportement. Puisque la performance locomotrice représente une « enveloppe » au sein de laquelle le comportement est exprimé, il est fort probable qu'une multitude de coadaptations existent entre ces traits. Récemment, un nombre croissant d'études ont identifiés des associations de force et de directions différentes entre performance locomotrice et comportement. Deux hypothèses ont reçu du support, d'un côté la performance locomotrice pourrait être « co-spécialisée » avec le comportement d'une manière que le comportement réduit le risque de prédation, ainsi, les animaux plus peureux, moins actifs et moins exploratifs devraient aussi être les meilleurs sprinteurs et les plus endurants. D'un autre côté, il aussi est possible que la performance locomotrice « compense » les comportements qui entrainent une augmentation du risque de prédation, ainsi les individus les plus téméraires, les plus actifs et les plus exploratifs devraient être capable de sprinter plus rapidement et d'être plus endurants. Dans ma thèse je présente une revue des études qui ont identifié des associations entre performance locomotrice et comportement et classifie chacune de ses associations selon leur support pour l'hypothèse de cospécialisation ou de compensation. Je mets aussi en évidence à quel point il est important d'utiliser des mesures répétées et de partitionner les (co)variances lorsque l'on s'intéresse aux corrélations entre des traits labiles. Enfin, je discute aussi l'un des principaux défis qui émerge lorsque l'on étudie performance locomotrice, à savoir l'importance de la variation en motivation, que ça soit d'un point de vue méthodologique, en utilisant des tests de performance différents, mais aussi d'un point de vue physiologique, en utilisant des mesures de corticostérone comme un indicateur potentiel de cette variation.

Statement of contributions:

Chapter 2 is a slightly modified version of the following published paper co-authored by Jennifer Thomson, Carsten Schradin and Vincent Careau:

Agnani, P., Thomson, J., Schradin, C., & Careau, V. (2020). The fast and the curious II: performance, personality, and metabolism in Karoo bush rats. Behavioral Ecology and Sociobiology, 74, 1-14.

The pronoun “we” is used in reference to the co authors (here Jennifer Thomson, Carsten Schradin and Vincent Careau), I designed the experiment, Jennifer and I collected the data, I analysed the data, wrote the manuscript and was provided comments by Carsten Schradin and Vincent Careau.

Chapter 3 is a slightly modified version of the following paper co-authored by Vincent Careau:

Agnani, P., & Careau, V. (2023). The fast and the curious III: speed, endurance, activity, and exploration in mice. Behavioral Ecology and Sociobiology, 77(2), 26.

The pronoun “we” is used to refer to the co-author (Vincent Careau), I designed the experiment, collected the data, analysed the data and wrote the manuscript and was provided comments by Vincent Careau.

Thesis overview

This thesis is organized in four separate chapters each tackling different aspects of coadaptation of locomotor performance and behaviour. The first chapter, which also serves as a general introduction, is a comprehensive review of studies reporting associations between performance and behaviour. I present there, key concepts of the field, identify different literature gaps that could benefit from additional research, and include a re-analysis of previously published data showing the benefits of (co)variance partitioning. The second and third chapters are published field and laboratory studies, respectively. Each expands on the avenues identified in my first chapter. In chapter 2, we partition correlations between behaviour and locomotor performance and provide a multilevel analysis of raw, unaggregated performance measurements. In chapter 3, we partition correlations between behaviour and two locomotor performance traits, one of which is based on the animal's survival instinct to elicit motivation instead of a human threat as typically used to prompt running. The fourth chapter represents an attempt at quantifying the impact of motivation on performance measurements relying on a human threat, using corticosterone levels in blood samples collected immediately after performance measurements. Chapters 3 and 4 are the result of a laboratory experiment that yielded a large dataset containing a range of different traits. Not included in this thesis, is the study of the relationship between the metabolic traits (resting metabolic rate, daily energy expenditure) and cage behaviours, which allowed us to study the energy management models. This work, spearheaded by Aly Abdeen during his honour's project, lead to a publication (not included in this thesis) on which I am co-author:

Abdeen, A., Agnani, P., & Careau, V. (2022). The active mouse rests within: energy management among and within individuals. Functional Ecology, 36(3), 526-537.

Finally, the order of the chapters does not reflect the chronological order in which they were published, which is why chapters 2 and 3 are included in the tables of published studies in chapter 1.

Chapter 1: A review of associations of locomotor performance and behaviour

Introduction

Variation among repeated performance measurements made on a given individual has historically been considered a nuisance to be discarded, with researchers typically preferring to retain only the maximum value per individual (i.e., "personal bests"). Maximum performance was measured because it was thought to be constrained by and determined by lower-level traits (which are not otherwise directly targeted by selection), therefore allowing the study of how sub-organismal traits evolve. Interestingly, this also meant that maximal performance should represent the theoretical maximal "power output" of an individual, thus giving researchers the upper limit of the possible behaviours that can be expressed by an individual. But in the more recent years, neighboring fields such as animal behaviour or energetics have embraced the full multi-level variability inherent to complex organismal traits. To help make connections among these fields, and to further our understanding of the multivariate evolution of performance, here I explore the potential linkages between locomotor performance and animal behaviour. I review the literature and classify studies according to their support for two competing hypotheses (trait compensation and co-specialization, see below). I also highlight how performance measurements are confounded with behaviour to various degrees, and how those terms are sometimes even used interchangeably. I propose a performance-behaviour continuum along which most traits measured using standardised tests can be positioned depending on the relative influence of stress and motivation in the measured output. Finally, I demonstrate how researchers interested in locomotor performance can obtain more accurate estimates of associations by partitioning correlations using a re-analysis of previously published data on performance measurements in mice.

Locomotor performance

Animal performance, defined as the ability of an organism to perform a task when maximally motivated, represents one of the main potential targets of natural selection (Arnold 1983). Because of clear potential links to Darwinian fitness, studies of performance have massively contributed to the field of evolutionary biology (Miles 2004; Husak et al. 2006; Irschick et al. 2008). Performance abilities can affect multiple daily tasks of an animal in its natural habitat, such as foraging (Miles et al. 2007), escaping predators (Domenici 2010), defending its territory, acquiring mates, and fighting conspecifics (Lailvaux et al. 2004; Lappin and Husak 2005; Husak and Fox 2008). As the list of performance measurements can be quite large, ranging from running speed, endurance, and grip strength in terrestrial animals (Garland 1999; Bonine and Garland 1999; Tulli et al. 2012; da Silva et al. 2014), to swimming in aquatic and flying abilities in aerial animals (Ellington 1991; Ghalambor et al. 2003), it is important to use operational definitions of the performance traits, ideally tied to specific standardised tests designed according to the species behavioural ecology (e.g., foraging mode, anti- predator strategies, intra-sexual competition) and preferred substrate (e.g., arboreal vs terrestrial). Moreover, biochemical and morphological properties of organisms generate trade-offs between performance traits (Wilson and James 2004; Gvož and van Damme 2006; Berberi and Careau 2019). Performance traits are also linked to multiple other biologically important traits such as morphology by which locomotor abilities are constrained (Bennett 1989; Garland and Losos 1994), but also to metabolism (Careau and Garland 2012). Finally, performance may define the limits within which an individual can behave [setting a “performance space”, see (Bennett 1989)], but behaviour should ultimately be a more direct target of selection than performance itself (Garland and Losos 1994).

Animal behaviour

Animal behaviour is one of the main domains within evolutionary biology, tracing its roots all the way back to the early 1920s with the behaviorists and ethologists. Since then, thousands of studies have focused on explaining the observed variation in animal behaviour. Behavioural ecologists have focused on different sub-topics which resulted in trends in the number of publications; from optimal foraging and mating systems in the 1980's and 1990's, to sexual selection and host-parasite interactions in the 1990's to early 2000's (Owens 2006). Perhaps the most recent revolution in the field of animal behaviour was the integration of the concepts and techniques from the field of quantitative genetics (Sih et al. 2004; Réale et al. 2007) to study behaviour, or more specifically animal personality [i.e., the repeatable differences in behaviour across context or time (Réale et al. 2010)]. Besides a clear focus on behavioural (co)variation at the among-individual level, a main feature of studies on animal personality includes the use of standardised tests to captures aspects of activity, exploration, boldness, aggressivity, and sociability depending on the social context of the tests, degree of novelty, and perceived risk to the animal.

The behaviour-performance continuum

Interestingly, tests used to measure performance and behaviour capture traits that are influenced by varying levels of stress and motivation. These traits can be positioned along a behaviour-performance continuum that ranges from what animals do when given the choice (i.e., behaviour) to what they can do when maximally motivated (i.e., performance). In addition to motivation, an individual's reaction to any novel and/or risky tests will be greatly influenced by its stress coping style (e.g., the novel environment test; Perals et al., 2017). While some tests clearly capture performance (e.g., endurance on a treadmill) or behaviour (e.g., activity in home cage over 24 hours), other tests may be harder to categorize. This is especially the case for performance tests relying on the animal's intrinsic motivation (e.g., bite force, see below) and for behavioural tests that

involve aspects of risk-taking (e.g., latency to exit a refuge). Some specific tests measure output traits that are even sometimes labelled as performance or behavioural traits depending on the context. Sprint speed, for example, when measured by chasing an individual along a linear racetrack in the laboratory, or upon release in its natural habitat, is often considered a measure of locomotor performance (Djawdan and Garland 1988; Irschick et al. 2008). One could also argue that running away from a threat (in this case a human threat) is a measure of escape behaviour, in a context of "fight or flight" response (Evans et al. 2019). In both cases, researchers are measuring the same thing: the speed at which the individual runs to escape. In practice, however, the interest of the researchers may define which label is used, which creates confusion and more importantly raises the question of where the line must be drawn along the behaviour-performance continuum. At which point is a sprint speed measurement considered a measure of behaviour versus performance? Is it sufficient to simply release an animal and measure its running speed or should it also be chased? Or, is it necessary to reach a point where animals show signs of distress or injuries to consider the measurement as performance? These questions raise important conceptual, practical, and ethical considerations.

Ecological performance

Considering the behaviour-performance continuum becomes even more important given the various contexts under which locomotor performance is measured. Performance is often measured in the laboratory and assumed to represent the ability of animals to conduct ecologically relevant tasks in their natural habitat (Irschick and Garland 2001). However, it appears that free-ranging animals use only a portion of their locomotor abilities, and that this proportion might vary depending on other factors such as sex and age (Irschick 2003). Perhaps in an attempt to specify the importance of the context within which performance is measured, the term "ecological performance" was coined to represent how organisms utilize maximal performance in nature while accomplishing an ecological

task (Irschick and Garland 2001; Irschick 2003; Husak 2006a). Yet, if discussing "ecological performance" should allow to better differentiate between laboratory and field measurements of performance, the line between performance and behavioural measurements might start to blur as a result. The main difference between performance and behavioural measurements seems to lie in the experimental protocol. When measuring performance, study organisms are subjected to a test with controlled settings, animals have very limited choice but performing the required task, and are "motivated" to do so in different ways [gentle electric shocks, chasing, taping of the tail, heating pads, etc.; (Bennett 1989; Castro and Kuang 2017)]. Whereas if the goal is to measure behaviour, animals can directly be observed in their natural (or artificial) habitat, or they might also be presented with a specific situation (open-field, maze, refuge, introduction of predator cues, etc.) to which their reactions are recorded. In behavioural observations or behavioural tests, animals are usually left with a wider range of potential actions they can take, and besides setting up the initial conditions, the observer has a very limited role, as opposed to performance tests where the observer actively ensures the execution of the desired task by the animal (e.g., running in a straight line at maximum speed). Ecological performance, however, is a dynamic measure (speed, force, stamina) taken on a free-ranging animal with as little human intervention as possible. As such, ecological performance traits may fall closer to the behavioural end of the continuum, but so far have not been integrated within personality studies and its (co)variance partitioning framework.

Variation in motivation among and within individuals

From a practical standpoint, measuring traits like jumping distance, flight velocity, and running endurance requires the appropriate equipment. Complications quickly arise because not all animals will perform the required task when prompted to do so. Studies linking performance and behaviour can also be particularly challenging for specific traits, such as bite force (Herrel et al. 2005). To measure bite strength, animals must bite on a dynamometer but will not do so if not intrinsically

motivated or aggressive enough (Freeman and Lemen 2008). In this case, differentiating between bite strength and aggressiveness seems complex, because they presumably both contribute to the measured output.

Losos et al. (2002) noted that motivation was a major problem "bedeviling studies of performance" and highlighted the dangers of discussing performance measurements when animals can vary in their propensity to perform. A traditional way of tackling this problem is to use "personal bests" values (i.e., selecting the trial with the highest output to eliminate sub-maximal trials). Although this greatly reduces or even eliminates within-individual variation in motivation, the possibility remains that individuals differ in their overall, average motivation when prompted to perform. Moreover, selecting maximal values out of repeated measurements introduces statistical bias (Adolph and Hardin 2007; Adolph and Pickering 2008). Because of its nature, the sample distribution of extreme values introduces bias (Head et al. 2012), and when the sampling is unequal between individuals, even greater bias emerges because more frequently sampled individuals are more likely to obtain higher values (Careau and Wilson 2017a).

Instead of selecting personal best values, one approach is to retain all of the repeated measurements made on every individual and use multi-level statistical tools to partition variance accordingly. This approach allows among-individual variation (how performance differs across individuals) to be separated from intra-individual variation (how the performance of an individual varies from a test to another), and even intra-trial variation (how performance varies from one repeated trial to another within a day) (Careau and Wilson 2017b, a; Berberi and Careau 2019; Lailvaux et al. 2019). Intra- trial variation (or residual variation in most cases) includes measurement error and some unknown amount of variation due to motivation and fatigue. Presumably, motivation also generates variation at the among- and within-individual levels, but evaluating this possibility remains a challenging task because it is almost impossible to quantify motivation separately from the performance measurements. If the importance of motivation differs across levels, however, then one could expect differences in the sign and strength of relationship with

behaviour (which, ultimately, is mostly determined by motivation).

Relationships between performance and behavioural traits

Multiple studies have found behaviour and performance to covary in different taxa (Garland 1988; Nelson and Formanowicz 2005; Seebacher et al. 2016; Piquet et al. 2018). Furthermore, because behaviour is constrained within a performance space (Bennett 1989; Bennett and Huey 1990), there are likely numerous co-adaptations between those traits. Dewitt and colleagues (1999) originally defined two hypotheses to explain the links between morphological defenses and anti-predator behaviour: the compensation and co-specialization hypotheses, but more recently these two hypotheses have been co- opted to study the links between behaviour and performance (Newar and Careau 2018; Agnani et al. 2020; Agnani and Careau 2023).

The compensation hypothesis

According to the compensation hypothesis, individuals that expose themselves to more risky situations should compensate with greater abilities to evade risky situations (or, put another way, individuals with greater locomotor performance can "afford" taking more risks). Assuming that 1) high levels of activity, exploration, aggressiveness, and boldness lead to a higher predation risk and 2) performance traits such as locomotor speed, agility, and strength increase the probability of escaping predators, then the compensation hypothesis predicts positive correlations between performance and behavioural traits. To my knowledge, the mechanisms at the origin of trait compensation have not yet been clearly identified, but various mechanisms could be involved. Trait compensation could result from correlational selection [i.e., non-linear selection on trait combinations: (Sinervo and Svensson 2002)]. As a simple way to illustrate correlational selection on behaviour and performance, I consider boldness and sprint speed in the context of predator-prey interactions (Fig. 1). By definition, bold individuals take more risks and should therefore be more

exposed to predators, in which case it would be advantageous to be equipped with a locomotor machinery (i.e., biochemistry, morphology) to escape predator encounters. By contrast, being able to run fast might be less important for shy individuals who take fewer risks and are presumably less exposed to predators. In this case, shy individuals might benefit from saving on the cost of building and maintaining the machinery required for higher sprint speed. Therefore, it is easy to imagine how these two combinations of boldness and sprint speed (bold and fast, or shy and slow) could yield high fitness, whereas the other two combinations (bold and slow, or shy and fast) could yield low fitness (Fig. 1). Such correlational selection, in which selection on one trait depends on the expression of another, should result in a positive (genetic) correlation between performance and behaviours.

The co-specialization hypothesis

According to the co-specialization hypothesis, both performance and behavioural traits are beneficial for reducing predation risks. Making the same two assumptions as above, the co-specialization hypothesis predicts a negative relationship between performance and behaviour. In other words, risk-averse individuals, who are shy and are less exposed to predators, are also expected to be equipped with better locomotor machinery to escape predator encounters. It is relatively easy to imagine how selection could favour "shy and fast" phenotypes, because such individuals would be well equipped both to avoid and run away from predators. However, the predicted negative correlation entails the persistence of "bold and slow" phenotypes. This particular trait combination is harder to explain as it seems disadvantageous in most situations. In their original study, Dewitt et al. (1999) suggested two proximate mechanisms underlying co-specialization between morphological defenses and anti-predator behaviours. First, developmental plasticity could generate individual (co)variation in both behaviour and locomotor performance. For example, predator encounters may result in stronger anti-predator behaviour and higher locomotor

performance in experienced versus naïve individuals; such correlated plastic responses would therefore generate a negative correlation between boldness and sprint speed. Second, an interesting possibility would be that individuals differ in their sensory ability to detect predators; bold and slow individuals might simply be bad at detecting predators (DeWitt 1998). Differentiating between those mechanisms seems challenging at this point, yet in some contexts it may be possible to design experiments to verify if one or both mechanisms are at play. For example, one could manipulate prior experience with predators and see if it generates a negative correlation between performance and behaviour (alternatively, individual differences in prior exposure to predators could be controlled for statistically to see if it explains part of the correlation between performance and behaviour). Another way would be to manipulate conditions that make predators more (or less) easy to detect; if individual differences in sensory ability to detect predators generates apparent co-specialization, then making predators easily (or impossibly) detectable should reduce the negative relationship between behaviour and performance.

The need for a literature review on behaviour and performance

The question remains: are performance and behavioural traits correlated, and how? In trying to answer this important and topical question, a significant hurdle presents itself (in addition to the conceptual and technical challenges identified above): inconsistent terminology. Although there is no lack of studies on locomotor performance itself, any attempt at synthetizing the field will face the problem that scientists from a wide array of disparate fields use the term “performance” to designate various traits deemed desirable in some way or the other. This tendency became clearer to me during my literature search, where a very large number of studies contained the term “performance”, but only a very small fraction of these was referring to the actions of an organism that is presumably maximally motivated to accomplish a task. Scientists usually strive towards clear and meaningful definitions because failure to do so might hinder the progression of research (see de

Queiroz and Donoghue 1988; Levitis et al. 2009 for examples), yet the range of meaning of "performance" is staggering. Apart from its main definition as "the ability of an individual to perform a task when maximally motivated" (Garland and Losos 1994; Careau and Garland 2012), performance is sometimes used to refer to the performance of reproduction (e.g., Das et al. 2016), including mating success (e.g., Byers et al. 2010), or reproductive success (e.g., Lane and Briffa 2022). However, using performance in this context adds the implied reasoning that individuals are always maximally motivated to mate and produce offspring as much as possible, which may not always be true (i.e., in many cases a greater lifetime reproductive success may involve producing fewer offspring than maximally possible on a given reproduction event). Performance is also used to refer to "how well" an individual behaves in its natural habitat in comparison to other individuals, for example courtship displays (e.g., Manica et al. 2017) or fighting (e.g., Edmonds and Briffa 2016). Performance also sometimes refers to regulatory performance, more specifically, the ability of animals to regulate physiological processes such as: the rate of growth (e.g., Coleman and Moore 2003; Sandberg et al. 2007), thermoregulation (e.g., Seebacher 2005), or even digestive capacity and production of gametes (e.g., see Husak et al. 2009). Because of the range of definitions, searching for studies on the relationship between locomotor performance and behaviour can be challenging and time consuming!

To my knowledge, there is currently no global and systematic overview of how locomotor performance and behaviour covary in animals, and I believe that such information would be useful to guide future research on locomotor performance and its relationship with other ecologically relevant traits. Here, I provide a review of the published research articles reporting any link (covariation, correlation, and linear regression) between locomotor performance and behavioural traits at the phenotypic and genetic levels. In doing so, I have realized that there is a growing list of studies reporting links between performance and behaviour across many taxa, that the vast majority of those studies report phenotypic correlations, and that only a handful of studies have explored potential correlated responses to selection on performance and behaviour. In light of these, I outline

a few recommendations to help guide future studies.

Literature survey/data collection

I conducted a literature survey of existing primary studies to assess the links between locomotor performance and behavioural traits in both field and laboratory settings. I only included studies which tested for links between performance and behaviour. The original literature search was conducted on July 8th 2022 on the Web of Science website in the "topic" field, including all available years. I used a list of keywords as follow: "performance, sprint speed, sprint, escape, endurance, swimming, running, jumping, jump, run" and "behaviour, exploration, shyness, boldness, activity, behavior, personality, temperament". My search was done using the "AND" argument to only return studies which included keywords in both categories (performance and behaviour). Once non-relevant areas of research were excluded (see supplementary material for the exhaustive list of categories excluded), the original search returned a total of 22,531 studies.

I proceeded in two steps; first, I screened abstracts to pre-select studies and then read all pre- selected studies to extract information about links between locomotor performance and behaviour. In the first step, abstracts were ordered by "relevance" according to web of science, and as I progressed and relevance decreased, fewer and fewer studies were pre-selected. After no studies could be pre-selected between study N°8,000 to 9,000, I only screened 100 abstract every 500, which did not result in any more studies being retained. A total of 337 studies were pre-selected in this first step and saved as a "first scan" list. In a second step, I read every pre-selected study to extract the information which resulted in a total of 43 research articles containing evidence of links between locomotor performance and behaviour.

Studies were grouped into three categories depending on the nature of the experimental protocol. First, the most common type of studies quantifies one or more correlations between locomotor performance and behavioural traits measured on a common set of individuals. From

these studies, significant effect sizes were extracted and reported in table 1 (when possible, simple linear regressions were transformed to correlations). Second, some studies quantified how performance and behaviour changed in response to a modification in the environment (e.g., temperature, substrate, see table 2). These studies are informative for the link between performance and behaviour because they provide evidence for correlated plasticity (i.e., when two or more traits change in response to a common environmental variable). Third, I compiled artificial selection experiments in which selection was applied on performance and resulted in a correlated change in behaviour, or vice versa (table 3).

For each study, I considered the traits measured and the sign of the relationship to interpret them as supportive of the compensation or of the co-specialization hypotheses. I also report the level at which the link was observed (i.e., interspecific, phenotypic, among- or within-individual levels) and, when available, indicated the strength of association reported. I chose to retain the vocabulary used by the authors to describe their measurement of performance and of behaviour, even if some measurements with different appellations are equivalent (for example “escape speed” and “sprint speed” are measured the same way).

Correlations between performance and behaviour

Of the 43 retained studies, 29 of them reported a total of 47 significant associations between performance and behaviour in various species (table 1). Most studies were conducted on reptiles, but table 1 also includes studies on a wider range of taxa such as birds, fishes, mammals, insects, and spiders. The performance traits mostly consist of classical measures of locomotor performance (e.g., speed, acceleration, endurance, and bite force) in relation to various behaviours such as activity, exploration, aggressiveness, and dominance. Of the 47 associations that were reported in the

published studies, I was able to extract the effect size for 32 of them and reported 29 correlation values, one non-linear association, and two genetic covariances. The remaining 15 entries in the table represent associations between performance and behaviour that were clearly reported by the researchers in their manuscripts, but for which effect sizes were not included. The majority (31 out of 47) of associations were observed at the phenotypic level, whereas only 7 were observed at the among-individual level, 2 at the residual level, and only three reported at the genetic level (the 4 remaining associations being observed at the inter-specific level). Twenty nine of the 47 associations (62%) between performance and behaviour were in support of the compensation hypothesis while only 18 supported the co-specialization hypothesis.

An apparent pattern emerging from table 1 is a difference in the hypothesis supported in reptiles vs rodents. Indeed, within the reptiles, 22 out of 28 associations were in support of the compensation hypothesis, whereas in rodents more than half (9 out of 16) of the associations found in support of the co-specialization hypothesis. Interpretation of this pattern should be done with extreme care because there is a potential infinite number of confounding factors when comparing two taxa in such a way (Garland and Albuquerque 2017).

Correlated plasticity in performance and behaviour

I found 9 studies that reported evidence of correlated plasticity in performance and behaviour for a total of 14 associations in various taxa such as crustaceans, insects, fish, amphibians, and reptiles (table 2). Phenotypic plasticity in performance and behaviour occurred in responses to different environments and treatments such as parasites, predator cues, temperature, diet, and substrate. The performance traits measured included different measurements of endurance, speed or jumping distance, whereas behaviours captured aspects of activity, aggressiveness, and risk-taking. Out of the 14 associations, the majority where in support of the compensation hypothesis (10 out of 14) and only four supported the co-specialization hypothesis.

Correlated responses to selection on performance or behaviour

I only found 4 artificial selection studies in which selection on behaviour produced significant correlated responses in performance, for a total of 7 associations in 3 species (table 3). Out of the 7 associations, 5 were in support of the compensation hypothesis, and only two were in support of the co-specialization.

Tables and Figures

Table 1. Significant associations between performance and behaviour reported to date (with one marginally significant genetic correlation indicated in bold). Only significant pairwise relationships are presented (studies in which relationships were studied but found not significant were not included). When more than two traits were significantly correlated, each relationship appears as a different row. Descriptions of performance measurements and behaviour were shortened but kept as close as originally described by the original authors. FID: Flight initiation distance, OF: Open field. Effect sizes, when available, were reported (covariances, correlations or non-linear regressions), simple linear regressions (R squared values) were transformed to correlations values for clarity purposes. For each study, I indicated if the relationship between performance and behaviour supports the compensation or cospecialization hypotheses (sensu Dewitt et al. 1999) and indicated at which level the relationship was observed (inter-specific, genetic, phenotypic, individual, residual).

Taxa	Species	Performance trait	Behaviour	Effect size	Hypothesis	Level	Reference
Bird	*Ficedula hypoleuca*	Weight-norm. flight force (+)	Tail spread (+)	NA	Cospecialization	Phenotypic	Tomotani and Muijres (2019)
Fish	*Danio rerio*	Critical swimming speed (+)	Voluntary speed in OF (+)	0.66	Compensation	Phenotypic	Seebacher et al. (2016)
Fish	*Danio rerio*	Critical swimming speed (+)	Voluntary speed in OF (+)	0.71	Compensation	Phenotypic	Seebacher et al. (2015)
Insect	*Lycaena tityrus*	Endurance (+)	Exploration (+)	0.19	Compensation	Phenotypic	Reim et al. (2019)
Insect	*Lycaena tityrus*	Endurance (+)	Exploration (+)	0.21	Compensation	Phenotypic	Reim et al. (2019)
Insect	*Teleogryllus commodus*	Jump distance (+)	Calling effort (-)	-0.014	Cospecialization	Genetic (cov)	Lailvaux et al. (2010)
Insect	*Teleogryllus commodus*	Jump power (+)	Calling effort (-)	-0.038	Cospecialization	Genetic (cov)	Lailvaux et al. (2010)
Reptile	*Chamaeleo calyptratus*	Bite force (+)	Aggressive response (+)	NA	Compensation	Phenotypic	Drown et al. (2022)
Reptile	*Chamaeleo calyptratus*	Sprint speed (+)	Fleeing response (+)	NA	Cospecialization	Phenotypic	Drown et al. (2022)
Reptile	*Leiocephalus carinatus*	Endurance (+)	Display of aggression (+)	0.55	Compensation	Individual	Diamond et al. (2014)

Reptile	*Phrynocephalus vlangalii*	Sprint speed (-)	Prob. of taking refuge (+)	NA	Compensation	Phenotypic	Qi et al. (2014)
Reptile	*Sceloporus undulatus*	Sprint speed (+)	Startle response (+)	NA	Cospecialization	Phenotypic	des Roches et al. (2013)
Reptile	*Aspidoscelis inornata*	Sprint speed (+)	Startle response (+)	NA	Cospecialization	Phenotypic	des Roches et al. (2013)
Reptile	*Sceloporus woodi*	Escape speed (+)	FID (+)	0.33	Cospecialization	Phenotypic	Stiller and McBrayer (2013)
Reptile	*Storeria dekayi*	Swimming velocity (+)	Death feigning duration (-)	R^2=0.724	Compensation	Phen. non linear reg.	Gerald (2008)
Reptile	*Crotaphytus collaris*	Escape speed (-)	FID (+)	-0.63	Compensation	Phenotypic	Husak (2006a)
Reptile	*Anolis cristatellus*	Endurance (+)	Displays of assertion (+)	0.31	Compensation	Phenotypic	Perry et al. (2004)
Reptile	*Podarcis muralis*	Escape speed (+)	Distance from refuge (+)	0.52	Compensation	Phenotypic	Braña (2003)
Reptile	*Lacerta vivipara*	Endurance (+)	Capture probability (+)	NA	Compensation	Phenotypic	Clobert et al. (2000)
Reptile	*Urosaurus ornatus*	Sprint speed (+)	Dominance (+)	NA	Compensation	Phenotypic	Robson and Miles (2000)
Reptile	*Urosaurus ornatus*	Endurance (+)	Dominance (+)	NA	Compensation	Phenotypic	Robson and Miles (2000)
Reptile	*Multiple*	Endurance (+)	Time spent moving (+)	0.80	Compensation	Inter-specific	Garland (1999)
Reptile	*Multiple*	Endurance (+)	Daily movement distance (+)	0.79	Compensation	Inter-specific	Garland (1999)
Reptile	*Multiple*	Endurance (+)	Time spent moving (+)	0.78	Compensation	Residual Inter-specific	Garland (1999)
Reptile	*Multiple*	Endurance (+)	Daily movement distance (+)	0.86	Compensation	Residual Inter-specific	Garland (1999)
Reptile	*Thamnophis sirtalis*	Crawling speed (+)	Antipredator display (+)	0.19	Compensation	Phenotypic	Garland (1988)
Reptile	*Thamnophis sirtalis*	Endurance (+)	Antipredator display (+)	0.20	Compensation	Phenotypic	Garland (1988)
Reptile	*Thamnophis radix*	Crawling speed (+)	Aggressive displays (+)	0.39	Compensation	Phenotypic	(Arnold and Bennett 1988)
Reptile	*Uta stansburiana*	Endurance (+)	Dur. aggressive displays (+)	0.49	Compensation	Phenotypic	Brandt (2003)
Reptile	*Sceloporus occidentalis*	Sprint speed (+)	Dominance (+)	NA	Compensation	Phenotypic	Garland et al. (1990)
Reptile	*Lacerta monticola*	Sprint speed (-)	Dominance (+)	NA	Cospecialization	Phenotypic	López and Martín (2002)

Reptile	*Gallotia galloti*	Bite force (+)	Dominance (+)	NA	Compensation	Phenotypic	Huyghe et al. (2005)
Reptile	*Anolis carolinensis*	Bite force (+)	Dominance (+)	NA	Compensation	Phenotypic	Lailvaux et al. (2004)
Reptile	*Anolis carolinensis*	Max. acceleration (+)	Dominance (+)	NA	Compensation	Phenotypic	Lailvaux et al. (2004)
Reptile	*Anolis carolinensis*	Max. jumping velocity (+)	Dominance (+)	NA	Compensation	Phenotypic	Lailvaux et al. (2004)
Rodent	*Myotomys unisulcatus*	Sprint speed (+)	Exploration (-)	-0.39	Cospecialization	Individual	Agnani et al. (2020)
Rodent	*Atlantoxerus getulus*	Escape speed (+)	Boldness (-)	-0.44	Cospecialization	Individual	Piquet et al. (2018)
Rodent	*Tamias striatus*	Sprint speed (+)	Distance moved in OF (-)	-0.59	Cospecialization	Individual	Newar and Careau (2018)
Rodent	*Tamias striatus*	Sprint speed (+)	Distance moved in OF (-)	-0.53	Cospecialization	Residual	Newar and Careau (2018)
Rodent	*Marmota flaviventris*	Escape speed (-)	Vigilance (-)	-0.09	Cospecialization	Phenotypic	Blumstein et al. (2010)
Rodent	***Marmota flaviventris***	**Escape speed (-)**	**Vigilance (-)**	**-0.57**	**Cospecialization**	**Genetic**	Blumstein et al. (2010)
Rodent	*Peromyscus leucopus*	Sprint speed (+)	Voluntary wheel running (-)	-0.52	Cospecialization	Individual	Agnani and Careau (2023)
Rodent	*Peromyscus leucopus*	Swimming performance (+)	Voluntary wheel running (-)	-0.37	Cospecialization	Individual	Agnani and Careau (2023)
Rodent	*Peromyscus leucopus*	Sprint speed (+)	Home cage activity (-)	-0.38	Cospecialization	Individual	Agnani and Careau (2023)
Rodent	*Peromyscus leucopus*	Sprint speed (+)	Voluntary wheel running (+)	0.33	Compensation	Residual	Agnani and Careau (2023)
Rodent	*Mus musculus*	Sprint speed (+)	Speed in open-field (+)	0.46	Compensation	Phenotypic	Friedman et al. (1992)
Spider	*Hogna carolinensis*	Sprint speed (+)	FID (+)	0.24	Cospecialization	Phenotypic	Nelson and Formanowicz (2005)

Table 2. Cases in which both performance and behaviour significantly changed in response to given experimental treatments. Only studies in which the authors found relationships are presented (see main text). Focal treatments are indicated in bold next to the control (or second treatment). When more than two traits were found correlated, each relationship appears as a different entry, therefore studies can appear multiple times. Descriptions of performance measurements and behaviour were shortened but kept as close as originally described by the original authors.

Taxa	Species	Focal treatment vs control treatment	Performance trait	Behaviour	Hypothesis	Reference
Amphibian	*Bombina variegata*	**Permanent breeding site** vs Ephemeral	Endurance (-)	Number of spontaneous jumps (+)	Compensation	Sinsch et al. (2020)
Amphibian	*Bombina variegata*	**Permanent breeding site** vs Ephemeral	Endurance (-)	Dist. covered in spontaneous jumps (+)	Compensation	Sinsch et al. (2020)
Amphibian	*Pleurodeles waltl*	**Snake kairomones** vs no kairomones	Escape speed (+)	Flight initiation distance (+)	Cospecialization	Javier Zamora-camacho et al. (2018)
Crustacea	*Nephrops norvegicus*	**Acoustic transmitter** vs no transmitter	C-start velocity (-)	Distance travelled (-)	Compensation	Newland and Chapman (1993)
Fish	*Pomacentrus amboinensis*	**Gnathids** vs no gnathids	C-start response speed (-)	Routine swimming distance (-)	Compensation	Allan et al. (2020)
Fish	*Poecilia reticulata*	**High water velocity** vs low water velocity	Critical swimming speed (+)	Mean duration of sigmoid displays (+)	Cospecialization	Nicoletto (1996)
Fish	*Poecilia reticulata*	**High water velocity** vs low water velocity	Critical swimming speed (+)	Total time spend displaying (+)	Cospecialization	Nicoletto (1996)
Insect	*Acheta domesticus*	**Performance enriching diet** vs normal	Jumping distance (+)	Total distance travelled (+)	Compensation	Tran et al. (2018)
Insect	*Tetrix subulata*	**27°C** vs 17°C	Jumping performance (+)	Jump initiation distance (+)	Cospecialization	Forsman and Appelqvist (1998)
Reptile	*Crotaphytus collaris*	**Gravid** vs non gravid	Escape speed (-)	Distance to refuge (-)	Compensation	Husak (2006b)
Reptile	*Anolis grahami*	**Reduced perch diameter** vs normal perch	Sprint speed (-)	Frequency of jumping (+)	Compensation	Losos and Irschick (1996)
Reptile	*Anolis gundlachi*	**Reduced perch diameter** vs normal perch	Sprint speed (-)	Frequency of jumping (+)	Compensation	Losos and Irschick (1996)

Reptile	*Anolis lineatopus*	**Reduced perch diameter** vs normal perch	Sprint speed (-)	Frequency of jumping (+)	Compensation	Losos and Irschick (1996)
Reptile	*Anolis sagrei*	**Reduced perch diameter** vs normal perch	Sprint speed (-)	Frequency of jumping (+)	Compensation	Losos and Irschick (1996)

Table 3. Cases in which selection on behaviour or performance resulted in correlated response to selection on performance or behaviour. Only studies in which the authors found relationships are presented. Traits selected for are indicated in bold. When more than two traits were found correlated, each relationship appears as a different entry, therefore studies can appear multiple times. Descriptions of performance measurements and behaviour were shortened but kept as close as originally described by the authors.

Taxa	Species	Trait selected for	Correlated change in	Hypothesis	Reference
Fish	*Danio rerio*	**Boldness (+)**	Fast start performance (+)	Compensation	Kern et al. (2016)
Fish	*Danio rerio*	**Boldness (-)**	Fast start performance (-)	Compensation	Kern et al. (2016)
Rodent	*Mus domesticus*	**Voluntary wheel running (+)**	Wheel running endurance (+)	Compensation	Meek et al. (2009)
Rodent	*Ratus ratus*	**Endurance (+)**	Voluntary wheel running (+)	Compensation	Waters et al. (2008)
Rodent	*Ratus ratus*	**Endurance (-)**	Voluntary wheel running (-)	Compensation	Waters et al. (2008)
Rodent	*Ratus ratus*	**Endurance (+)**	Novel environment activity (-)	Cospecialization	Waters et al. (2010)
Rodent	*Ratus ratus*	**Endurance (-)**	Novel environment activity (+)	Cospecialization	Waters et al. (2010)

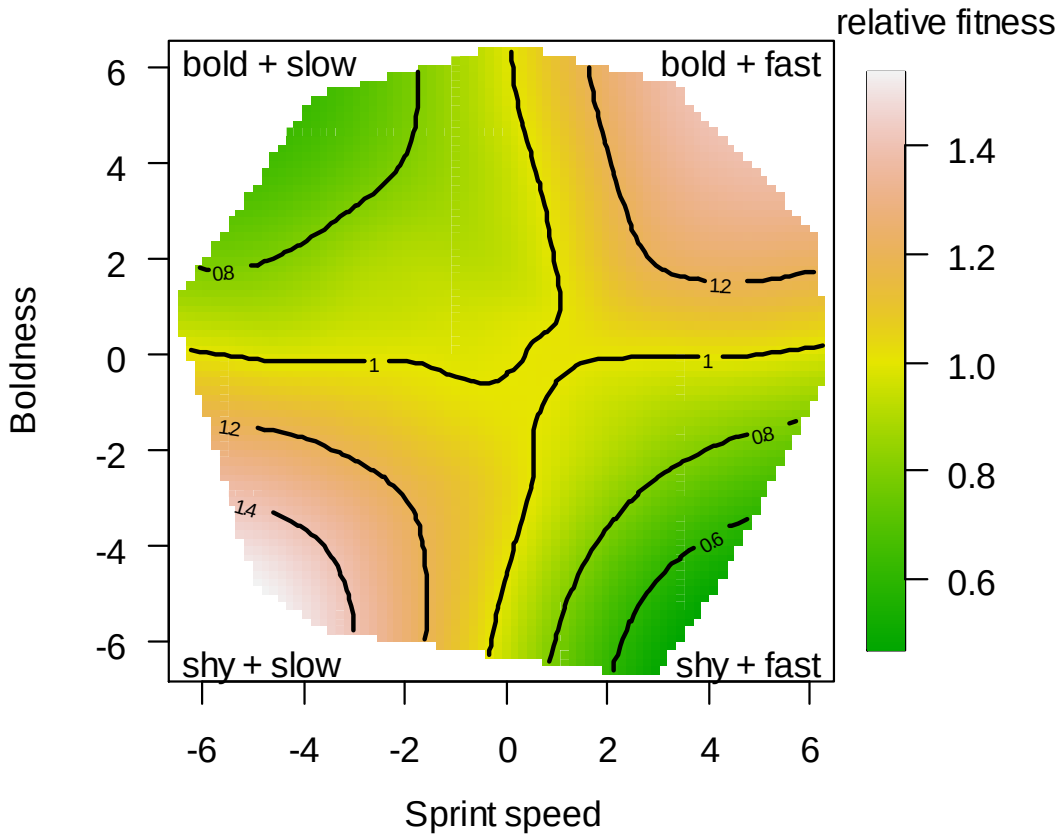

Figure 1. Hypothetical scenario in which correlational selection acts on boldness and sprint speed, yielding two trait combinations (bold+fast and shy+slow) with high relative fitness and two trait combinations (bold+slow and shy+fast) with low relative fitness.

Box 1. Application of the (co)variance partitioning approach.

Friedman et al. (1992) repeatedly measured several locomotor performance and behavioural traits in 35 male house mice (*Mus musculus*), including forced sprint speed, swimming endurance, five behaviours in the open field (defecations, movement latency, distance, maximum speed, sd of speed), and voluntary wheel running (over 7 days). Original data files and SPSS code where kindly provided by the authors for replication and re-analysis. Overall, the strength of the relationships between performance and behavioural measures was weak (mean $|r|$ = 0.151, Fig. I), and only one of the 14 performance-behaviour relationships was significantly different from zero (i.e., r = 0.467 between forced sprint speed maximum speed voluntarily expressed in the open-field test).

Inasmuch as the Friedman et al. (1992) study was innovative, standard statistical approaches at the time did not easily permit a full consideration of the repeated measures dataset, at least without violating some model assumptions. With their primary goal of testing "for correlations between putatively behavioral and physiological measures of locomotor capacities", Friedman et al. (1992) retained the higher of two replicate values for each performance trait. Moreover, the open-field test with the shortest movement latency was retained, which also had the advantage of eliminating non-independence in the reduced dataset (which also included voluntary wheel running on days 1 and 7 considered as separate traits). The resulting phenotypic correlations, however, were most likely attenuated by a certain degree of measurement error and within-individual variation, which represented about half of the phenotypic variation (i.e., the average repeatability was R = 0.56, range 0.29-0.73).

Here, I re-analyse data from Friedman et al. (1992) to potentially gain insight on the issue of correlation attenuation and illustrate the (co)variance partitioning approach. After replicating repeatability values using univariate mixed models, I ran a multivariate mixed model that included all of the repeated measurements made on each individual. Test sequence was fitted as a fixed effect to account for mean differences between the first and second tests. Individual identity (ID) was also included as a random effect fitted as an unstructured correlation matrix to estimate among-individual

variance and all possible among-individual correlations (r_{ind}). On average, the r_{ind} between performance and behavioural traits was noticeably stronger ($|r_{ind}| = 0.282$; see black diamonds, Fig. I). Four among-individual correlations were significantly different from zero and two more were approaching significance (Fig. I).

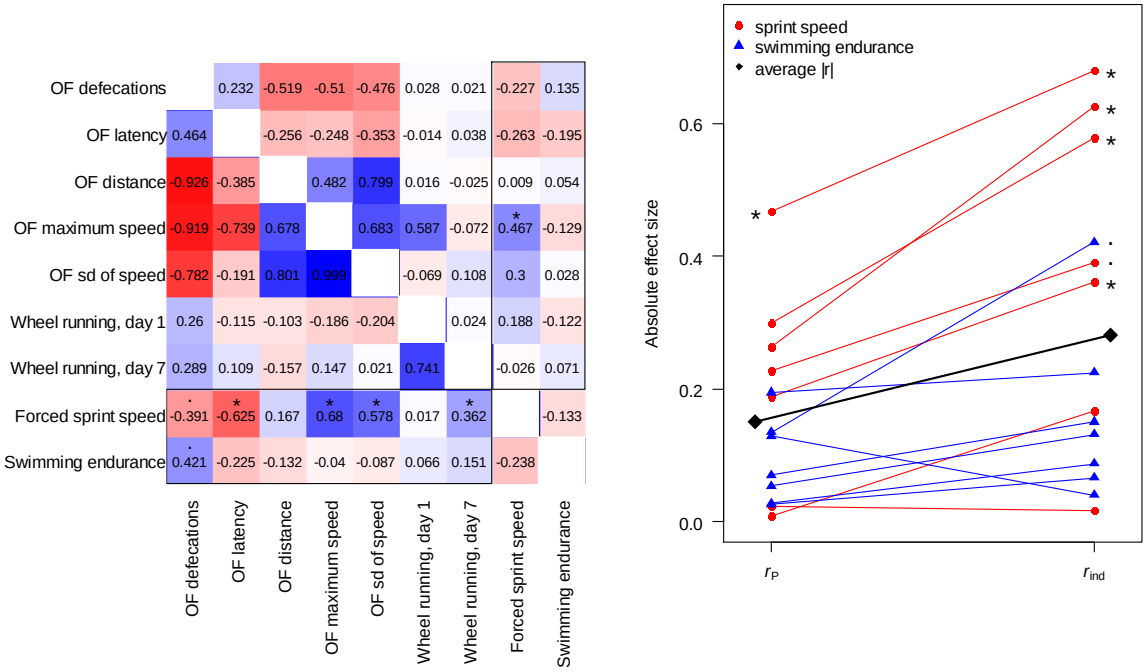

Figure I. Phenotypic correlations (r_P; upper right triangle) between two locomotor performance traits (spring speed and swimming endurance), five open-field test variables (OF), and two voluntary wheel running measures of as reported in Friedman *et al*. (1992) and among-individual correlations (r_{ind}; lower left triangle) as estimated using a multivariate mixed model that included all repeated measures. Asterisks (*) and dots (.) indicate $P < 0.05$ and $P < 0.1$ estimated using profile likelihoods (Wolak 2012).

Discussion

Overall, I show that most of the studies that report correlations, correlated plasticity, and correlated responses to selection between performance and behaviour are in support of the compensation hypothesis (29 out of 45 in table 1, 10 out of 14 in table 2, and 5 out of 7 in table 3). I also find that most of the correlations were found at the phenotypic level, and that most of the correlations and correlated plasticity were observed in lizards, potentially because of the high number of studies on performance in this group in the past four decades. In light of my literature review, I outline several recommendations to guide future research on locomotor performance and behaviour.

The need for more quantitative genetic studies

Associations between performance and behaviour can occur at multiple levels of (co)variation, including the genetic, phenotypic, among-individual, and the residual levels. A genetic correlation indicates the degree to which two traits share a genetic basis (Roff 1995) and is a key parameter of quantitative genetics with important potential causes (i.e., pleiotropy and linkage disequilibrium) and consequences (e.g., evolutionary constraint). Quantifying genetic correlations, however, requires large sample sizes and measurements on individuals from a known pedigree. I only found evidence of one marginally significant genetic correlation between performance and behaviour: escape speed was negatively genetically correlated with vigilance in wild marmots (Blumstein et al. 2010), and one negative genetic covariance, between jumping distance and calling effort in crickets (Lailvaux et al. 2010), both suggesting co-specialization between these traits at the genetic level. This is in clear contrast to the artificial selection experiments that lend support for compensation at the genetic level (table 3). Indeed, correlated changes in performance in response to selection on behaviour indicates a genetic correlation. Clearly, we need more quantitative genetic studies on locomotor performance and behaviour.

The need to move beyond phenotypic correlations

The vast majority of performance-behaviour correlations were estimated at the phenotypic level (31 out of 47, see table 1). These estimates, however, can be misleading because phenotypic correlations ignore measurement error and effect sizes are usually attenuated, with the size of the effect being closer to 0 than it really is (Trafimow 2016). Furthermore, correlations based on single measurements are a mix of among- individual and residual correlations and can sometimes be close to zero not because the studied traits are independent, but because the underlying correlations are of comparable strength but opposite directions (Careau and Wilson 2017b). Instead, when multiple measurements are taken, it is possible to partition (co)variances and estimate among-individual and residual correlations (Dingemanse and Dochtermann 2013). Among-individual correlations represent the association among individuals (i.e., within a sampled population are bolder individuals also faster sprinters) and residual correlations represent the association within individuals (i.e., if an individual is bolder according to his own average on a test day, is he also faster). Such studies, in which correlations are partitioned, are much needed to better understand the links between performance and behaviour, especially when correlations can be of opposite directions at those two levels (e.g., see Agnani and Careau 2023).

Only a fraction (5 out of 30) of the studies included in table 1 have partitioned (co)variances to estimate the among-individual correlation separately from the within-individual (residual) correlation Upon examination of the structure of the data collected, I believe that 10 studies would permit a (co)variance partitioning approach (Arnold and Bennett 1988; Garland 1988; Friedman et al. 1992; Brandt 2003; Lailvaux et al. 2004; Gerald 2008; Blumstein et al. 2010; Tomotani and Muijres 2019; Reim et al. 2019; Drown et al. 2022). Hence, re-analysing published datasets with multivariate mixed models would be an easy but potentially rewarding way to gain a more complete picture of the association between performance and behaviour.

Here, I include a re-analysis of one of the previously published studies that contained repeated measurements (Friedman et al. 1992; see box. 1). Reassuringly, the (co)variance

partitioning approach yielded relatively similar results and none of the conclusions made by Friedman et al. (1992) would dramatically change. However, the overall difference in average effect sizes (|rP| vs |rind| of 0.151 vs 0.282) most likely represents the level of attenuation that was present in the originally reported estimates (box 1). Clearly, (co)variance partitioning represents an efficient tool for testing trait correlations while accounting for the hierarchical structure of the data. Moreover, here we only focused on rind, but the approach can be extended to explore patterns of within-individual changes (phenotypic plasticity) over repeated tests and exposure to running wheels (e.g., Abdeen et al. 2022). Such a (co)variance partitioning approach has proven useful not only for detecting previously obfuscated patterns of trait correlations (e.g., Careau & Wilson 2017a; Careau & Wilson 2017b), but also for planning experimental designs that require repeated sampling of multiple traits (Dingemanse and Dochtermann 2013).

The need for multilevel analyses of variance in raw performance measurements

When repeated performance measurements on given individuals are recorded, quantifying the variation among and within individuals (residual variation) is possible. When multiple trials are performed within a day, it is also possible to estimate intra-trial variation (i.e., variation among repeated trials within the same day). If one cannot ignore the effect of measurement error, which should be limited by the experimental design and controlled for statistically as much as possible, intra-individual (or intra-trial variation) also captures variation in motivation and fatigue. This is particularly relevant because quantifying variation in motivation remains extremely challenging, if even possible. Properly accounting for the hierarchical nature of the data might represent a partial statistical solution to an inherently methodological problem and allow to estimate the extent of variation in performance measurements due to motivation (note though, that if the goal is to study performance, sub-maximal trials or trials in which animals exhibit “inappropriate behaviours” such as jumping out of a racetrack, or not performing as expected in the test, can, and arguably should,

still be removed).

Ponzi et al. (2018) proposed a way to estimate the variance in measurement error or "transient effects" using datasets that contain repeated measurements of traits at short-term (also called "measurement sessions") and long-term scales. When measurements are repeated at both temporal scales (multiple measurement within each measurement sessions, and multiple measurement sessions across a longer period of time), it is then possible to estimate the variance of the measurement error and calculate heritability with more accuracy. In a similar fashion, this rationale could be applied to raw repeated performance measurements where the "transient effects" represent the variance associated with the combined effect of the measurement error and of the trial-to-trial changes in performance due to variation in motivation. Accounting for trial order would allow detection of fatigue (decreased performance across repeated trials) or habituation (increased performance across repeated trials). With repeated measurements taken across two temporal scales, it is possible to calculate the variance of the measurement error (i.e., the error in measurement and the variation in motivation among the repeated trials) and estimate short-term and long-term repeatabilities. Such discussions about different repeatability estimates are sometimes reported (for examples see Berberi and Careau 2019; Lailvaux et al. 2019) and might help tackle the methodological problem of variation in motivation during performance measurements.

The need for a Performance-Behaviour continuum

Although great care must be taken to make sure performance is measured on a maximally motivated animal (Losos et al., 2002), there always remain the possibility that variation in motivation generates part of the variability observed in the performance measurement. To the extent that this occurs, then a covariance with behaviour might be due to a methodological bias caused by individual differences in stress coping styles (Koolhaas et al. 2007) that might determine the form and intensity of the reaction to the stimulus used (human, electric shock, etc.). Although motivational circuits in

the brain can also interact with many other parameters such as fear (Stankowich and Blumstein 2005), pain (Bank et al. 2013), or hunger (Burnett et al. 2016), studies in which performance is measured independently of an animal's reaction towards a human threat (i.e., not relying on escape) are greatly needed. Even after partitioning correlations and using a multilevel approach, motivation can remain a problem. Perhaps a way forward is for future studies to include multiple measurements of both behaviour and performance that vary in how much they might be influenced by stress and motivation. This can be done for example by placing an animal in a life-threatening situation such as into a cold environment to measure thermogenic capacity (Chappell 1984) or into water to measure swimming performance (Friedman et al. 1992; Huang et al. 2016).

Locomotor performance can be measured to study multiple aspects of an animal's phenotype. Although previous techniques of selecting maximal measurements of performance come with caveats, they might still be relevant to use when the ultimate goal is to study how selection acts on lower-level traits (i.e. physiology) which constrain performance. Yet, in order to study how complex traits coevolve, the use of more modern statistical approaches such as covariance partitioning should be favored.

Chapter 2: The fast and the curious II: performance, personality, and metabolism in Karoo bush rats

Preamble

From my review of literature studies linking performance and behaviour I identified multiple avenues which I believe would allow for a better understanding of the coadaptation of performance and behaviour. Namely, the field would greatly benefit from moving away from phenotypic correlations, and therefore studies in which among-individual, residual or even genetic correlations between performance and behaviour are greatly needed. Additionally, when it comes to performance measurements, multilevel analyses of variance were also needed because the use of personal best values introduces biases, whereas retaining multiple measurements can provide a more complete understanding of how performance varies across multiple levels. The second chapter, published in Behavioural Ecology and Sociobiology, tackles these two aspects. We provide empirical field-collected data of behaviour, performance, and metabolism in a new rodent species, use covariance partitioning to report among-individual and residual correlations, and include an additional multilevel analysis of sprint speed data.

But before continuing, I want to pause for a moment and provide some context, as this work holds a special place in my memory and played a crucial role in my career development. I first reached out to Dr. Careau in December 2015 while I was looking to do an internship to fulfil the requirements for the masters in Ecology at the Faculté d'Economie, Gestion et Sciences in my hometown of Lille in France. At the time, Dr. Careau convinced me to get some fieldwork experience that I was lacking and suggested a few researchers with whom I could potentially work with. After graduating from my masters, having done my internship in Dr. Carsten Schradin's lab, I decided to

volunteer in the Succulent Karoo Research Station, in Goegap, South Africa, that Dr. Schradin founded 22 years ago. There, I learned how to trap and handle small mammals with several other students and postdocs, and contributed to the long-term monitoring project of the striped mice (Rhabdomys pumilio) population. It was there that I saw an opportunity with the Bush Karoo Rats (Myotomys unisulcatus) that were increasingly abundant at the field site. These animals seemed to be a promising model to study suites of traits in the wild, while also guaranteeing that the same individuals could be repeatedly captured. In February 2018, now a first-year MSc student in Dr. Careau's lab, I returned to the Succulent Karoo Research Station on my own and collected repeated measurements of metabolism, behaviour, and performance which lead to the publication of this article co-authored by both my former and current supervisors. It is this work that sparked my interest in studying how locomotor performance and behaviour covary, and led me to continue onto a PhD with Dr. Careau. While this is the second chapter of my thesis, in reality this project is the first that I completed during my PhD in chronological order.

Introduction

The field of animal personality—defined as repeatable individual differences in activity, exploration, boldness, aggressiveness, and sociability—has experienced a major surge in interest over the last 20 years (Stamps 1991; Dingemanse and Réale 2005; Dingemanse et al. 2010; Stamps and Groothuis 2010; Carter et al. 2013). Salient features of animal personality studies are (1) the focus on individual variation and (2) the use of standardized tests to measure individual behavioural reactions to various situations (e.g. novel, familiar, and risky situations). Although behavioural tests are sometimes conducted in an unnatural context (e.g. the open-field test), individual differences captured in these tests are thought to relate to behaviours exhibited in various natural situations such as habitat use, predation avoidance, dispersal, or social behaviours (Dingemanse et al. 2003; Dall et al. 2004). Multiple studies have in fact illustrated how laboratory measurements can predict behaviours of

animals in their natural habitats (Fraser et al. 2001; Boon et al. 2008; Boyer et al. 2010; Van Overveld and Matthysen 2010; Yuen et al. 2016). As such, personality traits have been found to affect fitness components (Dingemanse and Réale 2005; Moiron et al. 2020 but see Smith and Blumstein 2008).

Personality traits are frequently correlated with each other, in which case they can be referred to as behavioural syndromes (Sih et al. 2004). Behavioural syndromes imply limited plasticity in behaviour (Sih et al. 2004). Although individuals may display different levels of aggressiveness depending on contexts, more aggressive individuals (as indicated by their agonistic behaviour during contests) typically remain more aggressive in all contexts (i.e. rank order maintained) and are also generally bolder than less aggressive individuals (Riechert and Hedrick 1993). Behavioural syndromes are especially relevant to the field of animal behaviour as they may help us understand the maintenance of inter-individual variation in behaviour. If personality traits are correlated across different contexts, personality traits may not evolve independently from each other, but instead as a suite of correlated traits (Price and Langen 1992). Furthermore, personality traits may not only correlate with each other but also with other traits of biological importance such as circulating hormones levels (Chang et al. 2012), aspects of the immune system (Butler et al. 2012; Monceau et al. 2017), locomotor performance (Careau and Garland 2012), and metabolic rate (Careau et al. 2008).

All biological processes in an organism require energy, for which individuals must match metabolic fuel supply (lipids, carbohydrates, and protein) to ever-changing energy demands (Weber 2011). The rate at which an individual oxidises substrates to produce energy has been defined as the metabolic rate. Due to the highly variable nature of metabolic rate in response to various factors (e.g. temperature, biosynthesis, activity), a certain degree of standardisation is required to compare metabolic rate among individuals. Basal metabolic rate (BMR)—the lowest rate at which substrates are oxidised by an endotherm to stay alive—is often measured as the O2 consumption of an animal that is alert but resting, fasting (i.e. post-absorptive), not reproducing or growing, within its thermal neutral zone, and is measured during the inactive part of the animal's daily cycle (Speakman 2013).

Despite the highly standardised nature of BMR measurements, there usually remains a large degreeof inter-individual variation within populations (Speakman et al. 2004). In many circumstances, especially in field studies, it is not possible to meet all of the criteria to measure BMR, in which case measurements are referred to as resting metabolic rate (RMR). BMR and RMR are nevertheless considered analogous traits (hereafter referred to as RMR).

The links between RMR and animal personality have been intensely explored over the last decade (Careau et al. 2011; Killen et al. 2011; Le Galliard et al. 2013; Bouwhuis et al. 2014; Gifford et al. 2014; Krams et al. 2017; Chen et al. 2019; Cornwell et al. 2020). According to the allocation model, which assumes that there is a finite amount of energy that an animal can spend on competing processes, behaviours such as activity should be negatively correlated with RMR, as a higher RMR will result in less energy available to spend on activity, and vice versa (Speakman 1997; Careau et al. 2008). Alternatively, according to the performance model, RMR and activity should be positively correlated because more active individuals sustaining higher levels of energy expenditure should require a larger "machinery" (larger organs) which, at rest, leads to a higher RMR (Daan et al. 1990; Careau et al. 2008). Finally, the independent model recognises that physical activity has a direct impact on daily energy expenditure, but assumes that RMR and physical activity are independent (Careau and Garland 2012).

The behavioural repertoire of an animal is, by definition, confined within a space set by its performance abilities (Bennett 1989). Performance—the ability of an individual to perform a task when maximally motivated—includes repeatable traits such as speed, strength, and endurance (Garland and Losos 1994). Intuitively, an individual that can run fast or has a strong bite could behave more boldly and aggressively than an individual that runs slow or has a weak bite (Herrel et al. 2009). This intuitive idea corresponds to the "trait compensation" hypothesis developed in the context of co-adaptations between anti-predator behaviour and morphological defence (Dewitt et al. 1999). With respect to locomotor performance, the trait compensation hypothesis received some empirical support. In zebrafish (*Danio rerio*), artificial selection for boldness resulted in the bold line

having higher locomotor performance than the shy line (Kern et al. 2016). In the delicate skink (*Lampropholis delicata*), bolder individuals with a "hot thermal type" had faster sprint speeds than shyer individuals with a "cold thermal type" (Michelangeli et al. 2018). In the Asian agamid lizard (*Phrynocephalus vlangalii*), time spent moving during a novel environment test was positively correlated with endurance (Chen et al. 2019).

According to the trait compensation hypothesis, a fast sprinter can take more risks (e.g. forage farther from refuge) because it could potentially escape a predator more easily than a slow sprinter. An alternative possibility is that both behaviour and performance enhance overall protection from predation, which corresponds to the "trait co-specialization" hypothesis that predicts a negative relationship between boldness and locomotor performance (Dewitt et al. 1999). We are aware of three studies that support the trait co-specialization hypothesis, all on rodents. In Barbary ground squirrels (*Atlantoxerus getulus*), escape speed was negatively correlated with time of entrance into the open field (i.e. faster sprinters were shyer; Piquet et al. 2018). Similarly, sprint speed was negatively correlated with distance moved during a novel environment test in Eastern chipmunks (*Tamias striatus*) (Newar and Careau 2018). In Yellow-bellied marmots (*Marmota flaviventris*), sprint speed was positively correlated with vigilance while foraging (i.e. faster sprinters were more vigilant; Blumstein et al. 2004). Given the contrasting results supporting both the trait compensation and co- specialization hypotheses, further research is needed on the links between personality and performance, and how these two aspects of the phenotype covary with metabolic rate.

Here, we test for associations between behaviour, metabolism, and performance in a wild population of Karoo bush rats (*Myotomys unisulcatus*) in South Africa. Karoo bush rats are diurnal (crepuscular) central-place foragers for which locomotor performance abilities such as sprint speed are important to rapidly travel across open spaces between bushes and therefore play a functional role in survival. At our field site (see below), Karoo bush rats are solitary living (JT and CS, unpublished data), which prevents an influence of social status on personality. We repeatedly

captured marked individuals and measured different aspects of their personality (exploration and docility), energy metabolism (RMR), and locomotor performance (sprint speed). Such repeated sets of measures of these traits also allowed us to partition phenotypic correlations into the among-individual correlations (rind; correlation between individual means) and within-individual correlations (re; correlation between deviations from the individual means). Although we found no relationship between personality and RMR (supporting the independent model), we found a negative rind between exploration and sprint speed, thus supporting the trait co-specialization hypothesis.

Methods

Study site

Karoo bush rats were monitored from February to April 2018 in an area surrounding the Succulent Karoo Research Station in the Goegap Nature Reserve, Northern Cape Province, South Africa (29° 41′ 56″ S, 18° 1′ 60″ E, altitude 912 m). The Succulent Karoo is an arid biodiversity hotspot with a mean annual rainfall of 160 mm; our study took place during a drought with ~ 76 mm of rainfall for 2018. At our field site, the minimum night-time and maximum daytime temperatures are respectively − 1.5 and 24 °C during winter and 4 and 42 °C during summer (CS, unpublished data).

Captures

Sherman-like traps baited with a mix of bran flakes, oil, raisins, salt, and freshly cut apples were used to trap individuals around their nests. Traps were set before the sun was illuminating the valley (6:00–7:00) and checked at first after 45 min and again after 90 min. For every capture done by one of the two observers (PA or JT), individuals were removed from the trap and a bag test was performed before any other handling occurred. The bag test consisted of transferring the individual from the

trap into a plastic handling bag, where it was suspended at an arm's length for 1 min while the number of seconds spent immobile was measured using two stopwatches (one for the duration of the test and another for the time spent immobile). This test was previously used to capture aspects of docility and freezing behaviour in the presence of humans (Martin and Réale 2008; Newar and Careau 2018). The bag test was not done when individuals were accidentally captured by members of another research team working on striped mice (47 out of 292 captures), in which case the individuals were identified and directly brought to the laboratory without bag test.

After the bag test, individuals were identified (or tagged if this was the first capture of an individual, see below), sexed, and weighed using a digital scale (KERN EMB 500, 0.1 g precision). Upon first capture, individuals were permanently marked using ear tags (National Band and Tag Co., Newport, KY, USA) for individual recognition, and immediately released. Ear tagging is a technique considered relatively harmless and more efficient than alternatives techniques of marking, but that can increase tick load (Wood and Slade 1990; Ostfeld et al. 1993; Kuenzi et al. 2005). After the bag test and standard manipulations were done, individuals were either released because they were brought to the lab on the previous day (107 out of 245 captures) or placed back into their traps and transported to an adjacent on-site laboratory where aspects of their behaviour, performance, and metabolism were measured. Up to 9 individuals per day were transported to the laboratory, but not all tests were conducted on all individuals on a given day to avoid rapid habituation to the novel environment and because our respirometry system allowed for measuring only 3 individuals per day. Individuals were released at their nest of capture after completing, one, two, or the three tests, depending on their last capture date, and were never kept longer than 7 h in the laboratory. It was not possible to record data blind because our study involved focal animals in the field.

Behavioural test

Once in the laboratory, the first test conducted was an open-field test (Archer 1973). All open-field tests were done between 9:00 and 10:30 prior to any other measurement, to try and limit carry-over effects. Karoo bush rats were released in a white-painted rectangle test arena (80 × 95 cm), where their behaviour was recorded with a camera (Microsoft LifeCam Cinema H5D-00018) for 5 min. Black curtains were placed around the arena to ensure that the individuals could not see the surroundings and were not disturbed. The software EthoVision XT (Noldus Information Technology, Wagenigen) was used to track the individual and extract the total distance moved.

Performance test

The second laboratory test, usually conducted between 10:30 and 11:30, was designed to measure sprint speed on a 6 m long by 12 cm wide plastic racetrack with marks at 1, 2, 3, 4, and 5 m allowing to measure the sprint speed over 4 m (starting at 1 and ending at 5). Individuals were released at the beginning of the racetrack and allowed to explore shelters (square metal box) at both ends of the racetrack where they could rest/hide in between trials. The individual was then chased by an observer who ran and screamed behind the rat using a piece of cardboard to scratch the surface of the racetrack behind the rat. Videos were recorded using a wide-angle camera at 30 frames per second (DanCoTech A9-DCH-BA). Trials in which the individuals did not run, stopped, or jumped out of the racetrack were discarded. A total of three trials per test were conducted on each rat on a given day. The status of the individual was closely monitored throughout the test to make sure the procedures did not induce any adverse effects (such as injuries or complete exhaustion that could lead to lasting effects after the test ended, which never occurred). Running speed was determined by using the Tracker software (Open Source Physics) by counting frames in between marks. For each test, we extracted the trial that yielded the fastest speed over 1 m; this value was retained as the maximum sprint speed for that individual on that day.

Respirometry

Respirometry tests were conducted after open-field and sprint speed tests, between 13:00 and 16:00. This period corresponds to the non-active phase of the diurnal cycle in Karoo bush rats (they are considered crepuscular and therefore lower their activity in the afternoon to avoid heat; du Plessis et al. 1991). Metabolic rate was measured using a flow-through respirometry system with three chambers and one baseline channel. Animals were placed in the chambers and allowed 30 minutes to habituate and for the air flow to stabilize. Ambient air was pumped and directed into a manifold where it was split into 4 different streams. Each individual air stream was directed through a mass-flow metre (FB8, Sables Systems) and sent into one of the 4 chambers (three chambers containing individuals and one empty chamber as baseline) at a flow rate of ~ 700 ml min−1. Outcoming air was directed through O2, CO2, and water vapour sensors (FoxBox, RH-300; Sable systems). After monitoring the baseline channel for 5 min, the system sequentially sampled the 3 chambers for 10 min each, after which another baseline measurement was taken. This process was repeated 3 more times, yielding a total of four 10-min samples for each individual on a given day. For each 10-min sample, we extracted the lowest mean O2 consumption over a period of 4:30 min. The lowest of these four estimates was retained as the RMR of that individual on that day. While in the chambers, individuals were monitored using a webcam (Microsoft Livecam HD-3000) to ensure that they were resting (i.e. not moving). We did not discard any estimates as individuals were always immobile for the timestamps corresponding to the lowest O2 consumption.

Statistical analyses

Statistical analyses were done using ASReml-R version 3 (Gilmour et al. 2009). All traits were standardised to a mean of 0 and a variance of 1. Before analysing sprint speed and RMR, we wanted to gain a better understanding of the variability in the raw measurements at various levels. Indeed, variation in running speed occurs at four different levels: among individuals, among tests within

individuals, among trials within tests, and among successive metres within trials. To capture variance at each of these levels, we used a multilevel approach, where random effects of (1) individual identity (ID) captured among-individual variance (V_{ind}), (2) ID combined with test captured variance among tests within individuals (V_{test}), and (3) ID combined with test and trial number captured variance among trials within tests (V_{trial}). After accounting for these levels of variance, the residual variance (V_e) represented the variance in running speed between successive metres within trials and measurement error. For metabolic rate, we took 4 repeated measurements per respirometry test; therefore, variation in metabolic rate occurs at three different levels: among individuals (V_{ind}), among tests within individuals (V_{test}), and among successive measurements within tests (V_e).

In a second step, we extracted daily maximum running speed (hereafter called sprint speed) and the daily minimum metabolic rate (hereafter called RMR) that we used to calculate repeatability and estimate among- and within-individual correlations. We first ran univariate mixed models to assess the effects of Julian day, sex, and test sequence (i.e. the number of times the individual passed the test) on body mass, docility, distance moved in the open field, sprint speed, and RMR. All univariate mixed models included body mass as a fixed effect (except the one in which body mass was the response variable) and included individual identity as a random effect to partition the phenotypic variance (after conditioning on the fixed effects above) into an among-individual variance (V_{ind}) and within-individual variance (V_e; residuals). Adjusted repeatability of sprint speed, RMR, time spent immobile in the bag test, and distance moved in the open field were calculated as the ratio $R = V_{ind}/(V_{ind} + V_e)$. The approximate standard errors (se) for R were calculated using the delta method using the pin function in nadiv (Wolak 2012).

We then used a multivariate mixed model to assess the among-individual correlations (rind) and the within-individual correlations (re) between docility, exploration, sprint speed, and RMR. The multivariate model included the same fixed effects as above (body mass, sex, test sequence, and Julian day) fitted to all traits. A 4 × 4 correlation matrix ("corgh" structure in ASReml-R) was fitted at the among-individual level (ID) to estimate Vind and rind between traits. A 4 × 4 correlation matrix

was also fitted at the residual level to estimate V_e and re between traits. The 95% confidence intervals for rind and re were calculated using profile likelihoods with the proLik function in nadiv (Wolak 2012). Best linear unbiased predictors (BLUPs) were extracted from the multivariate mixed model to obtain a graphic representation of rind and re.

ASReml-R uses a model-based missing data augmentation method (more specifically, "full information maximum likelihood") for the estimation of missing values in the response variables of a multivariate mixed model, which allowed us to fully consider all observations made on all individuals throughout the study in our multivariate mixed model. The missing data procedures in ASReml-R can improve the estimation of among-individual correlations, by increasing statistical power, and are preferable over "complete cases analyses" (Noble and Nakagawa 2018). Note that although traits were not always all measured on a given day, there was enough overlap between measurements to estimate re (e.g. sprint speed and RMR were measured 113 times on the same day, sprint speed and exploration were measured 110 times on the same day, and RMR and exploration were measured 87 times on the same day). Our protocol also means that individuals experienced different combinations of testing in the laboratory (animals sometimes were only subjected to 1 or 2 tests instead of 3 due to the limited space in the respirometry system, and if their exploration had been measured too recently). We tested the possibility of carry-over effects by creating a dummy variable indicating if the individual was tested in the open field prior to the other measurements, but this variable was not significant for sprint speed and RMR ($P > 0.05$ in both cases). Similarly, a dummy variable indicating if the individual was tested in the racetrack prior to respirometry did not have a significant effect on RMR ($P > 0.05$). Therefore, we are confident that the carry-over effects of sequential testing did not influence the results.

Finally, the multilevel analysis above revealed substantial variance in running speed from 1 m to the next within performance trials (see below). Therefore, we wanted to explore if behaviour and running speed covary differently at different points along the racetrack. We fitted a second multivariate model using the same fixed and random effects as above (body mass, sex, test

sequence, and Julian day, ID) in which the response variables were the two behavioural traits and running speeds at each metre (speed 1, speed 2, speed 3, and speed 4) of the racetrack. A 6 × 6 correlation matrix was fitted at the among-individual and residual levels to provide the rind and re between behavioural traits and running speed at each metre of the racetrack.

Results

Descriptive statistics

We captured and tested a total of 45 individuals (38 females, 7 males), of which 37 were repeatedly tested (8 individuals were only tested once). A total of 244 bag tests, 119 open-field tests, 123 respirometry tests, and 177 sprint speed tests were performed with repeated measures varying between 1 and 14 for each individual (see table 1).

Time spent immobile during the bag test averaged 53.7 s (see table 1 for descriptive statistics). While many individuals remained "frozen" (immobile) for the whole test, a few individuals were more active with the shortest time spent immobile being 5.8 s. During the open-field test, most rats moved around the walls of the arena, avoiding the centre and moved over relatively short distances (13 m) with some individuals showing higher levels of exploration than others (range 1–40 m). Sprint speed ranged between 1.5 and 4.3 m s−1 for an average of 3.2 m s−1 which is within the range of the sprint speed recorded on similar sized rodents (Djawdan and Garland 1988). RMR ranged from 0.62 to 2 ml O2·min−1 with an overall mean of 1.2 ml O2·min−1, which is in accordance with values measured for similar-sized grazing rodents (Bozinovic 1992).

Variance in running speed and metabolic rate

Considering all running speed tests and trials, we found that 35.1% of the variance was due to differences between individuals (as estimated by the Vind random effect), while 11.4% was due to

differences between days within individuals (as estimated by the Vtest component) (table S1). Additionally, only 6.2% of the variance was due to differences between trials within days (as estimated by Vtrial). Finally, 52% of the variance in running speed occurred among metres within trials (as estimated by V_e). The analysis of sprint speed below is based on the fastest speed recorded over 1 m on a given test (day).

Considering all respirometry tests, 31.7% of the variance in metabolic rate was due to differences between individuals (V_{ind}), whereas 36.2% was due to differences between days within individuals (V_{test}) (table S1). Finally, 31.9% of the variance occurred among successive measurements within trials, representing measurement error and variation in metabolic rate (probably due to activity and stress) when rats were restrained within metabolic chambers. The lowest metabolic rate value recorded over the four sampling periods on a given test (day) was extracted as RMR, and which always corresponded periods of inactivity.

Effect of mass, sex, and test sequence

Males were heavier than females (table 2 (A); Fig. 1a). Sex also had a significant effect on sprint speed with males showing slower sprint speed than females (table 2 (D); Fig. 1b). Body mass did not affect docility, exploration, and sprint speed (table 2), but as expected there was a positive effect on RMR (table 2 (E); Fig. 1c). Test sequence had a negative effect on distance moved in the open field (table 2 (C)), especially between the first and second measurements (Fig. 1d), suggesting a habituation effect. Test sequence also had a positive effect on sprint speed (table 2 (D); Fig. 1e), suggesting that individuals would perform better as they repeated the sprint speed test. Finally, Julian day had a positive effect on RMR (table 2 (E)).

Among- and within-individual correlations

After taking the above-mentioned fixed effects into account, all traits were significantly repeatable, with estimates ranging from R ± SE = 0.903 ± 0.021 for body mass to R ± SE = 0.203 ± 0.115 for RMR (Fig. 2). None of the within-individual correlations were significant (table 3). At the among-individual level, however, there were two significant correlations (table 3). There was a strong negative correlation between docility and exploration (r_{ind} ± SE = − 0.740 ± 0.216) indicating that individuals who spent more time immobile during the bag test moved over shorter distances during the open-field test (Fig. 3a). There was also a negative correlation between sprint speed and exploration (r_{ind} ± SE = −0.399 ± 0.213), indicating that the fastest sprinters moved over shorter distances during the open-field test (Fig. 3b).

Behaviour vs. running speed

Here, we consider all speed measurements from the trial for which sprint speed was extracted and look at the covariance with behaviour. As can be expected, consecutive speeds at each metre were correlated with each other with estimates ranging from 0.999 to 0.854 at the among-individual level and 0.563 to 0.230 at the within-individual level (table S2). At the among-individual level, exploration was negatively correlated with running speed at all positions of the racetrack, with significant relationships at 3 of the 4 positions (first, second, and fourth metre; table S2; Fig. 4). None of the within-individual correlations were significant between exploration and running speed (table S2; Fig. 4). By contrast, docility was not correlated with running speed at the among-individual level, but the within-individual correlations changed as function of the position of the racetrack; the only significant within-individual correlation was with running speed recorded over the first metre of the race track (table S2; Fig. 4).

Tables and Figures

Table 1. Descriptive statistics for body mass (measured in the field), docility (time spent immobile during a 1-min bag test), exploration (distance moved in a 5-min open-field test), sprint speed (fastest speed reached over 1 m on a given day), and resting metabolic rate (RMR; lowest O2 consumption over 4.5 min) in Karoo bush rats in the Goegap Nature Reserve (South Africa, 2018), including the number of individuals sampled (NID), total number of observations (nobs), units, mean, standard deviation (sd), and range (minimum to maximum).

	Body mass	Docility	Exploration	Sprint speed	RMR
N_{ID}	45	44	45	44	44
n_{obs}	285	244	119	177	123
Units	g	s	cm	m s^{-1}	ml $O_2 \cdot min^{-1}$
Mean	90.6	53.67	1309.65	3.22	1.2
sd	15.15	11.89	8.89	0.53	0.3
Min	50.2	5.8	1	1.54	0.62
Max	127.6	60	4008.02	4.29	2.03

Table 2. Effects of sex, test sequence, and Julian day on A) body mass, B) docility (time spent immobile during bag test), C) exploration (distance moved in a 5-min open-field test), D) sprint speed (fastest speed reached over 1 m on a given day), and E) resting metabolic rate (RMR) in 45 Karoo bush rats, as estimated in separate univariate mixed models. Body mass was also included as a covariate in B–E. Shown are the estimates (± SE), denominator degrees of freedom (df_{den}), Wald F-statistic, and P values. Italicized values are significant estimates (see table 1 for sample size, Fig. 2 for variance estimates, and table 3 for correlations obtained from the multivariate model).

Trait	Source	Estimate	±	se	df_{den}	F	P
A) Body mass							
	Intercept	0.199	±	0.190			
	Sex (m)	*0.958*	*±*	*0.395*	*42.5*	*5.9*	*0.0196*
	Test sequence	0.000	±	0.011	255.8	0	0.9895
	Julian day	− 0.002	±	0.002	259.2	1.5	0.2164
B) Docility							
	Intercept	0.314	±	0.635			
	Sex (m)	0.034	±	0.303	35.7	0	0.9118
	Body mass	− 0.005	±	0.006	73.1	0.7	0.4214
	Test sequence	− 0.005	±	0.094	100.3	0	0.8811
	Julian day	0.002	±	0.005	90.6	0.2	0.6752
C) Exploration							
	Intercept	1.111	±	0.622			
	Sex (m)	− 0.033	±	0.313	40.5	0	0.9171
	Body mass	− 0.003	±	0.006	101.3	0.3	0.6021
	Test sequence	*− 0.361*	*±*	*0.094*	*100.2*	*14.6*	*0.0002*
	Julian day	− 0.001	±	0.005	72.8	0.1	0.7951
D) Sprint speed							
	Intercept	− 0.185	±	0.346			
	Sex (m)	*− 1.043*	*±*	*0.339*	*40.3*	*9.5*	*0.0037*
	Body mass	0.003	±	0.002	147.1	1.6	0.2148
	Test sequence	*0.041*	*±*	*0.016*	*170.8*	*6.4*	*0.012*
	Julian day	− 0.003	±	0.005	167.4	0.3	0.5696
E) RMR							
	Intercept	− 4.685	±	0.499			
	Sex (m)	0.144	±	0.217	36.3	0.4	0.5129
	Body mass	*0.036*	*±*	*0.005*	*44.4*	*48.2*	*< 0.0001*
	Test sequence	− 0.053	±	0.078	114.3	0.5	0.4957
	Julian day	*0.021*	*±*	*0.004*	*71.5*	*30.2*	*< 0.0001*

Table 3. Among-individual correlations (r_{ind}) and within-individual correlations (r_e) between docility (time spent immobile during bag test), exploration (distance moved in a 5-min open-field test), sprint speed (fastest speed reached over 1 m on a given day) and resting metabolic rate (RMR) in 45 Karoo bush rats. All estimates were extracted from a single multivariate mixed model (see table 1 for sample size and Fig. 2 for variance estimates). Profile likelihoods were used to calculate the 95% confidence interval (CI) of r_{ind} and r_e.

		Among-individual correlations					Residual correlations				
					95% CI					95% CI	
Trait 1	Trait 2	r_{ind}	±	se	lower	upper	r_e	±	se	lower	upper
Exploration	Docility	*− 0.740*	*±*	*0.216*	*− 0.968*	*− 0.382*	0.078	±	0.104	− 0.060	0.211
Sprint speed	Docility	0.280	±	0.198	− 0.009	0.531	0.096	±	0.090	− 0.028	0.217
Sprint speed	Exploration	*− 0.399*	*±*	*0.213*	*− 0.659*	*− 0.076*	− 0.151	±	0.111	− 0.296	0.002
RMR	Docility	− 0.050	±	0.309	− 0.485	0.363	0.108	±	0.106	− 0.035	0.246
RMR	Exploration	− 0.247	±	0.337	− 0.666	0.254	− 0.085	±	0.126	− 0.250	0.087
RMR	Sprint speed	0.088	±	0.263	− 0.278	0.443	0.140	±	0.111	− 0.016	0.288

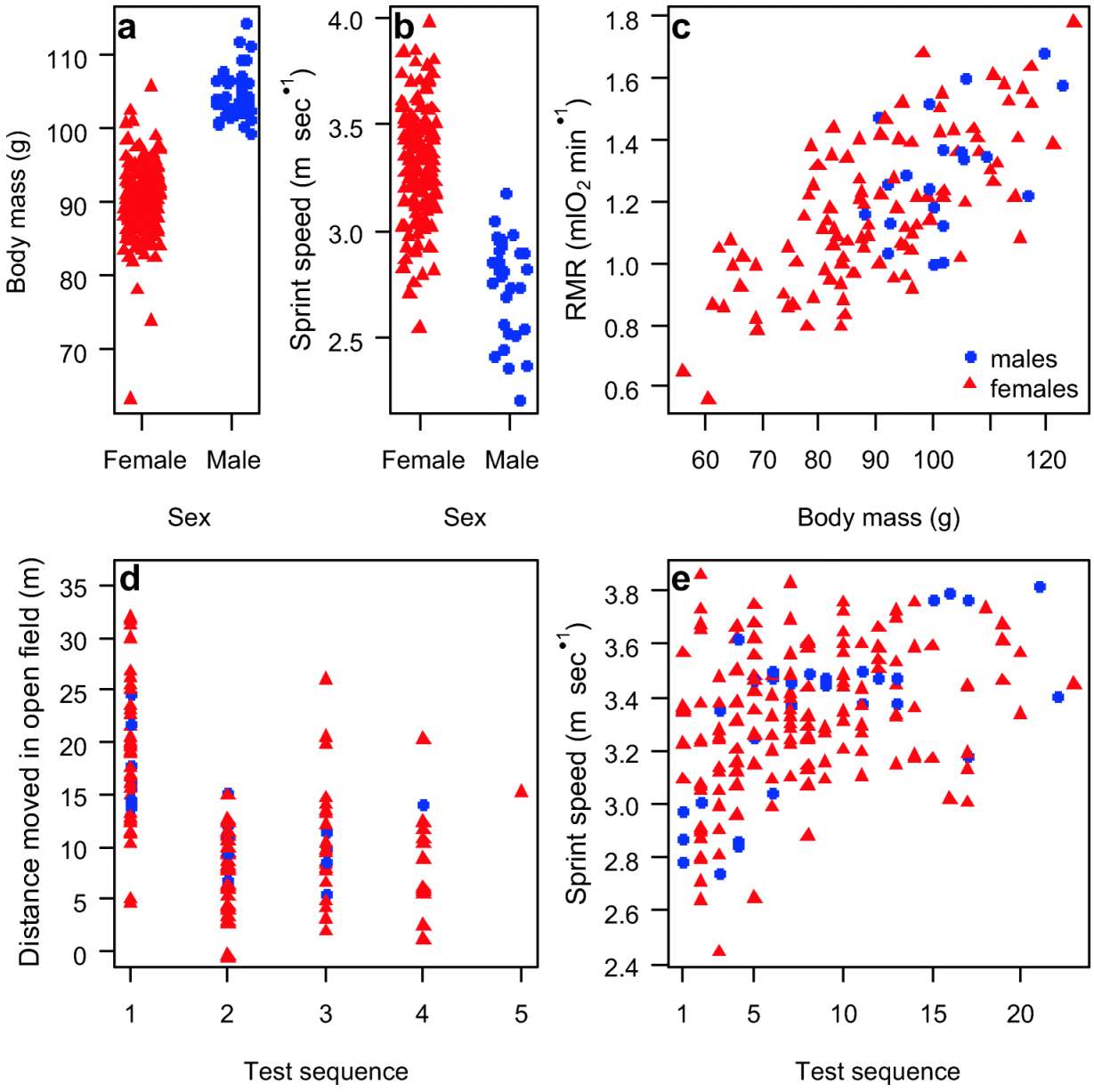

Figure 1. **a** Body mass (g) and **b** sprint speed (cm s−1) as function of sex, **c** resting metabolic rate (RMR; ml O2 min−1) as function of body mass, and **d** distance (m) moved during an open-field test and e sprint speed as function of test sequence in 7 males (blue dots) and 38 females (red triangles) Karoo bush rats. Shown are partial residuals (accounting for other effects in the models, see table 2).

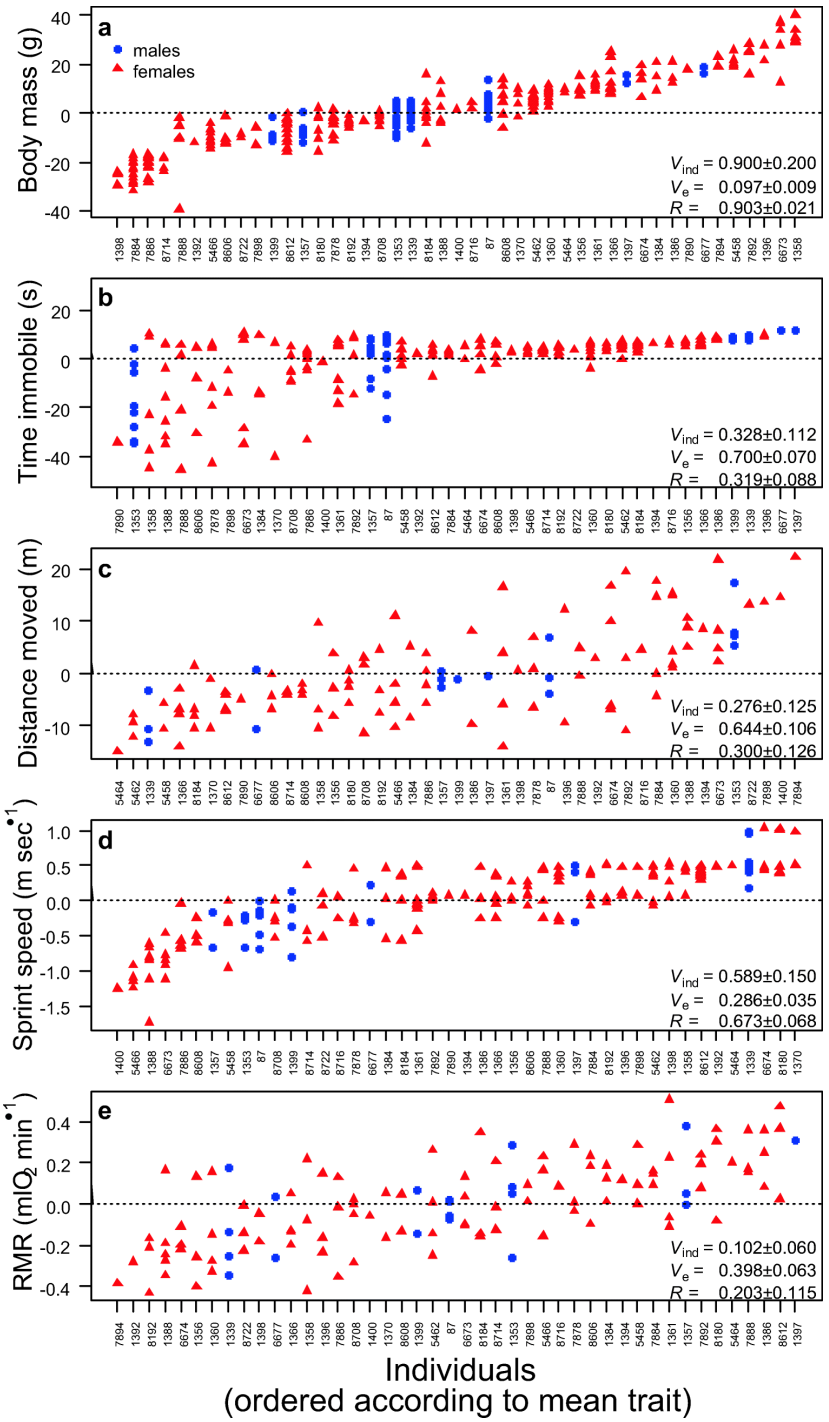

Figure 2. Among- and within-individual variation in **a** body mass, **b** time spent immobile during bag test, **c** distance moved in the open-field test, **d** sprint speed, and **e** resting metabolic rate (RMR) in 7 males (blue dots) and 38 females (red triangles) Karoo bush rats. Shown are residuals from a linear model on the raw scale that included body mass (except in **a**), sex, test sequence, and Julian day. In each panel, individuals are ordered along the x-axis according to their mean trait values. Among-individual variance (V_{ind}), residual variance (V_e), and repeatability (R) estimates with standard errors (SE) were extracted from linear mixed models on z-standardised traits (mean = 0, total variance = 1).

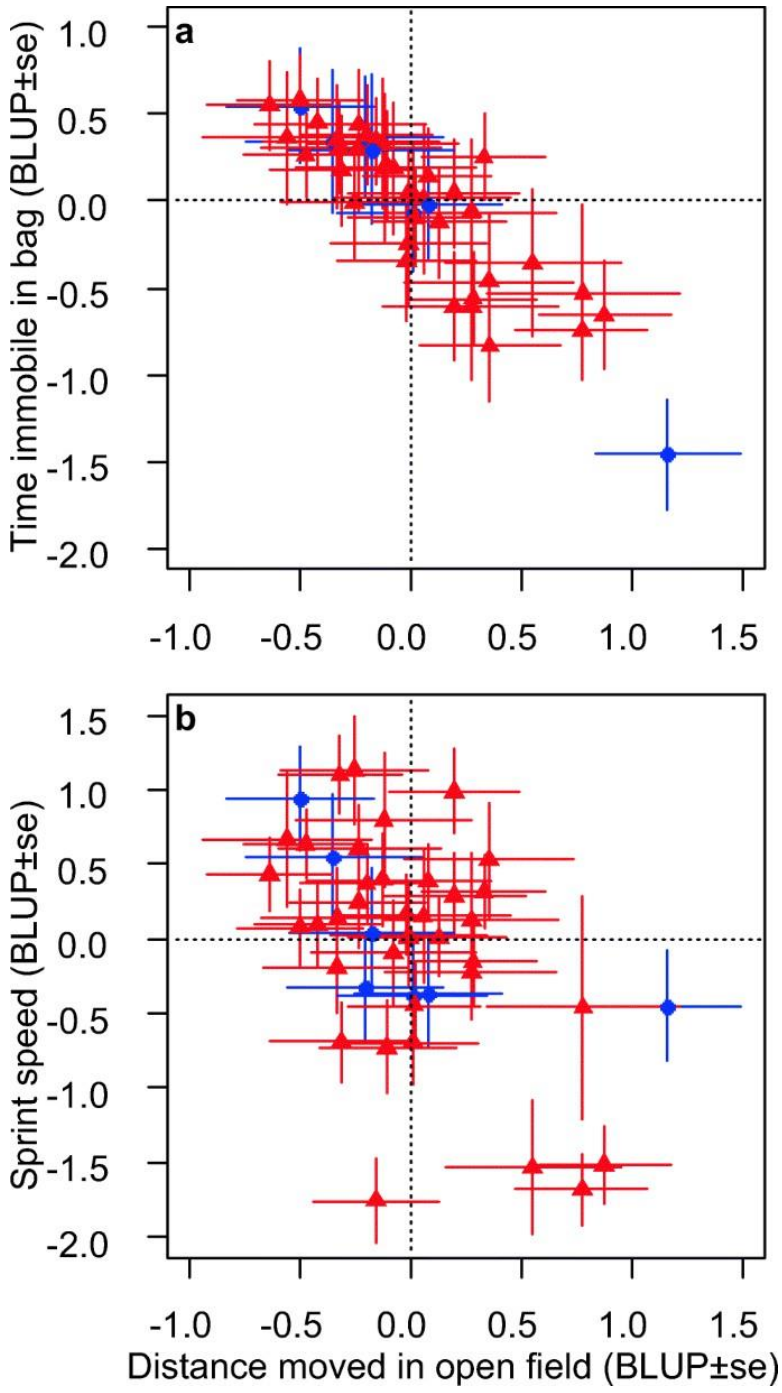

Figure 3. Representation of the among-individual correlations (rind) between a time spent immobile in the bag test and distance moved in the open field and b sprint speed and distance moved in the open field in 7 males (blue dots) and 38 females (red triangles) Karoo bush rats. Displayed are the best linear unbiased predictors (BLUPs ± SE) extracted from the multivariate model (see table 3 for correlation estimates) with z-standardised traits (mean = 0, variance = 1).

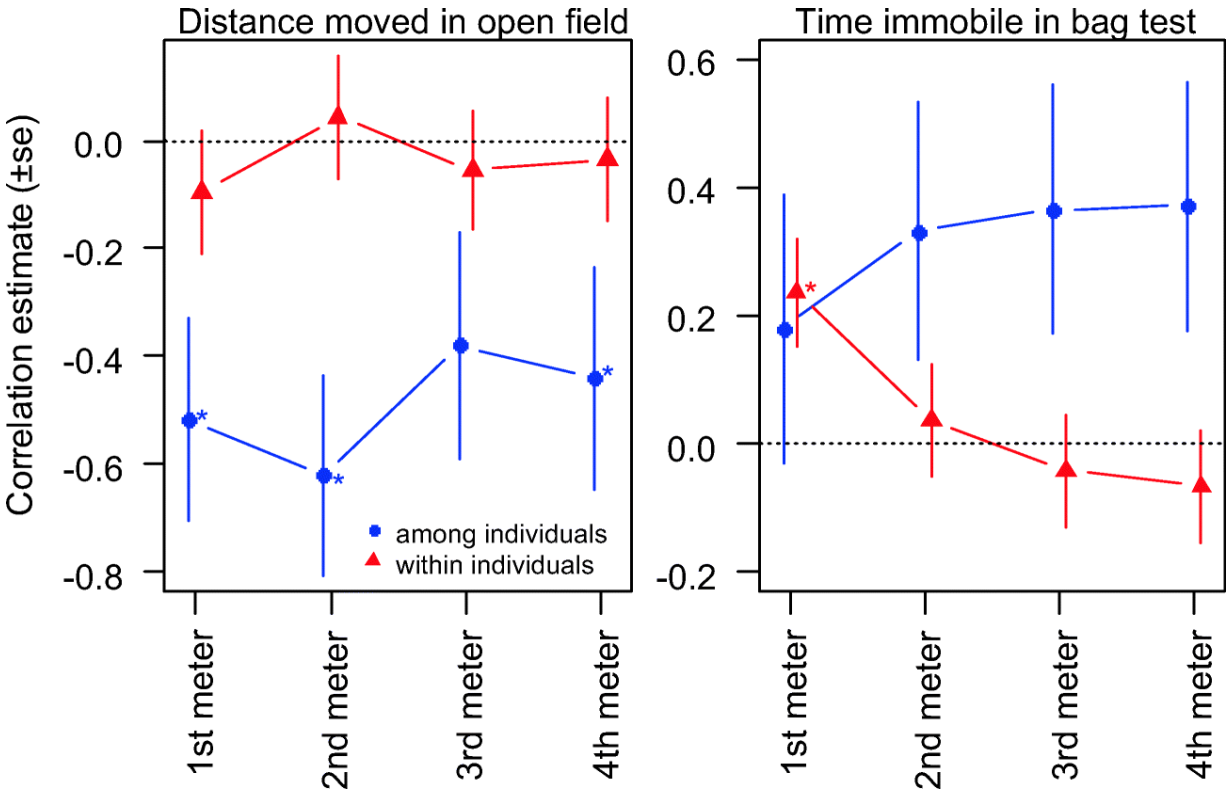

Figure 4. Representation of the among-individual correlations (r_{ind} ± SE, blue dots) and within individual correlations (re ± SE, red triangles) between distance moved in the open field (exploration), time spent immobile in the bag test (docility), and running speeds at each metre during the performance test for 7 male and 38 female Karoo bush rats. Correlation estimates were extracted from the second linear mixed models on z-standardised traits (total variance = 1). Asterisks denote estimates that are significantly different from 0.

Discussion

Our objective was to test for correlations between personality, performance, and metabolic rate at the among- and within-individual levels. As previously found in many studies on rodents, we found a behavioural syndrome in our population in which docility and exploration were negatively correlated at the among-individual level. We did not find correlations between RMR and any other traits, including docility and exploration. Therefore, our results support the independent model (Careau and Garland 2012). We found a negative correlation between exploration and sprint speed at the among- individual level. Altogether, our results and those from three other studies on rodents (Blumstein et al. 2004; Newar and Careau 2018; Piquet et al. 2018) provide empirical support for the idea that personality and performance traits are co-specialised.

The fast and the curious

If performance determines what individuals can do behaviourally, higher sprinting abilities should allow individuals to take more risks, because those individuals are better equipped to escape potentially dangerous situations (Careau and Garland 2012). Interestingly, however, we found that the individuals with the highest locomotor performance (fastest sprinters) were also less explorative in the open-field test. This is the opposite result of what is predicted from the trait compensation hypothesis (Dewitt et al. 1999). Instead, the negative correlation between sprint speed and exploratory behaviour lends support to the trait co-specialization hypothesis (see Dewitt et al. 1999), where both traits additively reinforce protection from predators. Although trait compensation seems to be one of the most commonly observed pattern among species between behavioural and morphological defences, whereby species with larger defences (e.g. shells) are bolder and/or more explorative (Mikolajewski and Johansson 2004 but see Hossie et al. 2017), studies conducted so far on the functional relationship between behaviour and performance at the individual level suggest that the prevailing pattern is trait co-specialization in mammals (this study; Blumstein et al.

2004;Newar and Careau 2018; Piquet et al. 2018). In other words, shy or less explorative individuals are less prone to encounter a predator, and in the event of an encounter, their higher sprinting abilities further increase the chance of successful escape, and therefore survival.

An alternative explanation for the negative relationship between sprint speed and exploratory behaviour could be that the stress response of the individuals covary with individual variation in motivation during the performance tests. For example, if shy individuals are more fearful and anxious than bold individuals, they might be less motivated to explore the arena during the novel environment test, but more motivated to run down the racetrack at their fastest when being chased. For that scenario to be true, however, there has to be individual variation in motivation, while performance protocols are typically designed to eliminate such variation. Nevertheless, Losos et al. (2002) identified motivation as "the major problem bedevilling studies of performance", as previous studies showed that some individuals do not use their maximal capacities during the tests (Pough 1989; Garland and Losos 1994). Sub-maximal trials—when animals are judged as not having performed to their maximum—are usually excluded from performance studies, but deciding which trials to exclude may vary across studies. Losos et al. (2002) pointed out that 49% of the 65 studies published between 1979 and 1999 on lizard sprinting abilities did not mention sub-maximal trials, and that only a few studies clearly stated that they excluded these trials. Although we excluded all sub-maximal trials, no individual was excluded from our study. Nevertheless, some variation in motivation must remain among and within retained trials, and, because of the potential covariance it can induce between performance and behaviour, a better appreciation of variation in raw performance measures is warranted (Berberi and Careau 2019).

Variation in running speed across levels

To our knowledge, only two studies so far have followed a multilevel approach (Araya-Ajoy et al. 2018) and used extra random effects to partition variance in running speed across different

temporal scales (Berberi and Careau 2019; Lailvaux et al. 2019). In these studies, 17–19% of the variance in running speed occurred at the among-individual level, whereas 35% of the variance occurred at that level in our dataset. These relatively low estimates of long-term repeatability imply that many repeated tests per individual are required to properly quantify individual differences in locomotor performance. In contrast to Berberi and Careau (2019) and Lailvaux et al. (2019), we retained running speed measures taken at each metre of the racetrack. Interestingly, the levels with the lowest amount of variance were V_{test} and V_{trial}. Therefore, running speed did not vary much between tests conducted on a given individual throughout the season and between trials conducted on a given day, which is quite encouraging because it suggests that variation in motivation was low at these levels.

About half of the variance in running speed resided in the Ve component, which consists of variation in motivation and measurement error. To reduce measurement error in our running speed measurements, we should have used a high-speed camera that records at a faster rate than 30 frames per second. Still, variation in motivation from metre to metre within trials was evident (PA, pers. obs.), which gave us the idea of exploring how behaviour covaries with running speeds recorded at different positions of the racetrack. We found that docility was correlated with running speed recorded over the first metre of the racetrack at the within-individual level. Therefore, on a given test day, an individual who remained more immobile during the bag test than its own average ran faster than usual on the first metre of the racetrack. This suggests that individuals who were, for unknown reasons, more fearful of humans on a given day, froze for a longer period during the bag test and displayed a higher acceleration when running away from the chaser. Given that docility and speed were measured in different locations and using different tools, correlated measurement error is unlikely to have caused the re between the two traits. Therefore, the r_e must have been caused by correlated phenotypic plasticity with respect to factors that were unaccounted for in our experiment (Brommer and Kluen 2012; Careau and Wilson 2017b, a). Note that the relationship between docility and speed was not apparent in the analyses of sprint speed (table 3) because the fastest running

speed is usually reached in the second and third metres of the racetrack. Hence, our results show the insights that can be gained from analysing raw running performance measurements, especially when trying to relate performance to behaviours that involve reactions to human presence.

Repeatability and behavioural syndrome

Repeatability of behavioural traits is generally higher in the field than in the laboratory, presumably because individuals live in heterogenous environments that permanently influence their behaviour, increasing among-individual differences and therefore repeatability (Bell et al. 2009). In our case, however, adjusted repeatability was R = 0.319 for docility and R = 0.30 for exploration, respectively, which is slightly under the reported average repeatability of behaviours (R = 0.37) (Bell et al. 2009). We also found that the more explorative individuals were less docile. Similar behavioural syndromes have been previously observed in a number of other populations of mammals between docility and exploration (Montiglio et al. 2012; Careau et al. 2015) and between docility, exploration, and activity (Petelle et al. 2015). A similar behavioural syndrome is also exhibited in birds, between docility (assessed with a "back test") and exploration (Hall et al. 2015) and between docility, aggressivity, and breathing rate (Brommer and Kluen 2012). Thus, the behavioural syndrome we found in bush Karoo rats is very similar to what has been reported for many other species.

These behavioural syndromes appear to be reflective of the "flight or fight" response and have been associated with the proactive-reactive continuum of coping style strategies (Koolhaas et al. 1999). According to the literature on coping styles, individuals consistently differ in their physiological and behavioural response towards stressful situations. Proactive individuals tend to interact with the stressor, while reactive individuals tend to avoid it. Applying this idea to our species, proactive individuals were likely those who moved more in the handling bag trying to escape (therefore being less docile), and who also travelled longer distances in the open-field test (being more explorative). By contrast, reactive individuals were likely those who froze during the bag

test and did not travel long distances in the open-field test. More stress-related measures are needed to confirm the associations between docility and exploration behaviour with coping styles in our species.

Lack of relationship with RMR

RMR has been previously found to be a repeatable trait in many taxa, with an average estimate of R =0.39 in mammals (White et al. 2013). In this study, the repeatability of RMR was R = 0.203 after controlling for date, body mass, sex, and test sequence. In other words, only ≈ 20% of the total variance in RMR was attributed to differences among individuals and ≈ 80% of the variance was due to within-individual variation (plasticity) and measurement error. Generally, the repeatability of metabolic rate declines with increasing time between measurement (White et al. 2013), but we think this is an unlikely explanation for the relatively low R observed in our study considering the number of repeated measures we took over a relatively short field season (February 2 to May 21). The relatively low repeatability of RMR might be explained by the fact that this study was conducted on wild-caught animals where natural conditions (i.e. food availability and predation risk) varying within individuals affect differently the expression of the metabolism, compared to more stable laboratory conditions (Dingemanse et al. 2009; Kontiainen et al. 2009; Auer et al. 2016). The field conditions also forced us to use relatively short respirometry tests (i.e. animals were only kept 4 h in the metabolic chambers), which might have amplified the influence of stress in our RMR measures.

We did not find correlations between RMR and any of the other traits we measured (docility, exploration, or sprint speed). Given the relatively low repeatability of RMR and number of individuals sampled, it is possible that we had low power to detect any relationship with RMR. Nevertheless, our results support neither the allocation nor the performance model; instead, it appears the independent model applies in our population, in which RMR and behaviours are not correlated but where non-resting measurements of metabolism (such as the daily energy

expenditure) are expected to be correlated to behaviour (Careau and Garland 2012). A recent meta-analysis on the covariance between RMR and behaviour pointed out that RMR was positively correlated with behaviours that likely have consequences for energy gain (e.g. foraging), or expenditure (e.g. sustained running speed), but was not correlated with behaviours that have uncertain energetic outcomes such as the two behavioural traits used in this study (Mathot et al. 2019). Although RMR and behaviours are not correlated in the independent model, behavioural traits still have an influence on the non-resting part of the energy budget (e.g. Careau et al. 2015). In such cases where the independent model applies, the energetic impacts of personality would only be detectable through the measurement of daily energy expenditure, which includes the energetic costs of activity in addition to basal costs of living (and other costs, e.g. thermoregulation).

Conclusions

Although it is intuitive to think that bolder and more explorative individuals should be better equipped to deal with the increased risks resulting from those behaviours (trait compensation), empirical evidence in rodents shows this is not the case. Instead, it appears that sprint speed is co-specialised with vigilance and exploratory behaviour in rodents (this study; Blumstein et al. 2004; Newar and Careau 2018; Piquet et al. 2018). Future research should tackle the implications of trait co-specialization for the pace of life, especially survival in prey species. Investigating how different predator types, diversity and density could also potentially shed light on how co-specialization and compensation occurs. It will also be important to evaluate the contribution of motivation underlying individual co-variation in personality and performance. Doing so might involve using methods for measuring performance with different levels of stress, for example tests that rely on an individual's survival instincts (i.e. forced swimming), tests with human-induced stress, and tests where individuals perform without being forced. Finally, it will be important to continue partitioning variance in raw performance measurements to get a better sense of where most of the variation occurs.

Chapter 3: The fast and the curious III: speed, endurance, activity, and exploration in mice.

Preamble

One of the main conclusions of my second chapter is that relationships between behaviour and performance might be influenced by the context in which traits are measured. Namely, when performance traits are measured in the lab or the field, a human threat (e.g., chasing individuals, tapping their tails, etc…) is often used to force individuals to perform the required task. This, however, relies on the assumptions that individuals react towards the human threat to the same extent they would towards a predator chasing them in their habitat, and that individuals perceive the human threat as an imminent danger, prompting them to use their locomotor abilities to the fullest.

In my third chapter, published in Behavioural Ecology and Sociobiology, I repeatedly measured a suite of behavioural and performance traits in Peromyscus leucopus, including different performance measurements with and without the human factor. To do so, I decided to run a laboratory experiment, for mainly two reasons: I was able to take repeated measurements on every individual, and I could use a forced swimming test, which does not rely on a human stressor, while also ensuring that individuals would recover correctly between measurements, a much harder task in the field.

Introduction

Performance, defined as the ability to perform a task when maximally motivated, encompasses multiple traits, such as sprint speed, running endurance, and bite force (Garland and Losos 1994). Performance traits are among the most ecologically relevant aspects of the phenotype to measure. For example, the maximum running speed of an animal, defines the upper limit to which an individual will be able to escape a predator. Therefore, it is expected that performance generally sets an "envelope" within which an individual's behavioural repertoire is limited (Bennett 1989; Garland et al. 1990a; Bauwens et al. 1995). Studies on performance have also noted the importance of behaviour in determining how wild animals use their performance in their natural habitat (Husak 2006a; Husak and Fox 2006; Irschick et al. 2008). Individuals may differ in their general activity and tendency to explore their environment, engage in a fight, and take risks while foraging, which in turn should influence how often and to what extent animals use their locomotor performance (Husak 2006a). Intuitively, we would expect that more active individuals would be able to run faster, or sustain running for longer, but this is not always the case (Friedman et al. 1992), and in fact the opposite has also been observed (Newar and Careau 2018; Agnani et al. 2020).

Two adaptive hypotheses have emerged to explain associations between animal performance and behaviour: the compensation and co-specialization hypotheses. Although both hypotheses have been originally developed to explain co-adaptations between anti-predator behaviours and morphological defenses (Dewitt et al. 1999), they have been recently transposed to explain why and how behavioural traits like exploration and boldness covary with locomotor performance (Newar and Careau 2018). In the compensation hypothesis, activity, exploration, and boldness are expected to be positively correlated with performance traits. In this scenario, bold individuals that have higher risks of encountering a predator would "compensate" with faster sprint speed. To put it another way, individuals with low performance would "compensate" by behaving in such a way as to avoid risk of encountering a predator. A potential ultimate explanation for trait

compensation is that it results from correlational selection in which particular combinations of characters are selected for (Sinervo and Svensson 2002). Bold and fast individuals are more exposed to predators but better equipped to escape them, whereas shy and slow individuals are less exposed but do not have to pay the costs of building up and maintaining the morpho-physiology needed to run fast. If both combinations yield high fitness, whereas other combinations (i.e., bold and slow, or shy and fast) yield lower fitness, then such a pattern of correlational selection should lead to the evolution of a positive genetic correlation between boldness and sprint speed. In zebrafish (*Danio rerio*), bi-directional artificial selection for increased/decreased boldness resulted in higher/lower fast-start escape performance (Kern et al. 2016). Other studies have found a positive phenotypic correlation (r_P) between performance and behaviour. In the delicate skink (*Lampropholis delicata*), bold individuals were able to sprint faster than shy individuals (Michelangeli et al. 2018). Finally, in the Asian agamid lizard (*Phrynocephalus vlangalii*), time spent moving in a novel environment was positively correlated with endurance (Chen et al. 2019).

By contrast, the co-specialization hypothesis predicts that activity, exploration, and boldness should be negatively correlated with performance traits because both behaviour and performance "enhance" protection from predation risk (Dewitt et al. 1999). If the benefits of being shy and fast are obvious when predation risk is high, it is harder to see the advantages of being slow and bold. It has therefore been suggested that co-specialization might result from a range of different mechanisms including individual differences in sensory ability to detect predators and prey experience in prior exposure to predators (Dewitt et al. 1999). Support for the co-specialization hypothesis comes mostly from studies on wild rodents. In Eastern chipmunks (*Tamias striatus*), sprint speed measured when releasing and chasing individuals in the field was negatively correlated with distance moved during a novel environment test (Newar and Careau 2018). In Yellow bellied marmots (*Marmota flaviventris*), faster sprinters upon field release were more vigilant while foraging than slower sprinters (Blumstein et al. 2010). Finally, in bush karoo rats (*Myotomis unisulcatus*), distance travelled during a novel environment test was negatively correlated with

maximal sprint speed measured on a racetrack in the laboratory (Agnani et al. 2020).

Considering the mixed results on the association between performance and behaviour, additional research is needed to document which performance and behavioural traits are correlated, and how. Almost all of the above cited empirical studies examined sprint speed or escape speed; therefore, we still do not know how other performance traits might be potentially co-adapted with behaviour (Djawdan and Garland 1988; Tang et al. 2002; Montiglio et al. 2010; Garland et al. 2011; Novak et al. 2012; Tatem et al. 2014). In particular, performance measures based on an escape response from a human threat might introduce a methodological bias and cause covariance with behaviours that are also measured under stressful situations. We thus need additional measures of performances that are elicited differently. Studies are also needed in which behavioural activity is measured in a non-risky and a non-novel environment (e.g., a home cage). In this regard, a noteworthy study on house mice (*Mus musculus*) examined the links between sprint speed, swimming endurance, exploratory behaviour, and voluntary wheel running (Friedman et al. 1992). Although Friedman et al. (1992) found that mice displayed consistent repeatable individual differences in all traits, none of the r_P between performance and behavioural traits were significant. Given their sample size (N = 35), Friedman et al. (1992) probably had acceptable power to detect moderate effect sizes (e.g., rP ≥ 0.45), yet they may have failed to detect a relationship because they did not use a (co)variance partitioning approach to separately estimate relationships at the among- and within-individual levels (Dingemanse and Dochtermann 2013). Indeed, multiple recent studies on labile traits such as performance and behaviour have shown how important relationships can be missed when estimated at the aggregated phenotypic level (Dingemanse and Dochtermann 2013; Brommer 2013; Careau and Wilson 2017b; Brommer and Class 2017; Careau et al. 2019). Therefore, it is imperative to estimate correlations separately at the among and within-individual levels to better understand relationships among labile traits.

Here, our main objective was to estimate the among- and within-individual correlations between multiple performance and behavioural traits. We repeatedly measured multiple aspects of

locomotor performance relying on either escape from a human (sprint speed) or survival (swimming performance) and behaviour in relatively new (exploration) and familiar environment (voluntary wheel running and home-cage activity) in 51 female white-footed mice (*Peromyscus leucopus*). Then, we used a multivariate mixed model to partition the phenotypic relationships between those traits at the among- and within-individual levels. Given past results in small rodents (Newar and Careau 2018; Agnani et al. 2020), we expected a negative among-individual correlation (r_{ind}) between sprint speed and exploration. However, if the negative r_{ind} obtained in rodents is due to individual differences in stress coping in response to novel environments and human threats, then we should find different relationships between performance and behavioural measures like swimming endurance and activity in a home-cage environment.

Methods

Study animals

Female *Peromyscus leucopus* were purchased from the Peromyscus genetic stock center, University of South Carolina, and housed in groups of 4 (with one group of 3) in standard rat cages. Only females were used in this experiment as they are easier to house in groups and exhibit much lower aggressivity than males. Individuals were kept at a room temperature of 21 °C with a 12–12 light cycle (lights on from 6am to 6pm). Mice were fed ad libitum and kept at the animal facilities of the University of Ottawa for the entirety of the experiment.

Experiment overview

Mice were tested in batches of 8. At the onset of each test period, each mouse was individually placed in 1 of 8 Promethion cages for measuring home-cage activity and voluntary wheel running over a 24-h period. Mice were then individually placed in temporary holding cages and transported to

a nearby room for behavioural and performance tests. Mice were first subjected to an open-field test for a duration of 5 min, then retrieved from the arena and placed on the racetrack for the sprint speed test, after which mice were put back in their communal cages where they were housed. This process was repeated three times for each mouse with an average of 32 days between measurements (each set of measurements beginning right after the previous one was completed) resulting in three repeated measurements for each test. Swimming tests were performed on different days and were not preceded or followed by any other behavioural or performance tests in order to allow individuals to rest and recover properly (minimum of one day of rest between swimming tests). Although observations were not blind, the observer was unaware of the behavioural tendencies of the individual mice until completion of the entire data collection (i.e., the observer's lack of attention to individual behavioural tendencies during data collection resulted in perceptual blindness).

Activity measurements

Home-cage activity and voluntary wheel running were measured using an 8-cage Promethion system (Sable Systems international, Las Vegas, NV, USA). Each cage (21 cm wide × 37 cm deep × 14 cm high) included a food hopper, a water bottle, a shelter, and a running wheel. Each cage was placed within a X-Y array of infrared beam break detectors (BX-1; physical beam spacing = 1 cm) that continuously detected movement inside the cage. Each cage also contained a WM-1 running wheel module (diameter = 11.43 cm) equipped with a reed switch to count wheel revolutions. Total daily distance run on the wheel and in the home cage were compiled over the entire 24-h period and converted in m h−1.

Open-field tests

Mice were released in a 1×1-m light grey squared test arena at ~ 500 LUX (Amprobe LM-120), where their behaviour was recorded with a camera (Akyta 1080P) for 5 min. Mice were transferred from

their temporary holding cages into the arena using a red plexiglass cylinder closed on one side. Individuals naturally entered the cylinder when presented, the cylinder was shifted upwards and the opening obstructed with the handler's hand (PA) before being released in the arena. To minimise disturbance, the examiner (PA) stepped out of the room for the duration of the test. In between tests, the arena was thoroughly cleaned using a light disinfectant. Open-field tests were conducted immediately after the end of the 24 h in the promethion cages and took place in between 12:30PM to 3PM. The software EthoVision XT (Noldus Information Technology, Wageningen) was used to track the individual and extract the area covered during the test by dividing the arena in 100 squares and recording the number of squares visited.

Sprint speed tests

The third test was designed to measure sprint speed on a 2-m-long by 10-cm-wide metal racetrack with marks at 0.5, 1, 1.5, and 2 m allowing to measure the sprint speed for each 0.5 m section over 2 m (for similar methodology see (Djawdan and Garland 1988; Garland et al. 1995). Individuals were released at the beginning of the racetrack and first allowed to explore the track and the two shelters (square plastic bin) at both ends of the racetrack where they could rest in between trials. To prompt running, mice were chased by an observer (PA) who ran and screamed behind them using a piece of cardboard maintaining contact with the surface of the racetrack and touching the tail of the mice. Videos were recorded using a wide-angle camera at 60 frames per second (Campark X20). Trials in which the individuals did not run, stopped, or jumped were excluded. Running speed was calculated using the Tracker software (Open Source Physics) by counting frames in between marks. A total of three trials per test were conducted on each mouse on a given day, which resulted in a total of 1670 running speed measurements (i.e., 51 individuals tested on three separate days, with three trials per test and one speed measurement across each of the four 0.5 m section of the trial, minus 166 observations missing). Berberi and Careau (2019) suggested that researchers consider the highly

hierarchical nature of raw running speed measurements and consequently report the relative amount of variation at each level. Following their approach (see also Agnani et al. 2020), we found that 26.2% of the variance in our raw running speed measurements was due to consistent differences among individuals across the study, while 35.4% was due to differences between days within individuals (from one test to another). Additionally, only 4.6% of the variance was due to differences between trials within tests. Finally, 33.7% of the variance in running speed occurred among 0.5 m sections of the racetrack within trials. For each test, we extracted the trial that yielded the fastest speed over a half-meter, and this value was retained as the maximum sprint speed for that individual on that day.

Swim tests

Swimming performance was measured using a modified forced swimming test (Friedman et al. 1992; Huang et al. 2016). Individuals were fitted with fishing weights attached to the base of their tail equaling 8 to 10.5% of their body mass (0.5, 1, or 1.5 g weights) and placed in a glass cylinder tank (height = 22 cm; diameter = 20 cm). The water in the tank was maintained at 30 °C to minimise heat loss during the test and two drops of hypoallergenic dog shampoo were added to prevent surface tension and minimise flotation. The test started when the mouse was introduced into the water, and swimming performance was recorded as the time during which individuals could maintain their head above water. The test ended when the mouse remained fully submerged for 7s (Wang et al. 2012; Huang et al. 2016), at which point the mouse was quickly retrieved to prevent drowning and placed on a heated pad until dry. No animals were lost during these measurements. One test was discarded because the animal refused to swim and instead repeatedly became submerged for an extended amount of time. A total of three tests were recorded for each animal, separated by a minimum of 1 day between tests.

Statistical analysis

Statistical analysis was done using ASReml-R version 4 (Butler et al. 2018). All continuous traits were standardised to a mean of 0 and a variance of 1. All parameters of interest were extracted from a single multivariate model that included voluntary wheel running, home-cage activity, open-field exploration, sprint speed, and swimming performance as response variables. Body mass (mean = 18.4 g, range 13.3 to 29.9 g), age (mean = 365 days, range 264 to 426 days), and test sequence (1 to 3) were fitted as fixed effects to all traits. Additional fixed effects were fitted to specific traits when relevant: cage identity (1 to 8) was fitted to wheel running and home-cage activity to control for potential mean difference between measurement cages (in both cases, $P > 0.05$, results not shown); "trial number" was fitted to sprint speed to account for potential differences between successive trials within a test; the mass of the fishing weight attached to the tail of the mouse was fitted to swimming performance.

For each trait, individual identity was fitted as a random effect to partition the phenotypic variance (not explained by fixed effects) into among-individual variance (V_{ind}) and within-individual variance (V_e; residuals). The adjusted repeatability of each trait was calculated as the ratio $R = V_{ind}/(V_{ind} + V_e)$. The approximate standard errors (se) for R were calculated using the delta method using the vpredict function in ASReml-R (Wolak 2012; Butler et al. 2018). The random effect of individual identity was fitted as a 5 × 5 unstructured correlation matrix to quantify the among-individual correlations (r_{ind}) between voluntary wheel running, home-cage activity, open-field exploration, sprint speed, and swimming performance. Residuals were also modelled as an unstructured correlation matrix to quantify the within-individual correlations (r_e) between all traits except swimming endurance (because swimming tests were not done on the same day as the other measurements to minimise stress, correlations were not estimable at the residual level and therefore were fixed to zero). The 95% confidence intervals for r_{ind} and r_e were computed using profile likelihoods with the proLik function in nadiv (Wolak 2012). Best linear unbiased predictors (BLUPs) and residuals were extracted from the multivariate mixed model to obtain a graphic representation of

r_{ind} and r_e.

Results

We repeatedly tested 51 females. Each trait was measured three times, resulting in a total of 153 tests of home-cage activity, open-field exploration, and sprint speed, except for voluntary wheelrunning and swimming performance for which one measurement was excluded due to technical problems (see table 1 descriptive statistics).

Effect of body mass, age, test sequence, cage, trial, and weight

Body mass and age had no statistically significant effect on any of the measured traits (table 2). We found a negative effect of test sequence on exploration in the open field (table 2C) and on swimming performance (table 2E). Test sequence had a positive effect on sprint speed, with individuals on average reaching slightly higher speeds with each consecutive tests (table 2D). Finally, weight had a strong and significant negative effect on swimming endurance, as individuals with heavier weights attached to their tail stayed afloat for shorter durations (table 2E).

Repeatability

All traits measured during the experiment were significantly repeatable, with estimates ranging from $R = 0.930 \pm 0.017$ for home-cage activity to $R = 0.334 \pm 0.105$ for swimming performance (Fig. 1).

Among-individual correlations

Although the r_{ind} between the three behavioural traits were all positive (Fig. 2), none were significantly different from zero (table 3). By contrast, the r_{ind} between the two performance traits was positive and significant (Fig. 2), with individuals that stayed afloat the longest during the

swimming test also reaching the highest speeds on the racetrack.

All of the six r_{ind} between the behavioural and performance traits were negative (Fig. 2), with three of the six significantly different from zero (table 3). Sprint speed was negatively correlated with both voluntary wheel running (Fig. 3A) and home-cage activity (table 3), such that mice that were faster sprinters travelled less distance on the wheel and in their home cage. Swimming performance and cage activity were negatively correlated (table 3), indicating that individuals that swam the longest also travelled less distance in their cage.

Within-individual correlations

We found two significant r_e (table 3). Home-cage activity and voluntary wheel running activity were positively correlated (table 3), indicating that on a given test day, individuals that were more active in the cage, were also more active on the wheel. Sprint speed and voluntary wheel running activity were also positively correlated (table 3), indicating that individuals that were also more active on the wheel than usual on a given day subsequently reached higher-than-usual speeds on the racetrack (Fig. 3B).

Tables and Figures

Table 1. Descriptive statistics for voluntary wheel running, home-cage activity, open-field exploration, sprint speed, and swimming performance in female white-footed mice (*Peromyscus leucopus*), including number of individuals sampled (N_{ID}), total number of observations (n_{obs}), units, mean, and range (minimum and maximum).

	N_{ID}	n_{obs}	Units	Mean	Min	Max
Voluntary wheel running	51	152	m/h	123.6	12.51	533.9
Home-cage activity	51	153	m/h	7.57	1.41	109.3
Open-field exploration	51	153	# squares	73.89	1	100
Sprint speed	51	153	m/s	1.58	0.68	2.50
Swimming performance	51	152	s	63.62	9.75	197.4

Table 2. Effects of body mass, age, test sequence, and body mass on A) voluntary wheel running, B) home-cage activity, C) open-field exploration (number of squares crossed), D) sprint speed, and E) swimming performance in 51 female white-footed mice (*Peromyscus leucopus*), as estimated in the multivariate mixed model. Shown are the estimates (±se), denominator degrees of freedom (df_{den}), Wald *F*-statistic, and *P* values (bold font indicates $P < 0.05$, see table 1 for sample size, Fig. 2 for variance estimates, and table 3 for correlations obtained from the same model). An additional covariate was fitted to sprint speed (trial number) and swimming endurance (weight attached to the tail).

Trait	Source	Estimate	±	se	df_{den}	*F*	*P*
A) Voluntary wheel running							
	Intercept	-0.061	±	0.203			
	Age	0.025	±	0.137	97.9	0.03	0.8552
	Body mass	-0.072	±	0.082	138.0	0.77	0.3804
	Test sequence	0.057	±	0.101	107.8	0.32	0.5751
B) Home-cage activity							
	Intercept	-0.068	±	0.168			
	Age	-0.053	±	0.099	130.6	0.28	0.5963
	Body mass	0.104	±	0.060	126.0	3.05	0.0834
	Test sequence	0.028	±	0.072	126.5	0.15	0.6974
C) Open-field exploration							
	Intercept	0.008	±	0.259			
	Age	0.179	±	0.176	69.6	1.03	0.3128
	Body mass	0.102	±	0.095	85.5	1.16	0.2855
	Test sequence	**-0.371**	**±**	**0.136**	**93.7**	**7.47**	**0.0075**
D) Sprint speed							
	Intercept	-0.056	±	0.132			
	Age	-0.109	±	0.152	65.9	0.51	0.4765
	Body mass	0.015	±	0.087	99.1	0.03	0.8599
	Test sequence	**0.438**	**±**	**0.131**	**99.6**	**11.08**	**0.0012**
	Trial	-0.049	±	0.075	156.2	0.42	0.5166
E) Swimming performance							
	Intercept	-0.068	±	0.166			
	Age	0.159	±	0.164	41.5	0.95	0.3365
	Body mass	0.276	±	0.161	98.8	2.95	0.0888
	Test sequence	**-0.175**	**±**	**0.079**	**125.0**	**4.88**	**0.0289**
	Weight	**-0.463**	**±**	**0.153**	**128.8**	**9.17**	**0.0030**

Table 3. Among-individual correlations (r_{ind}) and within-individual correlations (r_e) between voluntary wheel running (distance travelled on the wheel), home-cage activity (distance travelled in the cage), open-field exploration (number of squares crossed), sprint speed (maximum sprint speed along the racetrack), and swimming performance (time spent afloat) in 51 female white-footed mice (*Peromyscus leucopus*). All estimates were extracted from a single multivariate mixed model. Profile likelihoods were used to calculate the 95% confidence interval (CI) of r_{ind} and r_e (bold font denotes 95% CI that do not overlap 0).

		Among-individual correlations					Residual correlations				
					95% CI					95% CI	
Trait 1	Trait 2	r_{ind}	±	se	lower	upper	r_e	±	se	lower	upper
Home-cage activity	Voluntary wheel running	0.177	±	0.148	-0.039	0.378	**0.231**	**±**	**0.114**	**0.023**	**0.416**
Open-field exploration	Voluntary wheel running	0.033	±	0.182	-0.219	0.282	-0.125	±	0.102	-0.264	0.019
Open-field exploration	Home-cage activity	0.069	±	0.172	-0.170	0.299	0.020	±	0.104	-0.124	0.163
Sprint speed	Voluntary wheel running	**-0.522**	**±**	**0.148**	**-0.707**	**-0.296**	**0.337**	**±**	**0.093**	**0.201**	**0.459**
Sprint speed	Home-cage activity	**-0.379**	**±**	**0.152**	**-0.570**	**-0.155**	0.047	±	0.104	-0.097	0.189
Sprint speed	Open-field exploration	-0.267	±	0.197	-0.523	0.021	-0.056	±	0.102	-0.196	0.085
Swimming performance	Voluntary wheel running	**-0.372**	**±**	**0.176**	**-0.595**	**-0.109**					
Swimming performance	Home-cage activity	-0.153	±	0.184	-0.395	0.105					
Swimming performance	Open-field exploration	-0.155	±	0.219	-0.446	0.155					
Swimming performance	Sprint speed	**0.517**	**±**	**0.188**	**0.226**	**0.752**					

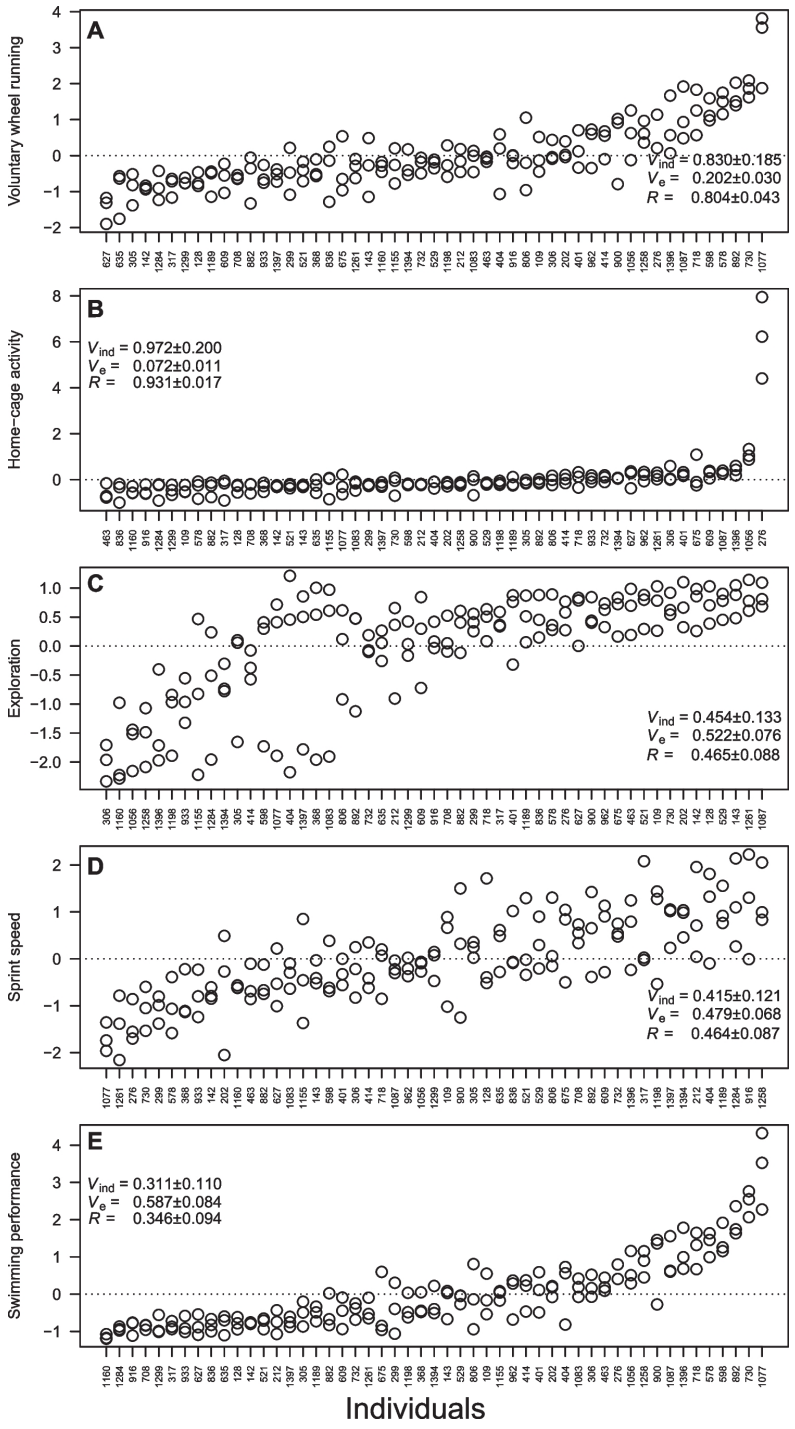

Figure 1. Among-individual variance (V_{ind}±se), residual variance (V_e±se), and repeatability (R±se) in A voluntary wheel running, B home-cage activity, C open-field exploration, D sprint speed, and E swimming performance in 51 female white-footed mice (*Peromyscus leucopus*). Shown are residuals from separate linear models on the raw scale that included body mass, age, test sequence, cage, trial, and body mass. In each panel, individuals are ordered along the x-axis according to their mean trait values. Variance estimates were extracted from linear mixed models on z-standardised traits (total variance = 1).

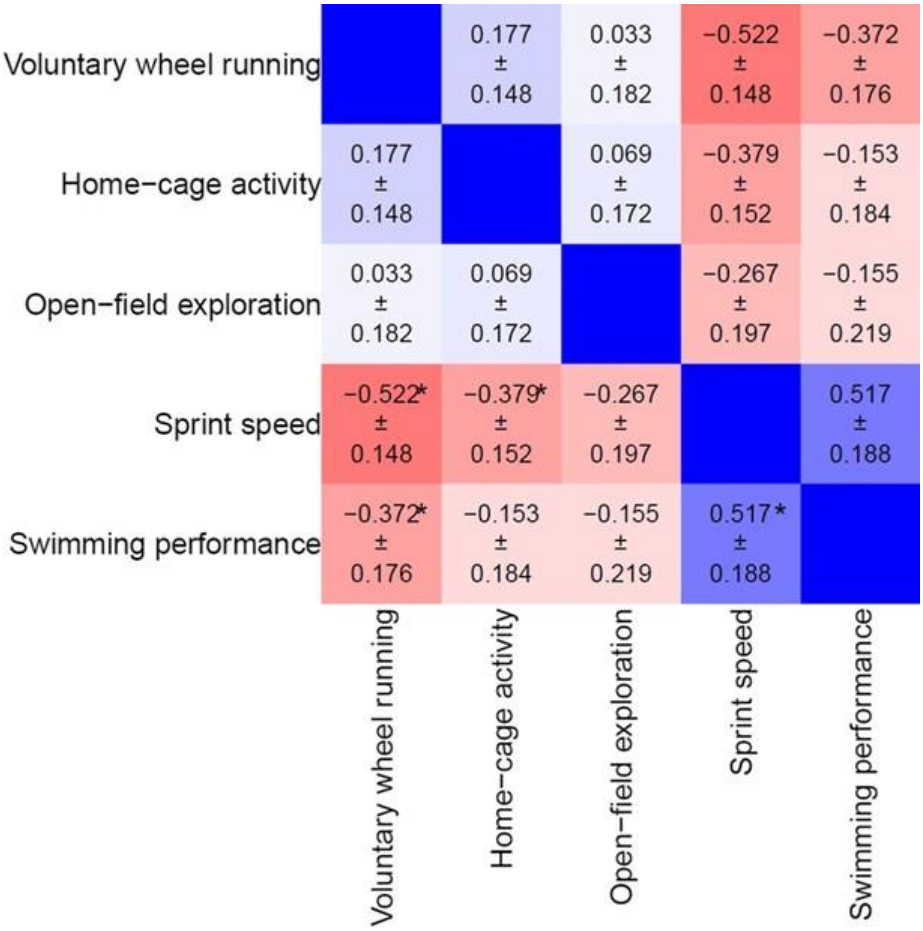

Figure 2. Heatmap showing the among-individual correlations ± se between voluntary wheel running, home-cage activity, open-field exploration, sprint speed, and swimming performance in 51 female white-footed mice (*Peromyscus leucopus*) (negative/positive correlations are in red/blue, with intensity proportional to the estimate, asterisks indicate significant correlations, see table 3).

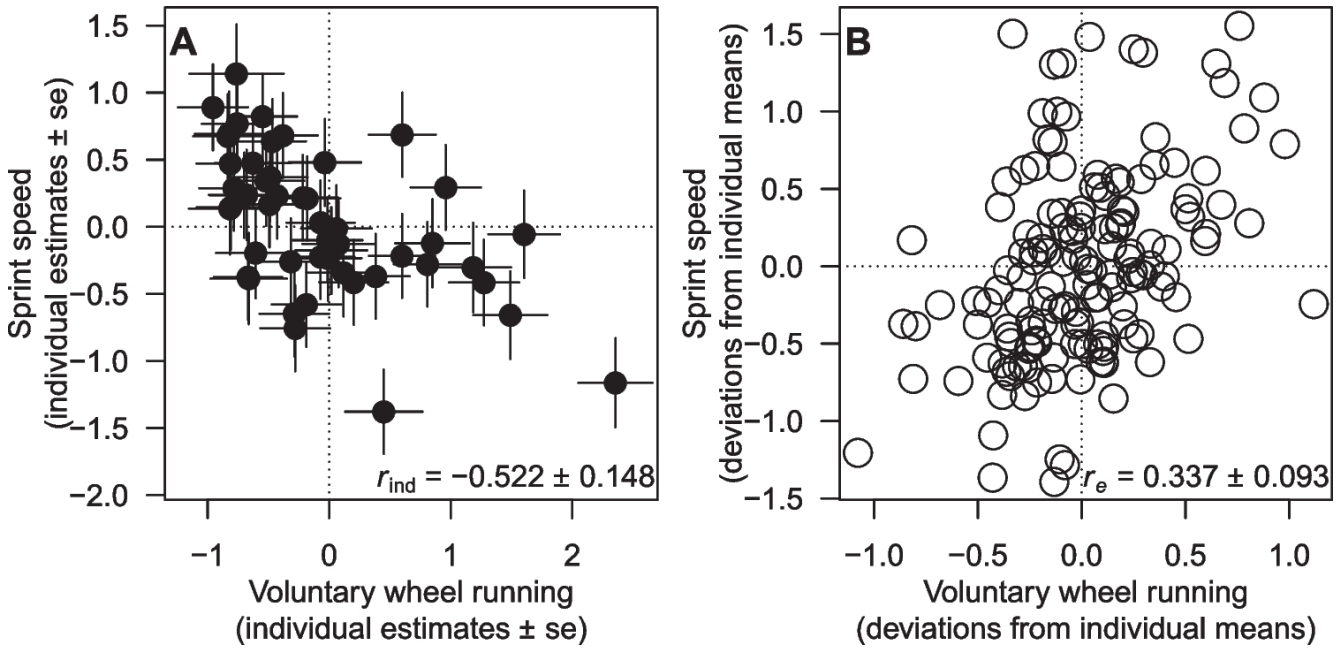

Figure 3. Representation of (A) the among-individual correlation (r_{ind}) and (B) the within-individual correlation (r_e) and between sprint speed and voluntary wheel running in 51 female white-footed mice (*Peromyscus leucopus*). Displayed are the best linear unbiased predictors (individual estimates ± se) and residuals extracted from the multivariate model (see table 3 for correlation estimates) with z-standardised traits (mean = 0, variance = 1).

Discussion

Using a repeated, paired-measures design and a (co)variance partitioning approach, we found three significant negative r_{ind} between performance traits and behavioural traits; swimming performance was negatively correlated to voluntary wheel running, sprint speed was negatively correlated to home-cage activity, and sprint speed was negatively correlated to voluntary wheel running. Altogether, our study combined with other studies in rodents suggest performance and behaviour are co-specialised. However, sprint speed and voluntary wheel running were also positively correlated at the within-individual level, which suggests plastic within-individual changes in performance and behaviour follow a compensatory relationship and illustrates the importance of (co)variance partitioning when studying links between complex traits.

Behaviour

The repeatability of our behavioural measurements all exceeded the average repeatability of behaviours, with exploration being closer to the average at R = 0.47 (average repeatability of behavioural traits: R = 0.37) (Bell et al. 2009), and our measures of activity being noticeably higher (R= 0.80 for wheel running activity and R = 0.93 for cage activity). Exploration was also not correlated with home-cage activity or voluntary wheel running. This is in accordance with previous findings on the links between traits measured in novel environments and other locomotor traits. Because behaviours measured in novel environments capture aspects of anxiety and reactions to novelty (Perals et al. 2017), activity in the open-field test is not representative of general locomotion due to its stressful nature (Stanford 2007), and should not be considered a measurement of activity per se (Walsh and Cummins 1975; Careau et al. 2012).

Although voluntary wheel running has sometimes been found to be linked to other activity traits (Mather 1981), consensus seems to emerge that voluntary wheel running and general activity are not the same (Sherwin 1998; Garland et al. 2011; Novak et al. 2012; Copes et al. 2015). Based on

detailed behavioural observations of lines of mice artificially selected for high voluntary wheel running, and finding that only a few (out of 27) behaviours differed between selected and control lines, Koteja et al. (1999) concluded that voluntary wheel running "is, at the genetic level, a largely independent axis of behaviour". Our results are consistent with this, in that there was no relationship between voluntary wheel running and home-cage activity at the among-individual level. In fact, when home-cage activity and voluntary wheel running are simultaneously recorded over an extended amount of time (6 to 10 days), they are often negatively correlated because access to a running wheel competes with time spent on other locomotor activities (Koteja et al. 1999; De Visser et al. 2005), even if access to a wheel increases total physical activity (Copes et al. 2015). In our study, however, we found that home-cage activity and voluntary wheel running were weakly but significantly positively correlated at the within-individual level (r_e ± SE = 0.23 ± 0.11, table 3). The underlying causes of this within-individual relationship remains to be determined, but since our study was conducted on females, variation in oestrus cycle might be at play (Abdeen et al. 2022). The oestrus cycle is known to have an effect on voluntary wheel running in rodents, including *Peromyscus leucopus* (Cushing and Cawthorn 1996; Basterfield et al. 2009), which could also generate within-individual variation with home-cage activity, therefore explaining the observed r_e.

Performance

Our measured sprint speed ranged from 0.62 to 2.5 m s−1 for an average of 1.58 m s−1 which is almost 40% below the sprint speed recorded on similar-sized rodents (Djawdan and Garland 1988) and 23% lower than for wild *Peromyscus leucopus* (average of 2.06 m s−1) (Berberi and Careau 2019). Even if both methods to measure sprint speed were very similar, our study only includes females, while the study of Berberi and Careau (2019) had both males and females (note, however, that no sex difference was detected in Berberi and Careau (2019)). It is also possible that the length of the racetrack influenced the speed measured if two meters were not enough for the mice to reach their

maximum speed. Nevertheless, our protocol detected significant individual differences in sprint speed with a repeatability of R = 0.46. Whereas sprint speed was found to be less repeatable than in other taxa such as lizards (Huey and Dunham 1987; Garland et al. 1990b), repeatability of sprint speed in our females in the lab was higher than in a wild population of *Peromyscus leucopus* (Berberi and Careau 2019).

Although swimming tests are widely used in order to study the effect of compounds on endurance (Ikeuchi et al. 2009; Yang et al. 2016) or depression-like behaviours (Porsolt et al. 2001; Strekalova et al. 2005), individual differences are rarely the focus and swimming tests are rarely repeated on the same individuals (but see Friedman et al. 1992). In our study, we found that swimming performance was repeatable at R = 0.35 and that that faster sprinters were able to stay afloat for longer during the swim test. To our knowledge, only two other studies, both in house mice, quantified the relationship between sprint speed and swimming performance, with one study reporting no phenotypic correlation (Friedman et al. 1992) and another reporting a negative genetic correlation (Dohm et al. 1996). Although these contrasting results might be due to correlations being quantified at different levels, we believe the most likely explanation is the difference in the swim test protocols. Friedman et al. (1992) and Dohm et al. (1996) used weights that represented 3–4% the body mass of the mice, and as a result female mice swam for a relatively long period (in Friedman et al. 1992, average of 5.6 min in second trial; in Dohm et al. 1996, average of 11.4 min in second trial). We used weights of ~ 10% the body mass, which resulted in much shorter swim tests (average ~ 1 min). Therefore, the relatively long swimming protocols used in Friedman et al. (1992) and Dohm et al. (1996) must have involved aspects of endurance, which is expected to trade-off against sprint speed (Wilson and James 2004), whereas our protocol triggered a short and intense swimming effort and therefore it is perhaps not surprising that it is positively correlated with sprint speed. On the one hand, the positive r_{ind} between sprint speed and swimming performance indicates that the two measures reflected general locomotory ability. On the other hand, the correlation was not perfect, and the moderate effect size (r_{ind} = 0.52) indicates that a large part of the variation swimming

performance is independent from variation in sprint speed. Part of this independent variation might occur because sprint speed involves a reaction to a human threat whereas swimming performance relies on the animal survival instinct.

Co-specialization of performance and behaviour

Our study included measures of behaviour recorded over a 24-h period (activity in the home cage and distance run on the wheel), which are presumably less influenced by the animal's stress response than behaviour recorded in a 5-min open-field test (see above). Nevertheless, all six r_{ind} between the performance and behavioural measures were negative. In particular, voluntary wheel running was significantly and negatively correlated with both sprint speed and swimming performance (table 2). Hence, co-specialization seems to occur even when performance is measured in a way that does not involve escape from humans, and when behaviour is measured in a relatively low-stress context.

Although our results suggest co-specialization irrespective of the trait measured, we observed some differences in effect size across trait combinations and the underlying mechanism remains unknown. In a laboratory study (such as ours), co-specialization cannot obviously arise from experience with predators. One remaining possibility, then, would be individual variation in overall risk sensitivity, which could be effective through sensory ability. Moreover, we cannot exclude stress effects entirely, because mice were isolated for an extended period (24 h) during activity measurements and they were subjected to a life-threatening situation during swimming performance measurements. Future analyses of blood samples will indicate which of the traits included in this study are most strongly correlated with stress hormones. In any case, mechanistic studies are most needed to prevent "oversteering" and "drifting" during the development of "the fast and the furious" framework.

Compensation of performance and behaviour

At the within-individual (residual) level, we found that sprint speed and voluntary wheel running were positively correlated (r_e ± SE = 0.337 ± 0.093, table 3), which is the opposite than observed at the among-individual level. Berberi and Careau (2019) found that failure to properly consider the hierarchical nature of performance data can lead to counterintuitive and biased conclusions [see also (Careau and Wilson 2017b)]. The contrasting relationships we found that the among- vs within-individual levels (Fig. 3) should encourage further research to adequately partition the (co)variance when studying locomotor performance and behaviour.

A r_e represents an association between deviations from individual averages observed within each individual across testing days. In our study, this signifies that individuals that were more active than usual on the wheel also ran faster than usual during the sprint speed test on the following day. This raises the interesting possibility that, although individual differences in risk sensitivity generates co-specialization between performance and behaviour (see above), within-individual plastic changes in performance and behaviour follow a compensatory relationship. This is similar to a compensatory mechanism observed in ectotherms predicted by the optimal escape theory (Ydenberg and Dill 1986) where, as temperature decreases, locomotor capacity decreases, but individuals increase their flight initiation distance presumably to compensate for lower escape speeds (Rand 1964; Rocha and Bergallo 1990). In endotherms, however, performance can be maintained across a much wider range of temperature (Seebacher and Little 2017); therefore, other factors must be responsible for the within-individual compensatory relationship between performance and behaviour.

Conclusions

Many studies have recently documented the relationship between locomotor performance and behaviour, yet these only represent the first step towards a full understanding of their correlated evolution within an integrated phenotype. One might expect differences in compensation vs co-specialization to occur according to the ecology and life history of the studied species. For example, most studies conducted so far were on prey species (marmots, chipmunks, mice, zebrafish, and small lizards) that are vulnerable to predators. To continue learning on the general conditions under which performance and behaviour might co-evolve, we need studies on less vulnerable prey species (e.g. those that have evolved efficient defenses such as armors and chemical defences) and in predator species. Studies in the wild are also needed to map the adaptive landscape of performance and behaviour. In particular, fitness measurements are required to test whether trait compensation may arise from correlational selection.

Chapter 4: Blood corticosterone and motivation during a performance test

Preamble

One of the major challenges that comes with studying performance traits and their relationships with other labile traits, is that measurements can be influenced by the motivation of the animals. Because of the complex nature of the motivational circuits of the brain, and the long list of potential systems that might influence it, measuring motivation seems an impossible task. Yet, it may be possible to control for among- and within-individual variation in stress response to the stimuli involved in the performance test.

This fourth chapter is an attempt at using blood corticosterone to estimate stress response to the sprint speed test, by measuring baseline blood corticosterone and elevated blood corticosterone immediately following the performance test. By measuring this glucocorticoid hormone repeatedly, I was able to study its among- and intra-individual variation and more importantly, look at the *elevation* of the hormone concentration by conditioning the correlations on the individual baseline measurements. This allowed me to highlight how individuals varied in their stress response and illustrates how informative it can be to include physiological measurements to studies on performance and behaviour.

Introduction

Animals must perform various tasks to survive and reproduce, with activities ranging from gathering food, finding or building a shelter, escaping predators, raising offspring, all of which are vital and necessary. Out of all the potential tasks an animal must accomplish in its daily life, locomotion is almost always involved and as a result, an animal's locomotor performance represents one of the

many traits selection can target (Arnold 1983; Jayne and Bennett 1990; Garland and Losos 1994; Irschick 2003; but see Careau and Garland 2012). Because natural selection might also act in discrete and intermittent events, rather than as chronic conditions (Vermeij 2023), a multitude of relevant locomotor performance traits, which animals rely on to face those events, can be of interest. One of such traits, perhaps the most commonly studied performance traits in terrestrial animals, is maximal sprint speed (also sometimes described as "escape speed") (Huey and Hertz 1982; Berberi and Careau 2019; Albuquerque et al. 2023) because of its importance in a context of predator escape or during prey capture. Sprint speed also has the advantage of being relatively easy to measure compared to other performance traits such as endurance, which requires more time, material, recovery, and more intense methods of prompting motivation (e.g., electric shock grids).

When measuring performance, one of the main challenges is to ensure that animals have attained their maximum. Unfortunately, animals might vary in their motivation to perform (Losos et al. 2002), which might result in variation in the performance measurements. Even more unfortunately, directly quantifying motivation seems challenging because of the complex and multidimensional nature of the brain chemistry and physiology involved. In response to those challenges, and to ensure that maximal performance is attained, multiple techniques have been used. Examples of these are, the use of gentle electric shocks to ensure that animals keep running on treadmills (Koch et al. 1998; Knab et al. 2009; Casanova-Vallve et al. 2022), or observers chasing the animals along racetracks or repeatedly stimulating them during a test (Hertz et al. 1983; Friedman et al. 1992; Blumstein et al. 2010). Ideally, these types of measurements should be accompanied by an evaluation of the quality of the motivational response by the animal to the test protocol, often using gate and stride to evaluate if animals ran to their fullest. Unfortunately, this type of control remains often specific to the species studied and the experimental protocol used to measure performance. Additionally, it is easy to suspect that the experience researchers have working with their study model would ultimately influence how strict the rules used become to separate trials in which maximum performance is attained versus sub-maximal.

Another useful way of estimating the extent to which individuals are performing during a test is through their behaviour and physiology. For example, during endurance measurements, while signs of exertion can be directly visible (fast breathing, collapsing, etc.) it is also possible to confirm that individuals have reached exertion by measuring physiological markers such as lactate levels in the blood (i.e. lactic acid) (Hill et al. 1924; Katz and Sahlin 1988; Cairns 2006). High lactic acid in the blood is the result of glucose breakdown during anaerobic activity, and is directly related to the effort, with high levels signalling that animals have been engaging in intense sustained exercise. Blood lactate level is also one of the most commonly measured parameters in humans, especially for athletes who can achieve higher blood concentration than most other people. Lactate levels can also be used to indicate exhaustion in short and extremely intense exercise (30 to 120 seconds), but the lactate peak is attained from 3 to 8 minutes following the intense effort (Goodwin et al. 2007). Most sprint speed measurements in animals, however, are made over a much shorter time period (in seconds), such that blood lactate levels cannot be used to evaluate motivation/exhaustion.

The problem remains: relative to their own true maximal performance, animals might not perform to the same extent when subjected to a test, which should generate variation in performance measurements not only in intensity of motor output (e.g., speed), but also in the general behavioural reaction to the stressor. For example, when measuring crawling speed in recently born garter snakes by chasing them around a rectangular track, Arnold and Bennett (1984) "discovered that they would spontaneously terminate such trials by adopting characteristic antipredator displays", which was scored on a defensive-offensive scale fine enough to detect significant individual differences. More recently, Fiedler and Careau (2021) found repeatable individual differences in the motivation to run to exhaustion in wild mice. It is interesting to see how, over the past three decades, consistent among-individual differences in stress physiology and behaviour, also known as coping styles, have been extensively illustrated within the context of research on performance. Classically, coping styles are defined along a continuum, ranging from reactive individuals to proactive individuals (Koolhaas et al. 1999). Reactive individuals are defined as

being shyer, taking less risk, and having higher physiological stress responses, while proactive individuals are bolder, take more risks, and have lower physiological stress responses. In a similar manner that consistent among-individual variation in stress response and behaviour has been described, it is possible to expect that individuals might not perceive or react to the same extent to the stressor used during a performance test, ultimately influencing the measurement of performance.

One of the most used metrics to quantify stress in rodents is corticosterone. This glucocorticoid hormone, released following the activation of the hypothalamic-pituitary-adrenal (HPA) axis, plays an important role in the physiology of both stress and exercise. In mice, corticosterone increases with the duration of a physical effort and facilitates the use of energy stores, by stimulating gluconeogenesis and enhancing mobilization of fat stores (Coleman et al. 1998). Mice selected for higher voluntary exercise tend to display higher post-exercise corticosterone serum concentrations than their control lines, suggesting a correlated change in the HPA axis as a response to selection. [note, however, that those changes occur at various levels within the HPA axis, seemingly underlying differential responses to either physical or emotional changes] (Girard and Garland 2002). Interestingly, while corticosterone is also released following acute stress, its circulating levels do not seem to be influenced by the amount of chronic voluntary exercise animals engage in on a daily basis (Girard and Garland 2002). Perhaps more importantly, the manipulation of the corticosterone receptors also results in the alteration of stress induced locomotor activation (Boyle et al. 2006), illustrating the central role of corticosterone in the regulation of physiological and behavioural stress reactivity.

Considering the importance of corticosterone in the physiology of stress, and because performance measurements rely on several different stressors, measuring corticosterone elevation during a performance test seems a promising way to (statistically) control for among- and within-individual differences in the intensity of the motivational response. If sprint speed and corticosterone elevations are not correlated, then one may infer that variability in the stress response did not have a

direct effect on the locomotor performance measurement, which, by definition, should reflect animals being motivated to (or all equally close to) their maximum. If sprint speed and corticosterone elevation are correlated, however, this would indicate a potential bias in the sprint speed measurement. Given that some of the variation in sprint speed would be caused by variation in the stress response of the animals, conditioning performance measurements on corticosterone levels would be the statistical equivalent of comparing individuals that are all equally motivated.

Here, I repeatedly measured behaviour (exploration in an open field), performance (sprint speed) and post-exercise serum corticosterone as well as baseline corticosterone in 51 female *Peromyscus leucopus*. I use a multivariate approach to estimate the among-individual and residual correlations between behaviour, performance, and corticosterone elevation. I do so with and without accounting for baseline corticosterone, to evaluate whether the relevant aspect/level/dimension of the HPA axis is the absolute level of corticosterone during performance measurements versus the relative increase in corticosterone from baseline condition.

Material and methods

The global experiment overview can be found in Agnani and Careau (2023) (chapter 3). Described below are the methods used to sample blood and obtain the corticosterone concentrations, and the statistical analyses used.

Sprint speed test and blood sampling

Blood was drawn from the saphenous vein three times for each mouse throughout the experiment. The first blood drawing occurred prior to any other measurements on the animals and within 3 mins of handling to assess a baseline blood corticosterone concentration. Blood was then drawn two more times immediately following the performance measurement (sprint speed test), right after, mice were chased along the racetrack (but after 3 minutes to allow for blood corticosterone elevation). This

yielded a baseline measurement and two repeated post-exercise measurements per individuals for a total of 29 baseline measurements and 100 elevated measurements (24 measurements were unsuccessful due to either mice not bleeding, or the blood being obtained after the 3 minutes mark, see table 1).

Corticosterone assays

After being drawn, blood was allowed to clot at room temperature and centrifugated to separate the serum. Serum was stored at -80°C prior to analyses. Serum corticosterone concentrations were obtained using I-125 RIA kits from MP Biomedicals (Solon, OH) with the serum diluted 1:500 because the higher corticosterone concentrations of *P. leucopus* compared to house mice (Pyter et al. 2005; Martin et al. 2006, 2007). The inter-assay coefficient of variability was 8.01% and the intra-assay coefficient of variability was 6.13%.

Statistical analysis

Statistical analyses were done using ASReml-R version 4 (Butler et al. 2018). All continuous traits were standardised to a mean of 0 and a variance of 1. I ran three separate univariate models that included exploration, sprint speed, and post-exercise blood corticosterone concentration as response variables. Body mass (mean = 18.9 g, range 13.3 to 29.9 g), age (mean = 365 days, range 264 to 426 days), and test sequence (1 to 3) were fitted as fixed effects to all traits. An additional fixed effect of "trial number" was fitted to sprint speed to account for potential differences between successive trials within a test. For each model, individual identity was fitted as a random effect to partition the phenotypic variance (not explained by fixed effects) into among-individual variance (V_{ind}) and within-individual variance (V_e; residuals). The adjusted repeatability of each trait was calculated as the ratio $R = V_{ind}/(V_{ind} + V_e)$. For the post-exercise corticosterone measurements, additional calculations of short term and long term repeatabilities were obtained by using each individual replicated tubeduring

the RIA (V_{test}) and by partitioning the variance at each hierarchical level as follow: $R_{short} = (V_{ind} + V_{test}) / (V_{ind} + V_{test} + V_e)$ and $R_{long} = V_{ind} / (V_{ind} + V_{test} + V_e)$. The approximate standard errors (se) for R were calculated using the delta method using the vpredict function in ASReml-R (Wolak 2012; Butler et al. 2018). In a second step, I ran a 4-traits multivariate model to quantify the correlations between exploration, sprint speed, post-exercise corticosterone concentration and baseline corticosterone (as measured at the beginning of the experiment prior to any other measurements, see table 1 for descriptive statistics). The goal of this model was to quantify the among- and within-individual correlations between exploration, sprint speed, and post-exercise corticosterone without conditioning on baseline corticosterone. Moreover, by including baseline corticosterone as a response variable in the model, and fixing its residual variances to zero (one baseline measurement was obtained per individual), it was possible to estimate the phenotypic correlation (r_P) between baseline corticosterone and the other traits.

Finally, a 3-traits multivariate model with the random effect of individual identity was fitted as a 3 × 3 unstructured correlation matrix to quantify the among-individual correlations (r_{ind}) and residual correlations (r_e) between exploration, sprint speed, and post-exercise corticosterone concentrations. This model included all of the fixed effects used for the univariate models with the addition of baseline corticosterone concentration (mean= 326 ng/mL, range 36.2 to 1098.2) in order to condition the elevated corticosterone measurements accordingly. Including baseline corticosterone concentration as a fixed effect is equivalent to modelling the change in corticosterone as a response variable. Residuals were modelled as an unstructured correlation matrix to quantify the within-individual correlations (r_e) between exploration, sprint speed, and post-exercise corticosterone. The 95% confidence intervals for both multivariate model's r_{ind} and r_e were computed using profile likelihoods with the proLik function in nadiv (Wolak 2012). Best linear unbiased predictors (BLUPs) and residuals were extracted from both multivariate mixed models to obtain a graphic representation of the r_{ind} between traits.

Results

I repeatedly measured behaviour, locomotor performance and serum corticosterone in 51 females. Behaviour and performance were measured three times, for a total of 153 tests of open-field exploration and sprint speed. Baseline corticosterone was measured for 29 individuals (the 22 missing measurements being cases in which mice did not bleed, or did not bleed within the 3 minutes). Post-exercise corticosterone was measured two times per individuals for a total of 100 measures (see table 1 for descriptive statistics).

Effect of body mass, age, test sequence, and trial

Body mass had no statistically significant effect on any of the measured traits (table 2). Age had a small yet significant on post-exercise corticosterone. I found a negative effect of test sequence on exploration and on post-exercise corticosterone, indicating a lower stress response during successive tests. Test sequence also had a positive effect on sprint speed, with individuals reaching slightly higher speeds with each consecutive tests (figure 1). Finally, trial had no effect on sprint speed.

Repeatability

All traits measured during the experiment were significantly repeatable, with estimates ranging from $R = 0.663 \pm 0.097$ for post-exercise corticosterone levels to $R = 0.454 \pm 0.098$ for exploration (table 3). When looking at each individual duplicated samples used during the RIA assay, I was also able to calculate short-term and long-term repeatability of post-exercise corticosterone and found $R_{short} = 0.742 \pm 0.054$ and $R_{long} = 0.480 \pm 0.098$.

Correlations

Model without conditioning

Without conditioning on baseline corticosterone, I found a positive among-individual correlation between post-exercise corticosterone and sprint speed (r_{ind}= 0.399, *LCL*= 0.126, *UCL*= 0.634; table 4), indicating that faster sprinters exhibited the highest corticosterone serum elevation following the sprint speed test. I also found a negative among-individual correlation between post-exercise corticosterone and exploration in the open field (r_{ind}= -0.290, *LCL*= -0.537, *UCL*= -0.015), indicating that individuals who exhibited higher post-exercise corticosterone covered less area in the open field. Finally, baseline corticosterone and post-exercise corticosterone were positively correlated (r_P= 0.488, *LCL*= 0.244, *UCL*= 0.676), with the individuals with higher baseline levels also having the highest post-exercise levels (see figure 2).

Model with conditioning

Both the r_{ind} between exploration and sprint speed, and between post-exercise corticosterone and exploration were negative but nonsignificant (table 5). Similarly to the non-conditioned model, post-exercise corticosterone was positively correlated with sprint speed at the among individual level (r_{ind}= 0.366, *LCL*= 0.082, *UCL*= 0.614) (see figure 2). None of the residual correlations were statistically significant (table 5).

Tables and Figures

Table 1. Descriptive statistics for open-field exploration, sprint speed, post-exercise corticosterone concentration, baseline corticosterone concentration, age, and body mass in female white-footed mice (*Peromyscus leucopus*), including number of individuals sampled (N_{ID}), total number ofobservations (n_{obs}), units, mean, and range (minimum and maximum).

	N_{ID}	n_{obs}	Units	Mean	Min	Max
Open-field exploration	51	153	# squares	73.97	1	100
Sprint speed	51	153	m/s	1.58	0.68	2.50
Postexercise corticosterone	51	100	ng/mL	1297.24	518.78	2264.84
Baseline Corticosterone	29	29	ng/mL	326.02	36.212	1098.23
Age	51	153	days	365.35	264	426
Body mass	51	153	g	18.99	13.3	29.88

Table 2. Effects of age, body mass, test sequence, and trial on A) open field exploration, B) sprint speed, C) post-exercise corticosterone concentration and D) Baseline corticosterone in 51 female white-footed mice (*Peromyscus leucopus*), as estimated in the non-conditioned multivariate mixed model. Shown are the estimates (± se), denominator degrees of freedom (df_{den}), Wald *F*-statistic, and *P* values (bold font indicates $P < 0.05$, see table 1 for sample size, and table 4 for correlations obtained from the same model).

Trait	Source	Estimate	±	se	df_{den}	*F*	*P*
A) Open-field exploration							
	Intercept	0.221	±	1.084			
	Age	0.001	±	0.004	82.6	0.141	0.708
	Body mass	0.107	±	0.105	103.5	1.042	0.309
	Test sequence	**-0.347**	**±**	**0.141**	**121.6**	**6.066**	**0.015**
B) Sprint speed							
	Intercept	0.127	±	1.333			
	Age	-0.002	±	0.004	59.2	0.233	0.631
	Body mass	0.131	±	0.103	98.3	1.643	0.203
	Test sequence	**0.153**	**±**	**0.052**	**88.3**	**8.794**	**0.004**
	Trial	-0.092	±	0.097	148	0.902	0.343
C) Post-exercise corticosterone							
	Intercept	-3.000	±	1.607			
	Age	**0.010**	**±**	**0.005**	**56.2**	**4.368**	**0.041**
	Body mass	0.058	±	0.123	90.2	0.223	0.638
	Test sequence	**-0.559**	**±**	**0.174**	**88.5**	**10.38**	**0.002**
D) Baseline corticosterone							
	Intercept	-0.009	±	0.175			
	Body mass	0.023	±	0.143	31.5	0.025	0.876

Table 3. Variance components (among individual V_{ind}, residual V_e), adjusted repeatabilities (R), and associated standard errors (se) from the non-conditioned multivariate model on exploration, sprint speed, and post-exercise corticosterone concentrations.

Trait	V_{ind}	±	se	V_e	±	se	R	±	se
Exploration	0.439	±	0.128	0.515	±	0.073	0.460	±	0.086
Sprint speed	0.394	±	0.116	0.484	±	0.069	0.449	±	0.088
Post-exercise cort.	0.689	±	0.187	0.345	±	0.072	0.663	±	0.085
Baseline cort.	0.977	±	0.257	NA	±	NA	NA	±	NA

Table 4. Among-individual correlations (r_{ind}), within-individual correlations (r_e), and phenotypic correlations (*) between open-field exploration (number of squares crossed), sprint speed (maximum sprint speed along the racetrack), post-exercise corticosterone (measured following the sprint speed test), and baseline corticosterone (measured once at the beginning of the experiment) in 51 female white-footed mice (*Peromyscus leucopus*). Profile likelihoods were used to calculate the 95% confidence interval (CI) of r_{ind} and r_e (bold font denotes 95% CI that do not overlap 0).

		Among-individual correlations					Residual correlations				
					95% CI					95% CI	
Trait 1	Trait 2	r_{ind}	±	se	lower	upper	r_e	±	se	lower	upper
Sprint speed	Exploration	-0.272	±	0.197	-0.528	0.016	NA	±	NA	NA	NA
Post-exercise cort.	**Exploration**	**-0.290**	**±**	**0.188**	**-0.537**	**-0.015**	-0.068	±	0.100	-0.205	0.072
Post-exercise cort.	**Sprint speed**	**0.399**	**±**	**0.181**	**0.126**	**0.634**	0.053	±	0.138	-0.140	0.241
Baseline cort.	**Exploration**	**-0.532***	**±**	**0.170**	**-0.734**	**-0.261**	NA	±	NA	NA	NA
Baseline cort.	Sprint speed	0.306*	±	0.214	-0.041	0.589	NA	±	NA	NA	NA
Baseline cort.	**Post-exercise cort.**	**0.488***	**±**	**0.160**	**0.244**	**0.676**	NA	±	NA	NA	NA

Table 5. Among-individual correlations (r_{ind}) and within-individual correlations (r_e) between open-field exploration (number of squares crossed), sprint speed (maximum sprint speed along the racetrack), and post-exercise corticosterone (measured following the sprint speed test) conditioned on baseline corticosterone in 51 female white-footed mice (*Peromyscus leucopus*). Profile likelihoods were used to calculate the 95% confidence interval (CI) of r_{ind} and r_e (bold font denotes 95% CI that do not overlap 0).

		Among-individual correlations					Residual correlations				
					95% CI					95% CI	
Trait 1	Trait 2	r_{ind}	±	se	lower	upper	r_e	±	se	lower	upper
Sprint speed	Exploration	-0.235	±	0.204	-0.501	0.060	-0.064	±	0.100	-0.201	0.076
Post-exercise cort.	Exploration	-0.169	±	0.205	-0.446	0.123	0.051	±	0.138	-0.142	0.239
Post-exercise cort.	**Sprint speed**	**0.367**	**±**	**0.191**	**0.082**	**0.615**	-0.080	±	0.141	-0.273	0.120

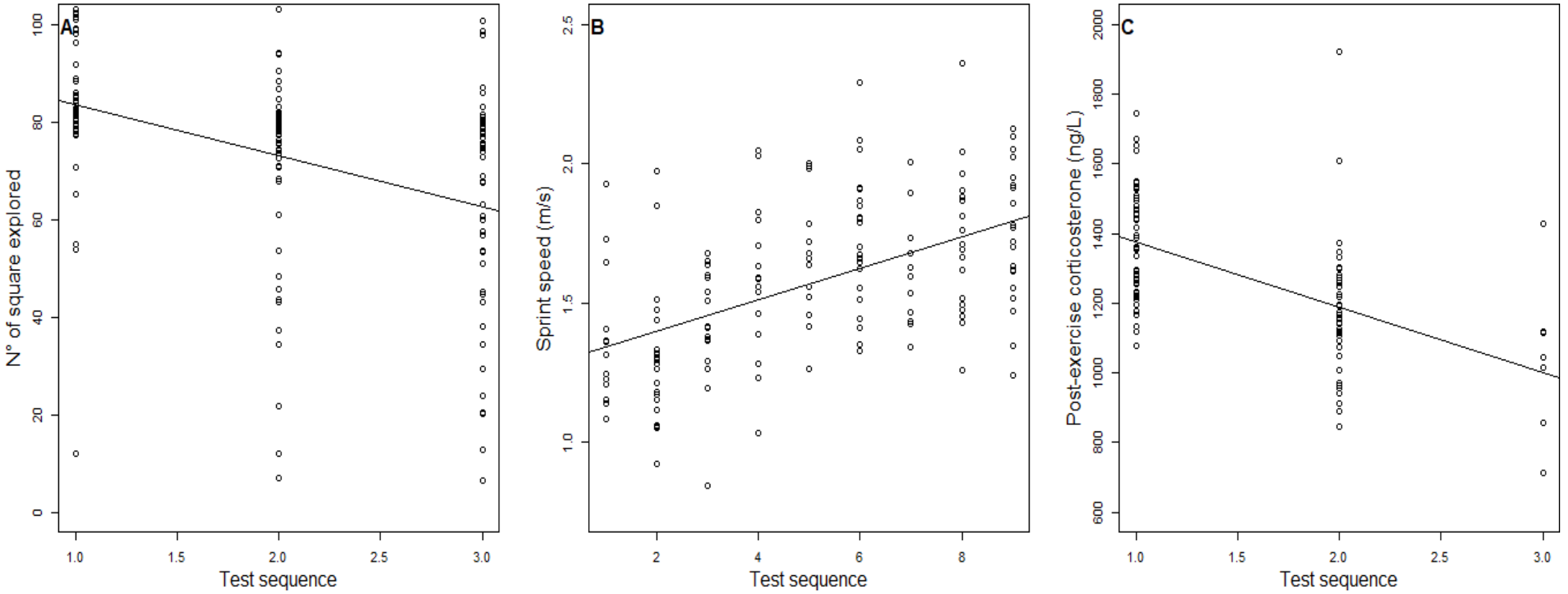

Figure 1. **A** Exploration (number of squares visited), **B** sprint speed (m/s), and **C** post-exercise corticosterone (ng/L) as function of test sequence in 51 female *Peromyscus leucopus*. Shown are partial residuals accounting for other effects as included in the multivariate models.

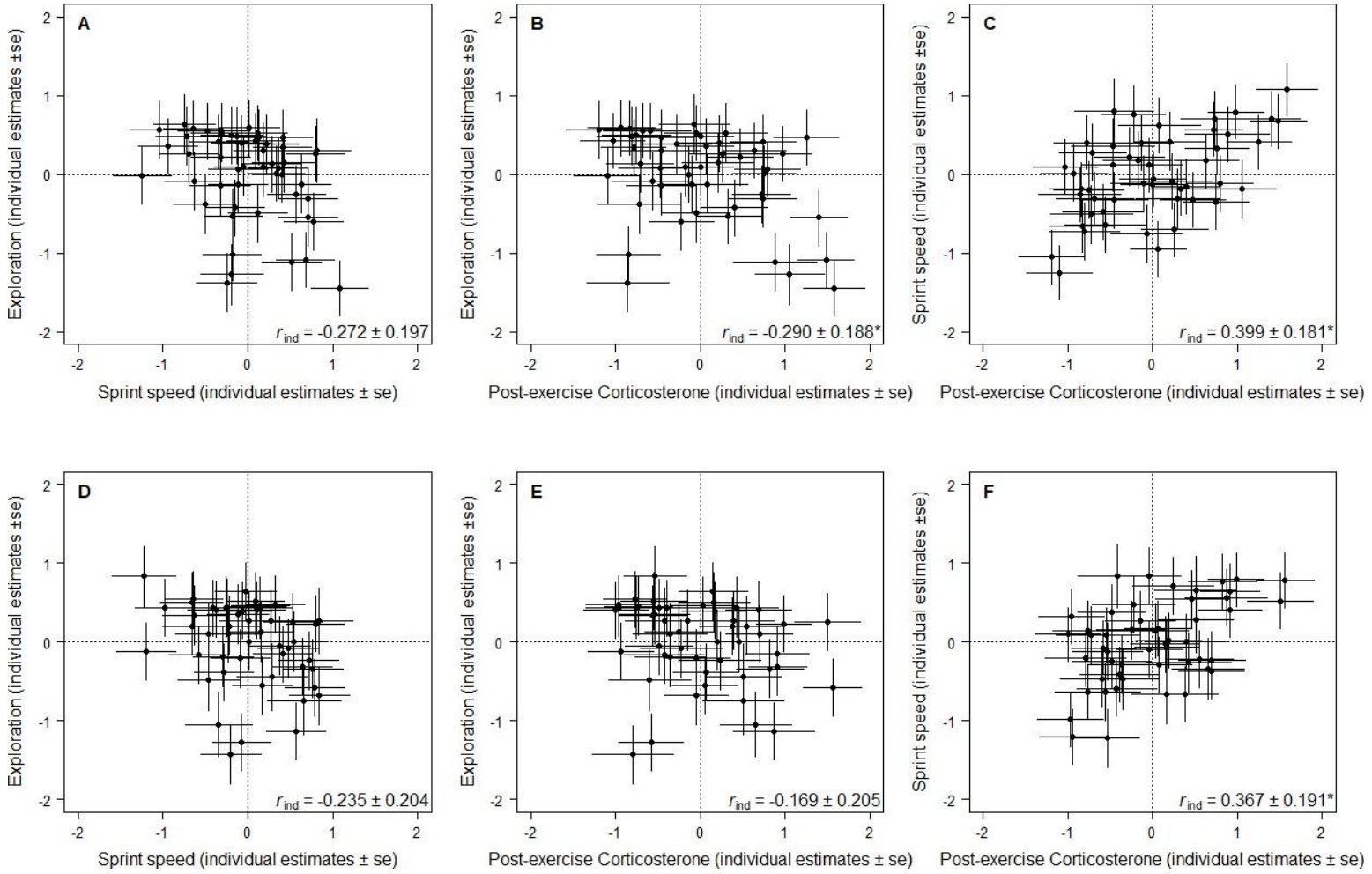

Figure 2. Representation of the among-individual correlation (r_{ind}) between exploration and sprint speed (**A**,**D**), exploration and post-exercise corticosterone (**B**,**E**), and sprint speed and post-exercise corticosterone (**C**,**F**) from the multivariate models not conditioned (top row) and conditioned (bottom row) on baseline corticosterone in 51 female white-footed mice (*Peromyscus leucopus*). Displayed are the best linear unbiased predictors (individual estimates ± se) extracted from both multivariate models (see table 4 and 5 for correlation estimates) with z-standardised traits (mean = 0, variance = 1), *indicate statistically significant estimates.

Discussion

By repeatedly measuring behaviour, performance, and post-exercise corticosterone levels, I found all traits to be repeatable, signifying the presence of consistent among-individual differences in these traits. Using a (co)variance partitioning approach, I found a positive and significant among-individual correlation between sprint speed and post-exercise corticosterone levels (with and without conditioning on baseline corticosterone). This indicates that individuals with the stronger stress response, as expressed by the elevation of corticosterone concentration in their blood, were also faster during the sprint speed test. This finding, paired with the current knowledge about the physiological role of glucocorticoids, indicates that individuals exhibited different stress responses during the performance test, and that part of the variation in locomotor performance measurements is shared with variation in stress reactivity.

Here I investigate the elevation of corticosterone following a sprint speed test which is designed to measure the maximal running speed of an animal. This type of performance test has been used extensively and relies on an observer either stimulating the animal or simply chasing the animal down a racetrack. Speaking to this, close to half (21 out of 45) of the published associations between performance and behaviour reported in the first chapter of this thesis included sprint speed (or escape speed) as their measure of locomotor performance (see chapter 1, table 1). Yet only rarely are these performance measurements accompanied by physiological measures of stress (but see Coleman et al. 1998; Dlugosz et al. 2012; Albuquerque et al. 2023). Following exercise and stress, elevation of blood corticosterone concentration is expected, and has been investigated in a variety of contexts (Borer et al. 1992; Stupnicki and Obminski 1992; Petrides et al. 1994; Herman et al. 2012; Cockrem 2013). Previous work on mice indicated that selection for high voluntary wheel running results in a two-fold difference in baseline corticosterone concentrations compared to control lines (Girard and Garland 2002). This difference between selected and control lines possibly suggests an adaptation to support higher physical activity since corticosterone is known to enhance the mobilization of energy reserves (Wasserman and

Halseth 1998). Interestingly, selected and control lines show similar levels of elevated corticosterone following a restraint test, in fact indicating a lower stress response (i.e., the difference between baseline and post restraint concentrations) from the high runners (Malisch et al. 2007). In Sprague-Dawley rats, exercised and sedentary groups also display similar corticosterone concentrations following a restraint stress, and exercised groups display higher baseline corticosterone levels only for the first weeks of access to their wheel, which decreases back to similar levels recorded in the sedentary groups after week five (Fediuc et al. 2006). These results suggest that baseline and post stress corticosterone concentrations are labile traits, and that baseline levels show both plastic responses to increase in locomotor activity as well as potential correlated responses to selection on locomotor activity.

In my experiment, mice showed a four-fold elevation in corticosterone concentration between baseline and post-exercise (see table 1). This high elevation represents a response to a severe stress (Hennessy 1997) potentially resulting from the carry-over effect of the open-field test in addition to the sprint speed test itself. Interestingly, baseline corticosterone was negatively correlated with exploration at the phenotypic level (table 4). This suggests that individuals displaying higher baseline levels of corticosterone were covering less area during the open-field test, and individuals with lower corticosterone levels were more explorative. This relationship corresponds to the reactive- to-proactive continuum and has been illustrated in previous research on coping styles (Koolhaas et al. 2010), but should nonetheless be interpreted carefully as it relies on non-repeated baseline measurements (Dingemanse and Dochtermann 2013; Trafimow 2016; Careau and Wilson 2017b). Post-exercise corticosterone concentration was also negatively correlated with exploration at the among-individual level (table 5), but interpretation of this result is dangerous, for both methodological and statistical reasons. First, post-exercise corticosterone concentration was measured directly following the sprint speed test, which was itself following the open-field test, meaning that more time had elapsed between the open-field test and the blood sampling, than between sprint speed and blood sampling. Additionally, the sprint speed test should arguably result in higher corticosterone release than the open-field test due to their different stressor intensity (new environment, as opposed to new environment plus being

chased by a yelling human tapping your tail), therefore rendering the detection of the smaller effect of the open-field test less likely, as compared to the effect of the sprint speed test on blood corticosterone. Second, because higher baseline levels were also associated with higher post-exercise concentrations (table 4) and that, when conditioning on baseline corticosterone levels in a second model, exploration and post-exercise corticosterone are not significantly correlated, this suggests that behaviour in a novel environment is more closely linked to baseline corticosterone concentration than stress-induced levels (see Umryukhin and Grigorchuk 2017).

Including baseline corticosterone levels as a fixed effect in a second multivariate model, namely conditioning the correlations on individual baseline measurements, allows me to estimate correlations between *corticosterone elevation* (i.e., the individual differences between baseline and post-exercise corticosterone) and performance. In both multivariate models, post-exercise corticosterone concentration was positively correlated with sprint speed (see table 4, table 5). This signifies that not only do faster sprints tend to display higher concentration levels following the effort, but they also have a greater increase in corticosterone. Because sprint speed represents a very short effort, it is most likely that mice rely on the high-energy phosphate stores in the muscles, as opposed to benefiting from the enhanced resource mobilization resulting form an increase in glucocorticoids (Tharp 1975). In humans, glucocorticoids do not increase during short (<10mins) intense exercise bouts, and even decreases before exhaustion is reached, only peaking 30 to 60 minutes after effort (Shepard and Sidney 1975). Similarly, in animals, glucocorticoids are mostly secreted during longer, strenuous, exercise bouts, for which their role might be more prominent, but not during short and intense activity (Terjung 1979). Therefore, finding a positive among-individual correlation between corticosterone elevation and sprint speed leads me to suspect that individual's performances are partially explained by their stress response during the sprint speed test.

This finding comes to challenge the assumption that individuals are maximally (or equally) motivated during the sprint speed test. As a general consensus, locomotor performance should be measured on ecologically relevant tasks, when animals are maximally motivated (Careau and Garland

2012). But performance tests generate different levels of stress and might be influenced by the animal's stress reactivity. This is perhaps most obvious when looking at tests such as bite force which relies on the intrinsic motivation of the animal to bite on the dynamometer during restraint. My results suggest that sprint speed tests are possibly falling into a similar category within the "behaviour-to- performance continuum" (see chapter 1). This should encourage future research on the links between performance and behaviour to include physiological measures of stress. These would ideally include measurements of baseline levels of stress as well as a "maximal stress ceilings" for each individual (i.e., maximal blood corticosterone concentration following ACTH injections see Dorin et al. 2012, Grota et al. 1997), this would in return allow to quantify how close to their maximal stress response animals get during performance measurement, a potential proxy for motivation in this context. Additionally, it is still possible that some of the associations between performance and behaviour are driven by among-individual differences in coping styles. Unfortunately, my methods did not allow me to test properly for associations between corticosterone elevation and behaviour, but studies including measures of stress reactivity to both behaviour and performance independently could help disentangle such relationships. Finally, studies designed to evaluate how stress reactivity covaries with performance measured with tests that do not rely on human stressors, such as with a forced swimming test, are also needed. Ideally, such studies would include paired measurements of stress reactivity and performance not relying on human stressors, to properly test for this association, as one could expect that stress reactivity is less likely to be associated with locomotor performance measured in such conditions.

General thesis conclusion

The main objective of my thesis was to study co-adaptations between locomotor performance and behaviour, with a primary focus on determining the sign and strength of covariance at the among- vs within-individual levels. Both suites of traits have been extensively studied over the years, and their associations have been highlighted in different conceptual frameworks such as the physiology-performance-behaviour-fitness (PPBF) and the pace-of-life syndrome (POLS). In the PPBF, originally defined by Arnold (1983) and expanded by Garland and Losos (1994), lower-level traits such as physiology and morphology determine locomotor performance which in turns sets the envelope within which behaviour can be expressed. The PPBF predicts that bolder, more active, or more explorative individuals are better equipped to deal with the risks associated with these behaviours and therefore have higher locomotor performance. In the POLS framework, which predicts co- adaptation of physiological and behavioural traits along a fast-to-slow continuum of life-history strategies (Ricklefs and Wikelski 2002; Réale et al. 2010), individuals with a fast pace of life are expected to be bolder, more explorative, more active, and have higher locomotor performance, as opposed to individuals with a slower pace of life, which are predicted to be shyer, less explorative, less active, and have lower locomotor performance.

These two frameworks both predict positive correlations between locomotor performance and behaviour, yet in recent years, a growing number of studies found opposite and counter-intuitive associations. This sparked my interest, as much discussion remains surrounding the causes of these observed associations. To my knowledge, a systematic review of all associations between locomotor performance and behaviour specifically was also not available, but would, in my opinion, allow to identify general patterns. After setting to compile this review, I also decided to evaluate the support for two competing hypotheses, namely the “trait compensation” and “trait co-specialization” which had recently been transposed to study the relationship between locomotor performance and behaviour. Overall, I found that most studies are in support of the compensation hypothesis (i.e., positive

association of behaviour and locomotor performance), which seems to align with predictions coming from the PPBF and POLS frameworks. Interestingly, and similar to what my preliminary literature search had indicated, I also found a non-negligeable number of associations (about 30%) supporting the co-specialization hypothesis, in which behaviour and locomotor performance are negatively correlated. So much work still remains in order to understand the complex nature of co- adaptations, including resolving the mechanisms at the origin of compensation and co-specialization hypothesis. Although this is beyond the scope of this thesis, a multitude of mechanistic studies are required, but those, to my knowledge, are not available yet. In fact, most of the studies that I could find in which associations of behaviour and locomotor performance were reported were not designed to test any of those associations in the first place, but happened to include measurements of these traits. Hopefully, with this thesis, I was able to draw attention to the complex compensatory and co-specialized nature of variation in behaviour and performance, which is a necessary first step to properly investigate the mechanisms responsible for the co-adaptations.

Perhaps one of the most important, yet simple, finding of this thesis, is the fact that the overwhelming majority of associations between locomotor performance and behaviour were reported at the phenotypic level. This has important implications, first, drawing conclusions based on these associations would signify taking the phenotypic gambit (i.e., assuming phenotypic correlations reflect among-individual correlations), which I am not willing to do, especially for correlations between labile traits with low repeatability like behaviour. Second, purely statistical biases are also in play. In my second and third chapters I try to highlight the importance of moving away from phenotypic correlations, instead using (co)variance partitioning to properly account for the hierarchical nature of repeated measurements data. This statistical approach has proven useful in unearthing performance trade-offs which otherwise remained hidden when only looking at phenotypic correlations (see yet another recent example; Lang and Gifford 2023), and so applying the same methodology to study the association of behaviour and locomotor performance seems relevant.

Another important point of this thesis, that emerged as I was measuring locomotor performance myself, is the importance of the context in which this trait is measured. Performance tests seem to rely on two assumptions, first, that animals when prompted to perform, react to the same extent as they would during a predator encounter. Second, that all animals react equally and to their fullest (i.e., animals are "maximally motivated"). Yet any researcher who has measured locomotor performance can probably attest to how much variation in motivation there seems to be. Some individuals are more inclined to engage fully, while others might not respond as well. This aspect of motivation probably represents, in my opinion, one of the hardest challenges to overcome in this field. In my third and fourth chapters, I try to tackle the problem of motivation in a few ways, first, by estimating the correlations between behaviour and performance measured with, and without a human stressor (chapter 3). Second, by using blood corticosterone, a glucocorticoid hormone involved in the stress response, to estimate its correlation with locomotor performance (chapter 4). The results of my fourth chapter seems to support some of the predictions from my previous chapters, as indicated by significant among-individual correlation between sprint speed and blood corticosterone, which indicates that individuals' stress's reactivity can influence performance measurements. While I only found an among-individual correlation, it is important to note that it is still possible to expect residual correlations between physiological stress responses and locomotor performance, which would have different implications if motivation not only varies among individuals, but also within individuals among repeated tests.

Overall, I hope that this thesis provides an updated context within which to study the covariance of locomotor performance and behaviour. Even if dozens, or hundreds of studies on locomotor performance and on behaviour already exist, I also hope that the future research looking at their associations specifically will be encouraged to adapt their experimental and statistical approaches to ensure that the estimates are not statistically biased, attenuated, or relying on potentially false assumptions. Furthermore, I hope that this thesis sparks interest in future young scientists to keep investigating the important role motivation plays on locomotor performance.

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