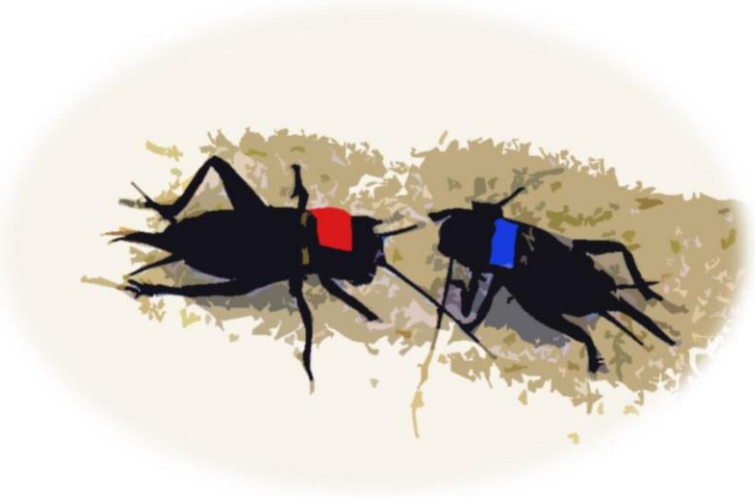
Consequences of social interactions on the evolution of individual differences in behaviour



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SUMMARY

Heritable variation in a trait is a key prerequisite for evolution by natural selection. However, when a trait is under stabilizing or directional selection, genetic variation may become depleted. Therefore, how variation among individuals in a species can persist is a crucial question in evolutionary biology. Behaviour represents a special type of 'labile' trait, because it is expressed multiple times throughout the lifetime of an individual and thus varies both within and among individuals. Over the past decades, behavioural ecology research has increasingly focused on the adaptive nature of repeatable among-individual variation in behaviour, called "animal personality". Understanding the processes generating and maintaining this among-individual variation, as well as its ecological and evolutionary consequences are hot current themes in this field of biology.

Social interactions have recently been suggested to represent an important factor shaping behavioural variation at both the within and among individual level. Behaviours and other types of traits that are expressed as part of social interactions are affected by phenotypes of conspecifics: these are known as 'indirect effects' of the social environment. Importantly, among-individual variation in social partners can be underpinned by genetic variation. Therefore, during social interactions the environment of one individual consists of genotypes of others, and thus can potentially also evolve. Quantitative genetic theory implies that social environments can have major evolutionary repercussions when the genes of an individual influence the (behavioural) expression of a trait in an interacting individual; such effects are termed 'indirect genetic effects' (IGEs). The consequences of indirect (genetic) effects for evolutionary processes are even more profound when we fully acknowledge the multivariate

nature of most phenotypes. Behavioural traits are often correlated with each other or with different types of traits, e.g. life-history traits. This may impose constrains on the evolution of behaviours because correlated traits may not be able to respond to selection independently when underpinned by tight genetic correlations.

Despite the potential for indirect (genetic) effects to shape within and among-individual behavioural variation, this mechanism is a largely overlooked in the field of behavioural ecology studying 'animal personality'. The main aim of my dissertation is to investigate the evolutionary consequences of personalities in the social environment and how they affect behavioural variation at the phenotypic and genetic level to explain why personality variation might persist. I do so by using large scale behavioural experiments in two species of field crickets, *Gryllus campestris* and *Gryllus bimaculatus*.

In **chapter one**, we lay the theoretical foundations and statistical framework on which we build up in the following chapters. In this chapter, we quantify the effect of the social environment on the aggressive behaviour of interacting individuals ('indirect' effects), and identify which traits are responsible for such effect. We do so in an empirical study on male European Field crickets (*G. campestris*). We find that individuals are consistently different in the aggressive responses that they elicit in others. These indirect effects are caused by their differences in behavioural traits (i.e. 'personality'), including non-social behaviours such as activity and exploration. The findings of chapter one imply that personality variation in the environment will have consequences for evolutionary trajectories of socially expressed behaviours, if representing heritable patterns of variation. In **chapter two** we therefore apply a similar design and analytical framework, but we study the effects of heritable phenotypes in the

social environment, i.e. the 'indirect genetic effects' (IGEs). We use a pedigreed lab population of Mediterranean field crickets (*Gryllus bimaculatus*) descending from wild-caught individuals in a large scale experiment. We find that indeed heritable personalities have evolutionary consequences, because IGEs affect genetic variation in behaviours expressed in both social and non-social contexts. In **chapter three**, we approach behaviours as part of an integrated phenotype with other traits. Trade-offs in the allocation of resources among multiple costly traits are often implied as a mechanism maintaining among-individual variation in life-histories. The 'pace-of-life' (POL) hypothesis suggests that alternative life-history strategies yielding equal fitness are associated with among-individual behavioural differences and thus may explain their maintenance. We embed our study in the POL framework, and test the predicted relationships between life-history traits (lifespan, development time, size at maturation) and 'risky' behaviours (aggression and exploration). We find that risky behaviours mediate genetic relationships (but not trade-offs) between life-history traits, suggesting their coevolution in natural populations. In **chapter four**, we show the broad applicability of our experimental design and conceptual framework to answer other questions related to the effects of the social environment on behavioural variation, in a different insect species. We study the indirect effects of the phenotypes of interacting partners on same-sex sexual behaviour (SSB) in water striders (*Gerris lacustris*) and identify the traits underpinning them.

My dissertation addresses questions currently raised by behavioural ecologists interested in adaptive individual variation with the tools developed by quantitative geneticists, bridging the two fields. By including indirect (genetic) effects in the study of social and non-social behaviours, we identify far-reaching effects of the social environment on behavioural

variation. Furthermore, we propose IGEs as an overlooked mechanism to explain the maintenance of personality, implying that genetic variation in indirect effects of the social environment can have consequences for the evolutionary trajectories of a wide range of traits.

GENERAL INTRODUCTION

Phenotypic variation is one of the pillars of Darwin's evolutionary theory (Darwin 1859). Natural selection acts on phenotypes, but its effect is translated from generation to generation at the genetic level. Thus, heritable variation in a trait is a key prerequisite for evolution by natural selection. However, when a trait is under selection, favoured alleles may get fixed in the population and genetic variation becomes depleted. Therefore, a central question in evolutionary biology is why genetic variation is maintained despite selection.

Traits such as adult height in humans, adult skull size in mammals, genetic colour morphs in birds, vary among-individuals within a species. These traits develop only once in an individual's lifetime and then become irreversibly 'fixed'. Explanations for the maintenance of genetic variation in such traits include mutation-selection balance, heterozygous advantage, antagonistic pleiotropy, frequency-dependent selection, and changing patterns of natural selection over time and space (Futuyma 2005). A classic example of the latter explanation is provided by work on Darwin's medium ground finches (*Geospiza fortis*), a species that shows substantial heritable variation in beak size and body size within populations. Birds with small beaks and small body size suffer selective mortality during severe drought periods, as they lack the mechanical power to crack the large and hard seeds which increasingly dominate the food supply. However, natural selection in the opposite direction, with small birds surviving disproportionately, occurs when abundant rain and high temperatures transform the vegetation and food supply (Grant and Grant 2002). A useful approach to understand the evolution of complex characters (e.g. that show continuous variation and multi-locus control) is

to analyse variation quantitatively by following changes in frequency distribution of phenotypes. Phenotypes are the product of environmental influences, genes, and their interaction. Thus, among-individual phenotypic variation can be underpinned both by heritable differences and differences that arise because animals can adapt to the changing environment through developmental plasticity (Pigliucci 2001). For these types of traits that become irreversible in adulthood, environmental factors can permanently affect the phenotype produced by a particular genotype ('permanent environment effects') during its development (Schlichting and Pigliucci 1998).

Other 'labile' traits, such as behaviour, physiology, etc. are typically expressed multiple times throughout the lifetime of an individual, and can vary both within and among individuals (Dingemanse et al. 2010). Within-individual variation in labile traits occurs because labile traits are susceptible to environmental factors with non-permanent effects (a form of phenotypic plasticity) and thus change over short time spans (i.e. they are reversible). For example, ectotherms such as reptiles and amphibians continuously modulate their activity levels to regulate body temperature according to the environmental temperature. The separation between the within and among-individual level is important, as only among-individual variation may represent heritable variation. Importantly, the 'classic' explanations for the maintenance of genetic variation in irreversible traits mentioned above do not explain why genetic, or among-individual variation as a proxy for genetic variation, should be maintained for labile traits. For example, although fluctuating selection can maintain variation in an irreversible heritable trait (e.g. the beak size example detailed above), this explanation does not readily hold for labile traits. This is because individuals are able to alter their phenotype to match the

current environment; thus, when plasticity is available to deal with environmental changes, it is unclear why consistent among-individual variation in labile traits would persist (Dall 2004).

Behavioural ecologists have traditionally focused on the within-individual level of variation and studied reversible phenotypic plasticity from an optimality perspective, where animals alter their behavioural phenotype to cope adaptively with changes in environmental conditions experienced within their lifetime (Krebs and Davies 1997). However, phenotypic plasticity comes with costs: maintaining the sensory and regulatory machinery necessary for expressing plasticity may require energy and material expenses; other costs that plastic individuals incur are time and energy spent on sampling the environment for cues. Furthermore, individuals are not infinitely plastic, because constrains to the production of the optimal phenotype in all situations are present (DeWitt et al. 1998). Thus, despite a certain degree of plasticity, at the same time individuals can also differ consistently from each other in their average behavioural expression (Gosling 2001, Sih et al. 2004, Réale et al. 2007, 2010a). For example, certain individuals tend to be repeatedly 'bolder' in risky situations than other 'shy' individuals. These consistent among-individual differences (also called 'personality') have been documented across a wide range of taxa (Bell et al. 2009, Garamszegi et al. 2012). Several adaptive explanations have been proposed for why selection might maintain among-individual behavioural variation. These explanations, while similar to those invoked for 'fixed' traits (detailed above), hinge on the assumption that individual variation rather than plasticity may be favoured due to costs or limits to the evolution of adaptive phenotypic plasticity (Nicolaus et al. 2016). Frequency dependent selection (Dall et al. 2004), temporal and spatial heterogeneity (Dingemanse et al. 2004, Réale and Dingemanse 2010) or life-history trade-offs (Wolf et al.

2007, Biro and Stamps 2008, Réale et al. 2010b), can all lead to the stable coexistence of different behavioural 'types' within populations. For example, in the great tit (*Parus major*) bold males and shy females fare better after favourable winters, while shy males and bold females profit from meagre winters. Mixtures of alternative strategies within populations can therefore evolve when the environment fluctuates regularly, and the best thing to do changes with each fluctuation (Dall 2004, Dingemanse et al. 2004). Understanding the processes generating and maintaining this among-individual variation (Wolf et al. 2007, Biro and Stamps 2008, Careau et al. 2008, Luttbeg and Sih 2010, Dingemanse and Wolf 2010, Réale and Dingemanse 2010), as well as its ecological and evolutionary consequences (Dall et al. 2012, Wolf and Weissing 2012, Dingemanse and Araya-Ajoy 2015), are currently central questions in the field of behavioural ecology.

Social interactions have recently been implicated as an important factor shaping behavioural variation at both the within and among individual level (Bergmüller and Taborsky 2010, Montiglio et al. 2013, Dingemanse and Araya-Ajoy 2015, Niemelä and Santostefano 2015, Santostefano et al. 2016). Social interactions are ubiquitous in nature: behaviours such as mating, courtship, communication, and different forms of cooperation are well studied examples of behaviours solely expressed as part of a social interaction (Székely et al. 2010, Westneat and Fox 2010). Behaviours and other types of traits that are expressed as a part of social interactions are particularly interesting when their expression is affected by phenotypes of conspecifics: these are called 'interacting phenotypes' (Moore et al. 1997, Wolf et al. 1999, McGlothlin et al. 2010). In this case, the phenotypes of conspecifics determine the environment experienced by an individual (Dingemanse and Araya-Ajoy 2015). For example, an individual

may decide to engage in a fight if the opponent is smaller and the chances of winning are higher, but decide to instead retreat (and thus stay unharmed) if the opponent is larger than itself (Huntingford and Turner 1987). Other examples of behaviours that are affected by social partners include female choice based on male ornaments and mating displays (Andersson 1994), or how parents change their provisioning behaviour according to offspring begging (Kölliker et al. 2000). In other words, individuals respond plastically (i.e. adapt their behavioural expression) to their partner identity and phenotype. These are known as 'indirect effects' of the social environment.

Importantly, behavioural variation will have evolutionary consequences when repeatable differences in phenotypes of social partners (indirect effects) are underpinned by genetic variation. Quantitative genetic theory implies that social environments can have major evolutionary repercussions when the genes of an individual influence the expression of a trait in an interacting individual; such effects are termed 'indirect genetic effects' (IGEs) (Wolf et al. 1998, 1999, McGlothlin et al. 2010). Notably, IGEs can explain the maintenance of (genetic) variation and lack of (phenotypic) evolution even in cases where there is strong directional selection acting on the focal trait (see below). During social interactions, the environment of one individual consists of genotypes of others (Moore et al. 1997), and thus can potentially also itself evolve (McGlothlin et al. 2010). Evolution in the presence of IGEs is studied widely in animal breeding, animal welfare, and forestry, to predict evolution in response to artificial selection on traits expressed as part of social interactions, such as feather pecking behaviour, tree growth or egg reproduction (Wade et al. 2010, Bijma 2014). IGEs can greatly influence evolutionary processes particularly when they are correlated with direct genetic effects (DGEs)

of an individual's genes on its own phenotype. In other words, when an individual is genetically predisposed to express a trait, it may also be genetically predisposed to increase or decrease the expression of that trait in others due to the occurrence of positive vs. negative genetic correlations between DGEs and IGEs, respectively. Positive genetic correlations between DGEs and IGEs acting on a trait are predicted to greatly speed up the response to directional selection compared to predictions derived from classic evolutionary theory (e.g., McGlothlin et al. 2010). Negative genetic correlations between DGEs and IGEs acting on the same trait can instead impose evolutionary constraints. For example, in gulls, the same genes that contribute to early egg laying when expressed in females, when expressed in males actually delay the lay date of their partners (Brommer and Rattiste 2008). Such negative correlations reduce heritable variation available to selection in a trait and thus the potential for phenotypic change (Bijma 2011, 2014). Therefore, IGEs arising from the social environment can both provide a major source of heritable variation on which selection can act, as well as represent a widespread source of potential evolutionary constraint (Wilson et al. 2011). However, indirect (genetic) effects are still an overlooked mechanism shaping variation in labile traits such as animal behaviour.

The consequences of indirect (genetic) effects for evolutionary processes are even more profound when we fully acknowledge the multivariate nature of most phenotypes (Moore et al. 1997, Blows 2007, McGlothlin and Brodie 2009, Alemu et al. 2014, Araya-Ajoy and Dingemanse 2014). Behavioural traits are often structured into syndromes (Garamszegi et al. 2012), that is, whole suites of behaviours are correlated at the among-individual level. For example, individuals who are bolder towards predators are often also more aggressive towards

conspecifics and more prone to explore a novel environment (Sih et al. 2004, Garamszegi et al. 2012). Such syndromes may impose constrains on the evolution of behaviour (Dochtermann and Dingemanse 2013) because correlated behaviours may not be able to respond to selection independently when underpinned by tight genetic correlations. It is likely that indirect effects, which are expected to exist for behaviours such as aggressiveness and dominance (Moore et al. 1997, 2002), are also integrated as parts of syndromes. However, despite evidence that social partners can directly and indirectly influence key behavioural traits, to date, social partner effects have not been incorporated in 'animal personality' research.

Recent theory poses that other important traits connected with social behaviours (and behaviours more in general) are life-history traits (Duckworth 2009, Réale et al. 2010b, Morrissey 2014). Life-history traits are those traits that affect the survival and fitness of individuals (e.g. age at sexual maturation, reproductive lifespan and ageing, number and size of offspring) (Stearns 1992). Therefore, the covariation of social behaviours with life-history traits can potentially affect the evolutionary trajectories of populations (Stearns 1989, Morrissey 2014). Importantly, alternative life-history strategies linked with behavioural differences may yield equal fitness and thus their association could explain the maintenance of individual variation in behaviour.

My dissertation sits at the interface of two fields, addressing questions currently raised by behavioural ecologists interested in adaptive individual variation in labile behavioural traits with the tools of quantitative geneticists. Including indirect (genetic) effects into the study of individual behavioural variation, I am able to integrate distinct areas of evolutionary biology. By doing so, I address outstanding questions regarding the maintenance of (genetic) variability and

evolution of behaviours. Furthermore, with a multivariate view of the phenotype, this framework can be broadly applied to all traits involved in social interactions, and explain the maintenance of their (genetic) variation.

Dissertation overview

The main aim of my dissertation is to investigate the evolutionary consequences of social interactions and how they affect and maintain behavioural variation at the phenotypic and genetic level. I do so by using large scale behavioural experiments in two species of Field crickets, *Gryllus campestris* and *Gryllus bimaculatus*.

In **chapter one**, we lay the theoretical foundations and statistical framework upon which we build in the following chapters. In this chapter, we aim at (i) quantifying the effect of the social environment on the behaviours of interacting individuals ('indirect' or 'opponent' effect), as well as (ii) including the social environment in a multivariate view of the phenotype, to identify which traits of the opponent are responsible for such effects. We do so in an empirical study focused on the individual level, where we repeatedly measure aggressiveness (a social behaviour) as well as other non-social behaviours (activity, exploration), and morphology (size) on male European Field crickets (*G. campestris*). With a specific experimental design, where every individual plays repeatedly the role of the 'focal' and the 'opponent' in dyadic fights, we can estimate not only the repeatabilities of all behaviours ('personality'), but also the indirect effects on social behaviours, that is, if individuals are consistent in the behavioural responses that they elicit in others. We can address this question by using mixed-effects models, which partition the total phenotypic variance in the population and explain variation in a trait due to among-individual differences (the focal identity, as well as the opponent identity). This allows us to quantify whether individuals are repeatable in the responses that they elicit in their social partners, i.e., whether the opponent itself represents an important source of indirect effects on the focal individual's behaviour. This potentially unaccounted source of variation has rarely

been addressed in studies on social behaviour. Expanding this framework, we further investigate which traits of the interacting individuals are responsible for such effect. Is it the same behaviour (aggression) in the opponent, a physical trait (size), or another behavioural trait expressed in another context (e.g. exploration, activity)? In statistical terms, this means estimating the correlation between the focal and opponent effects (on the same or different traits). Furthermore, we apply a structural equation modelling approach, which allows us to test explicit predictions, based on literature, on the correlation structure among behaviours. We can therefore identify explicitly which traits are responsible for the 'opponent effect'. This extension to a multivariate framework represents a novelty in behavioural ecology studies of social behaviour and allows us to embed the social environment in a broader version of the phenotype, considering the complexity of relationships among traits. Including indirect effects in the multivariate phenotype helped us identifying far-reaching effects of the social environment on other traits that would otherwise be missed and greatly improves our ability to understand the ecology and evolution of behaviour.

The findings of chapter one implies that personality variation in the environment may have consequences for evolutionary trajectories of socially expressed behaviours, though this is only the case when such individual differences represent heritable patterns of variation – this is the focus of **chapter two.** In this chapter, we apply a similar design and analytical framework, but this time we study the effects of *heritable* phenotypes in the social environment, i.e. the 'indirect genetic effects' (IGEs). This allows us to explicitly address whether IGEs can maintain genetic variation in behaviours expressed in both social and non-social contexts and thus the evolutionary consequences of personality. We use a pedigreed lab population of

Mediterranean field crickets (*Gryllus bimaculatus*) descending from wild-caught individuals, in a large scale experiment. Because of the half-sib full-sib design we used to breed the population, the relatedness of all the individuals is known and therefore we can estimate which proportion of the indirect effects is heritable, as well as the correlation with the heritable part of other behavioural phenotypes. Again, mixed-effects models provide a useful tool to address these questions, by implementing information on relatedness among individuals in the 'animal model' (Kruuk 2004). With this approach, we could identify IGEs as an overlooked mechanism to explain the maintenance of personality, implying that genetic variation in indirect effects of the social environment can have consequences for the evolutionary trajectories of a wide range of traits.

In **chapter three**, we follow up the theme introduced in the previous chapters of viewing behaviours as part of an integrated phenotype with other traits. Specifically, we embed our study in the 'pace-of-life' (POL) framework outlined by behavioural ecologists, where specific associations between life-history traits and 'risky' behaviours are predicted. The POL hypothesis has primarily been proposed to explain the maintenance of individual variation in behaviour by suggesting that their associated alternative life-history strategies may yield equal fitness. Specifically, individuals with a 'fast' lifestyle should develop faster, reproduce at an earlier age but live less long (Stearns 1989) compared to those with a 'slow' lifestyle. Fast individuals are also expected to show increased expression of behaviours that facilitate resource acquisition at the cost of reduced longevity ('risky' behaviours) such as aggressiveness, boldness, exploratory tendency, or foraging activity (Stamps 2007, Wolf et al. 2007, Réale et al. 2010b). In this chapter, using the same individuals as in Chapter 2, we focus on relationships between

behaviour (Chapter 2) and life-history traits (lifespan, development time, size), and ask whether trade-offs between these traits are mediated by risky behaviours. Importantly, genetic trade-offs included in the POL hypothesis are often hidden at the phenotypic level by environmental effects. We therefore focus on the estimation of genetic correlations. Again we can address this comparison across levels of variation by using multivariate mixed-effects models, which allows us to extract the individual, genetic, and environmental correlations among traits. Furthermore, in this chapter we apply path analysis, a useful tool to test, and compare, alternative hypotheses concerning the structure of multivariate phenotypes. This tool allowed us to test for the support of alternative *a priori* hypothesized models with different causal and temporal relationships between traits. In addition, path analysis helped us uncover relationships that would have remained undetected by observing correlations alone. Overall, the genetic integration of behaviour and life-history identified in this chapter implies a major role of behaviour in life-history evolution and suggests that both aspects co-evolve in natural populations. However, because this integration does not involve trade-offs, alternative life-history strategies do not explain the maintenance of behavioural variation.

In **chapter four**, we show the broad applicability of our experimental design and conceptual framework to answer other questions related to the effects of the social environment on behavioural variation, in a different species. We investigate the effect of the phenotypes of interacting partners on same-sex sexual behaviour (SSB) in water striders *(Gerris lacustris*). Males interacted repeatedly in dyads (in a similar scheme as the one used in the other chapters), on which we measured SSB, along with body size. We apply both a variance partitioning and a trait-based approach. Doing so allows us to estimate the indirect effects of

social partners on SSB (the equivalent of indirect effects on aggression in Chapter 1) with the variance partitioning approach, and identify which phenotype of the social partner is responsible for this effect, with the trait based approach. However, with the latter, we can also further ask whether this effect is due to the repeatable part of the phenotype of the partners (their 'personality') or their plastic part instead (the 'day-to-day' change). This distinction has important evolutionary implications, as only the repeatable part is potentially heritable, but has rarely been addressed in this context.

Study species

To address the main questions of this thesis, I used two species of field crickets, the European field cricket (*Gryllus campestris*) (Chapter 1) and the Mediterranean field cricket (*Gryllus bimaculatus*) (Chapters 2 and 3). Both species are abundant in Europe and their behavioural repertoire, especially aggressive behaviour, is well studied (see below). Furthermore, *G. bimaculatus* is multivoltine (having more than one generation per year), and easy to keep and breed in laboratory conditions, which makes it ideal for large scale artificial breeding studies.

Life history

The European field cricket, *G. campestris* (Linnaeus, 1758), is a flightless, comparatively large cricket species (17–26 mm) characterized by its distinctively large head and shiny black body coloration (Marshall 1974). It occurs in central and southern Europe, western Asia and North Africa and mainly inhabits dry grasslands with short vegetation. The reproductive season lasts from May to July, when males attract females with mating calls which they produce in the close vicinity of their burrows. Burrows can be recognized from their vegetation-free entrances, as both sexes clear the area in front of their burrows. After mating, females lay eggs and the nymphs hatch usually within 3 to 4 weeks and undergo 10 to 11 instar stages before becoming adults. When reaching their penultimate instar, nymphs dig individual burrows where they over-winter and emerge in March. Because of the overwintering stage as nymphs, this species is not suitable for breeding in laboratory conditions.

The Mediterranean field cricket, *G. bimaculatus* (De Geer, 1773), is morphologically very similar to *G. campestris* and can be discriminated by the two dot-like marks on the base of its wings. This species is widely used as live food for pet and zoo animals, as well as in several lab-based behavioural, physiological, and neurobiological studies, however surprisingly little is known about its natural history in the wild. *Gryllus bimaculatus* occurs in Southern Europe throughout the Mediterranean, where it inhabits pastures, scrub, dunes, grasslands and rural terrain. Individuals do not dig their own burrows, but hide under logs, grasses, and in crevices; nonetheless, males are believed to be territorial and sing from their refuges (Simmons 1986, 1988). Adults appear from summer to autumn, and females lay eggs in the ground throughout the mating season. After hatching, nymphs undergo several instar stages before maturation within 2-3 weeks.

Figure 1. Left to right: Adult male *Gryllus bimaculatus* and *Gryllus campestris*.

Behaviour

Males of both species frequently engage in agonistic contests to monopolize resources such as burrows or mates (Ritz and Köhler 2007). If neither of the males retreats during an encounter, they engage in a fight consisting of a sequentially escalating series of behaviours. The aggressive repertoire of field crickets is well studied in the lab, where males fight as soon as placed together (Alexander 1961, Hoy and Adamo 1995, Hack 1997, Stevenson et al. 2000, Hofmann and Stevenson 2000). Furthermore, repeatable among-individual differences in suites of traits such as activity, boldness, and exploratory tendency (i.e., 'personality') have been found both in G. *campestris,* G. *bimaculatus,* and other closely related species (Rodríguez-Muñoz et al. 2010, Wilson et al. 2010, Dochtermann and Nelson 2014, Fisher et al. 2015a, 2015b, Niemelä et al. 2015).

Collection sites

G. campestris used for my studies were collected from a meadow adjacent to the Max Planck Institute for Ornithology (Seewiesen), Germany (47°58'35.5'N 11°14'04.5'E). We collected individuals close to adulthood with funnel traps set at the burrow entrance. Following capture, crickets were placed in individual containers and transported to a climate room at the Ludwig Maximilians University of Munich.

The parental generation of *G. bimaculatus*, used for quantitative genetics studies, was collected from a tomato field of approximately 2500 m^2 near Capalbio, Italy (42°42'46.7' N 11°33'99.3' E). Collected individuals were part of a large meta-population. We collected a total

of 100 individuals, which constituted our parental generation. Following capture, crickets were transported to a climate room at the Ludwig Maximilians University of Munich.

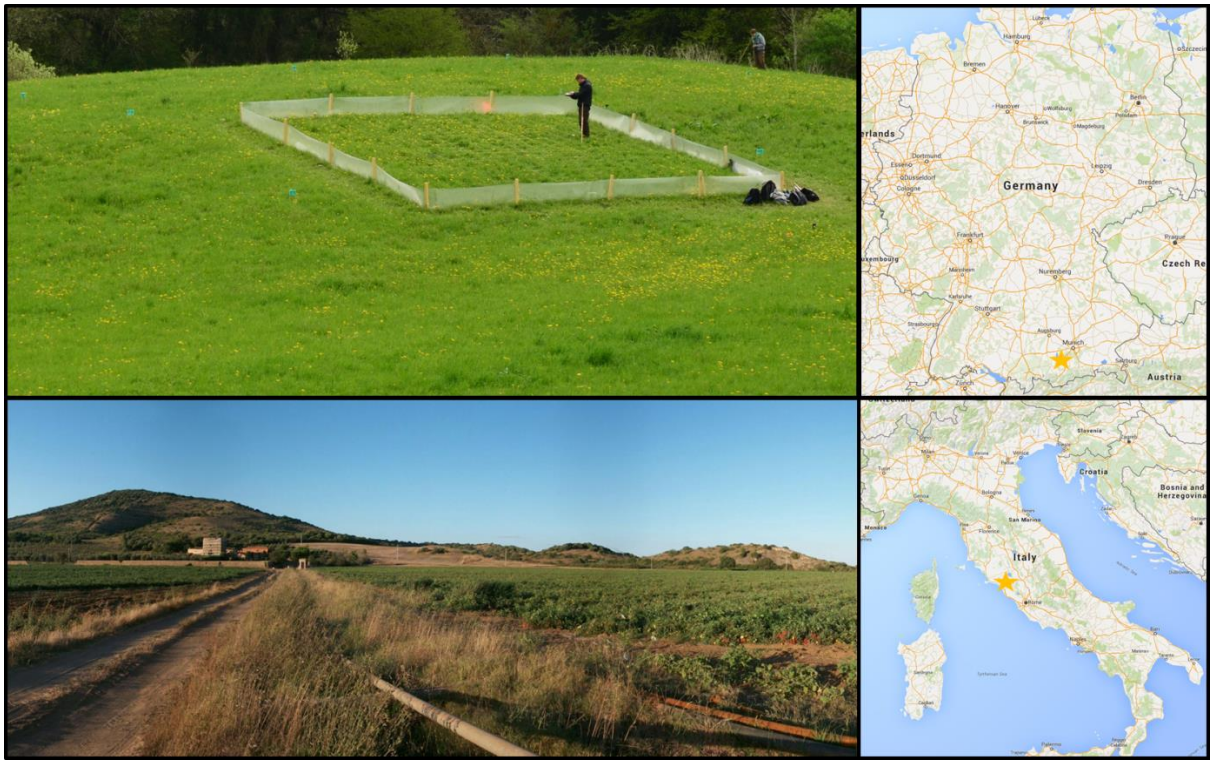

Figure 2. Collection sites for *G. campestris* (top) and *G. bimaculatus* (bottom)

Housing and breeding

For both species, individuals were housed in a climate room at 26°C (±0.5) and 65% (±0.5) humidity, under a light:dark photoperiod that wild crickets experienced at the time of capture (14:10 h). Each individual was housed alone in a plastic container which included an artificial, half-cylindrical shelter, food and water.

Wild-caught individuals of *G. bimaculatus* were used to establish a pedigreed population in the lab. Sexually mature individuals from the parental generation were randomly paired after arrival in the laboratory to produce the F1. We adopted a full-sib/half-sib breeding design

(Lynch and Walsh 1998) on the F1 and F2 generations by having each male fertilize the clutches of each of two females. Adult males of the F1 and F2 were used in our behavioural assays.

Figure 3. Left to right: pair of adults in the mating container; freshly hatched nymphs; adult in isolation container.

Experimental setup

A similar scheme for behavioural trials was adopted for both species. Each individual was repeatedly assayed for each of 3 behaviours (2 for *G. bimaculatus*, which did not have activity assays) on the same day, in the same order: activity, exploration, and aggression (described in detail below). The same individual was assayed for each behaviour either 12 (*G. campestris*) or 6 times (*G. bimaculatus*), with measurements taken one day (*G. campestris*) or one week (*G. bimaculatus*) apart.

In *G. campestris*, 32 males were divided into groups of 4 individuals. For *G. bimaculatus*, 455 males were divided into groups of 40 individuals. All individuals within a group were tested on the same day (8 individuals simultaneously). Dyads of males paired for the aggression tests were randomly assigned amongst the non-related individuals within the same group to produce social environments that were homogenous with respect to relatedness.

All trials were performed in the same climate room where the individuals were housed and recorded using high-resolution digital video cameras fitted above each testing arena.

Behavioural trials and scoring

The general level of activity in a familiar environment (*G. campestris* only) was recorded automatically in the individual's home container for 60 minutes. Following the activity test, we gently moved the subject to the exploration arena, where exploratory activity was recorded automatically for 60 (*campestris*) or 30 (*bimaculatus*) minutes. After the exploration test, the divider between two exploration arenas was then lifted, after which we filmed each dyad engaging in social interactions for a period of 10 minutes.

Activity and exploration videos were analysed using Ethovision X 10.0 (Noldus, the Netherlands). This software package enables tracking of isolated individuals and extracts the spatial coordinates for each video frame. For both the activity and exploration tests, we used the total distance moved, in the familiar environment (activity test) viewed as a measure of 'activity'. The total distance moved in the novel environment (exploration test) is viewed as a measure of 'exploratory behaviour' (following Réale et al. 2007). The aggression test was scored manually in G. *campestris* and automatically in G. *bimaculatus.* In the manual scoring, we scored an individual as 'approaching' during an interaction when it moved towards the other individual until they came into contact. For the aggression test in G. *bimaculatus*, we calculated the total time each individual spent moving towards the opponent ('relative movement' for simplicity), by summing up only the consecutive samples (frames) where the relative distance between subjects decreased.

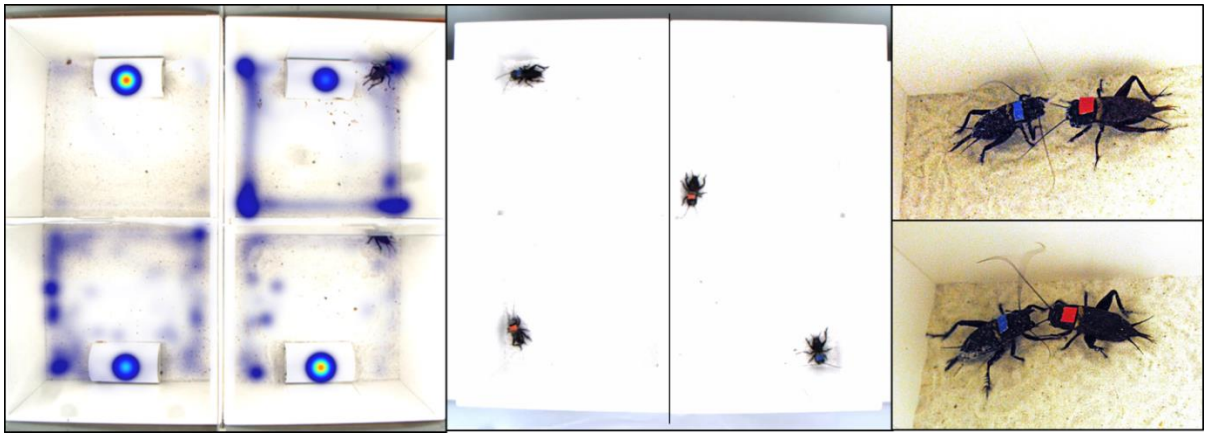

Figure 4. Left to right: Exploration test with heath map; aggression test; close up of different stages of an escalating interaction.

Life-history and morphological traits

We recorded in both species the right hind femur (to the nearest 0.05 mm) as measure of structural adult size (Simmons 1988). We also recorded two key life-history traits in G. *bimaculatus*: developmental time, and lifespan. We calculated developmental time for each individual as the difference between the individual's date of final moult and the date of hatching for the egg clutch from which it emerged. We calculated an individual's adult lifespan as the difference between the death date and the date of final moult.

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CHAPTER 1.

Interacting with the enemy: indirect effects of personality on conspecific aggression in crickets

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ABSTRACT

In animal contests, individuals respond plastically to the phenotypes of the opponents that they confront. These 'opponent' – or 'indirect' – effects are often repeatable, e.g., certain opponents consistently elicit more or less aggressiveness in others. 'Personality' (repeatable among-individual variance in behaviour) has been proposed as an important source of indirect effects. Here, we repeatedly assayed aggressiveness of wild-caught adult male field crickets *Gryllus campestris* in staged dyadic fights, measuring aggressiveness of both contestants. Measurements of their personality in non-social contexts (activity and exploration behaviour) enabled us to ask whether personality caused indirect effects on aggressiveness. Activity, exploration, and aggressiveness were positively associated into a behavioural syndrome eliciting aggressiveness in conspecifics, providing direct evidence for the role of personality in causing indirect effects. Our findings imply that a multivariate view of phenotypes that includes indirect effects greatly improves our ability to understand the ecology and evolution of behaviour.

KEY WORDS: animal personality, behavioural syndrome, aggression, indirect effects, social behaviour, crickets

INTRODUCTION

Over the past decades, research has increasingly focused on the adaptive nature of repeatable among-individual variation in behaviour, called 'animal personality' in the behavioural ecology literature (Dall et al. 2004; Sih et al. 2004a,b; Réale et al. 2007, 2010). This field of evolutionary biology thereby increasingly embraces the notion that natural selection can favor behavioural variation at multiple hierarchical levels, such as within *and* among individuals (Dingemanse et al. 2010b; Araya-Ajoy and Dingemanse 2014). While the existence of animal personality is increasingly viewed as representing a ubiquitous feature of natural populations (Bell et al. 2009; Dingemanse et al. 2012b; Garamszegi et al. 2012), recent focus is shifting from questions regarding adaptive causation towards those concerned with the consequences of personality variation for ecological and evolutionary processes (Dall et al. 2012; Wolf and Weissing 2012; Dingemanse and Araya-Ajoy 2015).

Social interactions have recently been implicated as an important factor shaping behavioural variation at both the within- and among-individual level (Bergmüller and Taborsky 2010; Montiglio et al. 2013; Dingemanse and Araya-Ajoy 2015; Niemelä and Santostefano 2015). Variation within individuals may partly occur because individuals modify their behaviour as a function of the phenotypes expressed by conspecifics (so-called 'indirect effects'; see below). Individuals may alter a particular behaviour either in response to the same trait or a different trait expressed by conspecifics. An example of the former effect is provided by reciprocal aggression in escalating fights, while an example of the latter is provided by aggression in response to opponent body size (Moore et al. 1997).

Indirect effects can have major consequences for ecological and evolutionary processes especially when caused by repeatable differences in phenotypes of social partners that are underpinned by genetic variation. In such cases, the genes of an individual influence

the (behavioural) expression of a trait expressed by an interacting individual; such effects are termed 'indirect genetic effects' (IGEs) (Wolf et al. 1998, 1999; McGlothlin et al. 2010). IGEs can greatly influence evolutionary processes particularly when they are correlated with direct genetic effects (DGEs) of an individual's genes on its own phenotype. In mice, for example, aggressiveness is heritable (a DGE), aggressiveness elicited in conspecifics is heritable too (an IGE), and aggressive genotypes elicit greater levels of aggressiveness in conspecifics. Such positive genetic correlations between DGEs and IGEs are predicted to speed up the response to directional selection compared to predictions derived from classic quantitative genetics theory (e.g., McGlothlin et al. 2010). By contrast, negative genetic correlations between DGEs and IGEs might instead impose evolutionary constraints. In gulls, for example, genes expressed in females that contribute to early egg laying (a DGE) actually delay the egg laying date of other females when expressed in their male partners (an IGE) (Brommer and Rattiste 2008). Obviously, phenotypes that are relatively repeatable (e.g., body size) and affect fitness in interaction partners are most likely to induce indirect effects in nature.

The consequences of indirect (genetic) effects for evolutionary processes are even more profound when we fully acknowledge the multivariate nature of most phenotypes (Moore et al. 1997; McGlothlin and Brodie 2009; Araya-Ajoy and Dingemanse 2014; Bijma 2014). Behavioural traits are often structured into syndromes (Garamszegi et al. 2012), i.e., whole suites of behaviours are correlated at the among-individual level; such syndromes may impose constrains on micro-evolution of behaviour (Dochtermann and Dingemanse 2013). This would, for example, occur when selection favors multivariate behavioural phenotypes that are not present in the population due to strong syndrome structure. To date, studies of syndromes have considered only correlations among 'direct' sources of

behavioural variation. However, it is likely that indirect effects are also integrated as parts of syndromes. This would occur if, for instance, opponent personality is itself an important source of indirect effects on focal behaviour. Indirect effects are expected for behaviours such as aggressiveness and dominance (Moore et al. 1997, 2002), which are themselves often correlated with other behaviours such as boldness, exploration and activity (Garamszegi et al. 2012). Social partner effects should therefore be incorporated as part of behavioural syndromes to better understand the evolution of correlated traits.

Aggressiveness represents a good example of an 'interacting phenotype' studied both in quantitative genetics and behavioural ecology (Wilson et al. 2009; Briffa et al. 2015). Aggressiveness is interesting because of its reciprocal nature and documented occurrence of opponent (also called 'social partner') effects (Wilson et al. 2009, 2011, 2013; Camerlink et al. 2012, 2013; Alemu et al. 2014). Interestingly, contest theory has traditionally focused on the role of morphological traits such as weapons and body size in shaping the intensity of aggressive interactions (Arnott and Elwood 2008). In empirical studies, however, morphological traits often explain only a moderate portion of the variation in contest behaviour (Briffa et al. 2015). The behavioural stress physiology literature implies that behavioural traits may also play an important role because 'proactive' (active, aggressive, explorative, bold) individuals are less responsive to intentions signaled by interaction partners compared to 'reactive' (less active, aggressive, explorative, bold) individuals (Koolhaas et al. 1999; Coppens et al. 2010). While effects of personality on social responsiveness are predicted by game theory (Maynard-Smith 1982; Dall et al. 2004), the idea has received little empirical testing to date (Briffa et al. 2015; Dingemanse and Araya-Ajoy 2015).

Crickets are an ideal system to address the occurrence of indirect effects of personality on the expression of aggressiveness. Males of the European field cricket (*Gryllus campestris*) are territorial and frequently engage in agonistic contests to monopolize resources such as burrows or mates (Ritz and Köhler 2007). Wild European field cricket populations show repeatable among-individual differences in suites of traits such as activity, aggressiveness, boldness, and exploratory tendency (Rodríguez-Muñoz et al. 2010; Fisher et al. 2015a,b; Niemelä et al. 2015).

Specific study designs are required to quantify variation in individual-level traits where both direct and indirect effects are targeted (Moore et al. 1997; McGlothlin and Brodie 2009; Bijma 2014). We focus here on a design where the same individual has social interactions both as a 'focal' and as an 'opponent', and where each individual plays each role repeatedly across behavioural trials (Wilson et al. 2009, 2013; Dingemanse and Araya-Ajoy 2015). Such a setup enables the partitioning of the total phenotypic variance in behaviour expressed in focal individuals to variance attributable to (i) the focal individual's identity (a direct effect), (ii) the opponent's identity (an indirect effect), and (iii) residual within-individual variance (see Wilson et al. 2009, 2011, 2013 for worked examples). Moreover, because each individual is both used as a focal individual and as an opponent, we can additionally ask whether individuals that (on average) have a high value for a particular behaviour also elicit this behaviour in conspecifics. Statistically this is measured as the correlation between direct and indirect effects (i.e., the phenotypic components of the genetic correlation between DGEs and IGEs detailed above). Application of this design has, for example, demonstrated that mice with aggressive personalities also elicit aggressiveness in their interaction partners (Wilson et al. 2009). When other traits are additionally measured, their effects may readily be incorporated to ask which (combination of) traits

exactly induce social responsiveness in interaction partners (McGlothlin and Brodie 2009; Bijma 2014).

Here, we applied this paradigm to the study of aggressiveness expressed in pairwise interactions. We asked whether the personality of an individual (measured by its average level of activity, exploratory behaviour, and aggressiveness across repeated observations) affects the aggressiveness expressed in conspecifics, thus integrating the indirect effects in a multivariate view of the phenotype. We repeatedly assayed activity, exploratory tendency, and aggression to determine the presence of repeatable among-individual variation in these traits ('personality'). To address their indirect effects, we applied a design where each male cricket fought repeatedly in dyadic interactions, equally often as a focal and as an opponent. We then quantified (i) whether activity, exploratory behaviour, and aggressiveness harbored repeatable among-individual variation, (ii) whether individuals were also repeatable in the level of aggressiveness elicited in opponents, (iii) whether an individual's average level of aggressiveness predicted aggressiveness elicited in opponents or (iv) whether other key individual-level characteristics (activity, exploratory tendency, body weight) did so instead. Our second objective was to integrate indirect effects into our description of behavioural syndrome structure. We therefore tested the relative fit of nine alternative hypotheses (models) concerning syndrome structure based on literature, with a structural equation modelling approach (Dochtermann and Jenkins 2007; Dingemanse et al. 2010a); this allowed us to test explicit predictions on the correlation structure among behaviours (detailed in Figure 1). Doing so enabled us to determine the nature of associations between behavioural traits and indirect effects in contest behaviour, and thereby forcefully address the consequences of variation in 'personality' during social interactions.

METHODS

Cricket collection

Crickets were collected from a meadow adjacent to the Max Planck Institute for Ornithology (Seewiesen), Germany (47°58'35.5'N 11°14'04.5'E), between the 2nd week of May and the 1st week of June 2013. The main collection site was a south-west facing slope within the meadow. From the 4th week of April onwards, we searched the field daily for burrows of newly emerged individuals and marked each with a numbered flag. Our aim was to trap individuals close to adulthood because our interest was in quantifying adult behaviour (detailed below). We attempted to catch crickets in this stage with funnel traps (detailed in Niemelä et al. 2015) set at the burrow entrance between 10h00-17h00, for up to 30 minutes and up to 4 times per day per burrow, until the individual was caught. We succeeded in catching most crickets within the first two days of attempted catching. Because we monitored burrows on a daily basis, we could track each individual's developmental stage; all captured adults were no more than 1 day post-molting, implying that they lacked experience with mating and fighting. Following capture, crickets were placed in individual containers and transported to a climate room at the Ludwig Maximilians University of Munich. We collected a total of 57 males of which we randomly selected 32 at the onset of the experiments.

Housing

All individuals were housed in a climate room (dimensions: 4.5 l x 3.6 w x 2.7 h m) at 26°C (±0.5) and 65% (±0.5) humidity, under a light:dark photoperiod that wild crickets experienced at the time of capture (14:10 h). Each individual was housed alone in a plastic container (10 x 10 x 9 cm) with a sand-covered floor and a flow-through plastic netted lid

that prevented escape but allowed air circulation. Each container included an artificial, half-cylindrical shelter (6 x 3.5 x 2 cm), a petri dish (3.5 cm diameter) with food, and another petri dish with water held within a cotton-plugged vial. Individuals were fed with a mix of dry bird food (Aleckwa Delikat, Germany) and fresh food (carrot and apple) *ad libitum*. Food and water were replaced every 3-4 days. Containers were checked daily for newly molted adults, enabling us to assign age since final molting for each individual not yet molted.

Experimental protocol

Behavioural trials were conducted between the 26th of May and 24th of June 2013. Each individual was repeatedly assayed for each of 3 behaviours (activity, exploration, aggression; described in detail below), on each of 12 consecutive days. Because individual identification is required for the aggression test, subjects were marked with colored tape on the pronotum (red or blue, randomly assigned each time) the day before a trial. The three tests were always done sequentially and in the same order (table 1, figure 2); carry-over effects (from one test on the next) could therefore not be modelled. We chose this set-up because it ensured that all individuals were given the exact same treatment since this greatly facilitates comparison between individuals (Dingemanse et al. 2007; Dochtermann 2010).

We initially selected 32 individuals randomly from the 57 collected individuals. These were divided into eight groups of four individuals according to their estimated age (i.e., days post-molting) to ensure that all the individuals of the same group were approximately the same age. It has been shown that adult crickets within one week post-molting do not show fully developed aggressive behaviour (Hofmann and Schildberger 2001); the behavioural assays were therefore conducted when individuals were at least 7 days post-molting. Groups were on average 9.8 days (standard deviation (SD): 2.6) post-molting at the onset of

their behavioural trials. Six individuals died before the end of the experiment and were replaced, increasing the total number of tested individuals to 38. For data analysis (see below), we used all data, including behavioural trials of individuals that died during the experiment and of their replacements.

Within each group of four, each individual was subjected to an aggression test once on each of 12 consecutive days. From previous work, we know that this inter-test interval prevents carry-over effects caused by winner or loser effects (Khazraïe and Campan 1999). To maximize the number of unique pairwise dyads, and sample size per dyad, each individual fought four times with each of the three interaction partners within its group. All individuals within a group of four were tested on the same day, and each of two groups were assayed at the same time (i.e., 8 individuals simultaneously, one group per shelf). To minimize within-group variation in micro-environmental effects, each group was assayed at a fixed time of day over the 12 consecutive days (either between 9h00-12h00, 12h00-15h00 or 15h00-18h00). After each group was tested, walls and dividers of the testing arenas were thoroughly washed with warm water; sand was raked and mixed in a stock container in order to minimize the presence of contact pheromones that could otherwise affect future contests (Judge and Bonanno 2008).

All trials were performed on a rack fitted with two shelves, each equipped with a camera, in the same climate room where the individuals were housed. Arenas were not acoustically isolated from the rest of the room such that all the individuals were exposed to abundant background noise typical of conditions experienced in the wild. To prevent any visual disturbances, the shelves were isolated from the climate room by white curtains; entrance to the room was not permitted during trials. All trials were recorded using high-resolution digital video cameras (Basler GenICam, Germany) fitted 43 cm above each testing

arena. The cameras were connected to a computer outside of the climate room and managed using the software MediaRecorder (Noldus, Netherlands). Videos were recorded at 27.81 frames per second and 1600 x 1200 pixels resolution.

A small number of trials were excluded from the final dataset: 32 of 382 activity and 29 of 382 exploration trials (8.4% and 7.6% respectively) due to technical problems with data recording, and 24 of 191 aggression trials (12.6 %) because males failed to engage in aggressive interactions. Note that the total number of aggression trials is approximately half of that of other trials since two individuals are involved in each aggression test. The final sample size was therefore 350 tests for activity (mean number per individual: 9.2, SD 3.7), 353 for exploration (mean number per individual: 9.3, SD 3.4) and 167 for aggression (mean number per individual: 8.8, SD 3.8).

Behavioural trials and scoring

Activity: The general level of activity in a familiar environment was recorded in the individual's home container. Those home containers were large enough to allow free movement reflecting its routine activity. Prior to the onset of the behavioural trials, selected individuals were carefully moved to the recording shelves inside their home container and given 30 minutes to acclimatize. To optimize the automated video tracking, the lid, water vial, food and petri dishes were removed from the home containers. Activity was recorded automatically for 60 minutes (Table 1; Figure 2a).

Exploration: Following the activity test, we gently moved the subject (inside its home container) to the side of the shelf where it had previously been filmed, after which we placed two arenas under each camera. These arenas (29.5 l x 15.5 w x 9 h cm) had floors covered with white sand and an opaque removable divider in the middle. When the divider

was lowered, the two halves (14.5 l x 15.5 w x 9 h cm) of a single arena could be used as arenas to simultaneously screen, in isolation, exploratory behaviour of each of the two individuals that would later meet in an aggression test. At the onset of the exploration test, each individual was moved (inside its own shelter) from its home container to a (randomly allocated) half of the arena. We ensured that all individuals were inside their shelter at the onset of the exploration trial, after which exploratory activity was recorded automatically for 60 minutes (Table 1; Figure 2b).

Activity and exploration videos were analysed using Ethovision X 10.0 (Noldus, the Netherlands). This software package enables tracking of isolated individuals and extracts the spatial coordinates for each video frame. For both the activity and exploration tests, we used the Pythagorean equation to calculate the distance moved from one frame to the next, using the X and Y coordinates of the individual's center point at each sampling point (User manual of Ethovision X 10.0, Noldus Information Technology 2013). The frame rate at which the videos were analysed was adequately set to 6.95 per sec, and we therefore acquired 25020 positions per individual for these 60-min trials. We then summed up all distances to calculate the total distance moved, in the familiar environment (activity test) viewed as a measure of 'activity'. The total distance moved in the novel environment (exploration test) is viewed as a measure of 'exploratory behaviour' (following Réale et al. 2007).

Aggression: Following the exploration test, the shelters were removed and the individuals given a further 10 minutes to acclimatize to their half of the arena. The divider was then lifted, after which we filmed each dyad engaging in social interactions for a period of 10 minutes (Table 1, Figure 2c). At the end of each aggression test, each individual was weighed on an electronic balance (precision: 0,001 g). We then changed each individual's

color tag according to the next day's schedule, and returned it to the home container in the allotted housing slots within the climate room.

We assayed three behaviours that represented an individual's willingness to engage in aggressive interactions: 'approaching', 'singing', and 'chasing' (defined below). An interaction was defined as starting when any part of one individual touched any part of the other (Bertram et al. 2011), and ended if the contact was interrupted for more than two seconds. Within the 10-minute trial, for each interaction, we scored for each member of the dyad whether it did or did not perform each of these three behaviours. We subsequently counted how often a behaviour occurred per individual over the entire trial, resulting in three count variables for each combination of individual and trial. The total number of interactions observed over all 167 trials was 2589 (mean number of interactions per trial = 15.05, min = 1, max = 52). To acquire the behavioural data, each video was played in slow motion and scored blindly by one of three observers.

Approaching*:* We scored an individual as 'approaching' during an interaction when it moved towards the other individual from any angle until they came into contact. When only one individual was actively approaching the other (i.e. the other cricket sat still), we assigned the behaviour to that individual alone. In cases where both contestants approached each other at the same time, we assigned the behaviour to both. The mean number of approaches per individual per trial was 9.64 (min = 0, max = 48). Approach behaviour has been used by several studies quantifying aggressiveness in male crickets, sometimes called 'initiating first contact' or 'initiating first aggression' (Brown et al. 2006, 2007).

Singing: Male crickets produce songs by rubbing their wings together when they encounter rivals and this has been reported as an indication of male aggressive intent

(Alexander 1961). Singing involves wing movements and is visually easily distinguished from courtship songs and victory songs, the latter produced only by the winner *after* an interaction. The mean number of times singing recorded per individual per trial was 1.73 (min = 0, max = 19).

Chasing: A chase was recorded when one of the contestants (namely, the winner) actively pursued its opponent immediately after the interaction had ended. We defined chases as pursuits in the direction of the loser initiated within 2 seconds after termination of the physical contact. Chasing represents a behaviour indicating aggressiveness (i.e. the motivation to continue the fight after contact has ended), and has also been used as a measure of aggressiveness in several other studies (e.g. Jang et al. 2008; Bertram et al. 2011; Fitzsimmons and Bertram 2013). Unlike the other two behaviours, chasing after an interaction is mutually exclusive for the two individuals, i.e. only the winner ever chases the opponent away. The mean number of chases recorded per individual per trial was 1.46 (min = 0, max = 22).

Statistical analyses

We conducted two sets of statistical analyses. First we partitioned the variation for each measured trait into within- and among-individual variance using a univariate mixed-effect modelling framework. Univariate models allow a straightforward interpretation of fixed effects, and allow for testing repeatabilities of single behaviours. As a second step, we estimated patterns of among-trait covariance at each estimable hierarchical level using a multivariate extension of this framework. All models were fitted using restricted maximum likelihood; dependent variables were mean-centered and variance standardized to facilitate comparison of variance components across traits. Throughout, we assumed a Gaussian error

distribution, which was confirmed for all response variables after visual inspection of model residuals.

Univariate mixed-effects models

Sources of variation in traits repeatedly measured in a non-social context (i.e., activity, exploration, and body weight) were estimated by fitting the focal trait as the response variable into a univariate mixed-effect model. To statistically control for potential sources of variation not relevant to our biological hypotheses we included the following fixed effects: test sequence (covariate, range 0-11), time of the day (minutes from midnight, covariate), shelf (categorical variable with two levels), and location of the arena within a shelf (categorical variable with four levels). Test sequence was coded as the progressive order of repeats for the same assay (0 to 11), such that the fixed effect intercept of the model was estimated for the first test (e.g. Dingemanse et al. 2012a). Time of day was mean-centered, such that the fixed-effect intercept of the model was estimated for the phenotype expressed on the average testing time (following Dingemanse and Dochtermann 2013). We fitted random intercepts for group (8 levels), date (28 levels), and the focal individual's identity (38 levels). Effects of variables fitted to control for variation induced by the experimental design (time of day, shelf, location, group, and date) were unimportant (results not shown) and are not discussed further, except for the variable test sequence which explained significant variation.

Prior to the analysis of the traits repeatedly measured in a social context (i.e., approaching, singing, and chasing), one individual in each trial was randomly assigned the focal and the other the opponent role. Since we hypothesize that focal behaviour will depend on opponent phenotype, random intercepts for both focal and opponent identity are included in the univariate model of the focal individual's behaviour. Furthermore,

because the same individual is the focal in some aggression trials but the opponent in others, the model can be expanded to estimate the covariance (or correlation) between focal and opponent identity effects on focal behaviours. If positive (vs. negative), individuals that are on average aggressive as focal animals also tend to induce (vs. reduce) aggressiveness in others when acting as the opponent. If opponent identity effects are present but this correlation is zero, individual-level traits of the opponent other than its aggressiveness affect the focal individual's aggressiveness.

While previous studies applying this design have analysed sources of variation in the focal individual's behaviour alone (e.g. Wilson et al. 2009), we were able to record detailed behaviours performed by both individuals in each dyad (i.e. the designation of focal and opponent identities as described above was arbitrary). In order to fully utilize all available behavioural data for parameter estimation we therefore modified the modelling strategy described above as follows. First, we estimated focal and opponent identity effects (as well as their correlation) for the behaviour expressed by the focal individual. Second, we structured the dataset in a way that enabled us to simultaneously estimate focal and opponent identity effects (and their correlation) in the behaviour of the opponent (as detailed in the Supplementary Text). Third, because focal and opponent roles were randomly assigned, (i) focal identity effects in the focal individual's behaviour should equate opponent identity effects in the opponent's behaviour, (ii) opponent identity effects in the focal individual's behaviour should equate focal identity effects in the opponent's behaviour, and (iii) the covariance between focal and opponent identity effects as well as any other fixed-effect (e.g. time of day) and random-effect (e.g. group, date) estimates should be the same for the focal's and opponent's aggressive behaviour. We thus implemented a bivariate mixed-effect model with the focal's and opponent's behaviour

fitted as the two response variables where parameters that were logically identical were constrained to be identical. This bivariate implementation thereby enabled us to utilize all data at hand while simultaneously avoiding pseudo-replication and biasing effects of arbitrary assignment of focal versus opponent roles. Because of the imposed model constraints, the bivariate model effectively represents a univariate model, and is treated as such in the remaining text.

Adjusted individual repeatability was estimated for each trait by calculating the proportion of the total phenotypic variance not attributable to fixed effects that was explained by individual identity (Nakagawa and Schielzeth 2010). For behaviours expressed in a social context, we additionally calculated the proportion of the total phenotypic variance that was explained by the identity of the opponent and was not attributable to fixed effects, which we call 'adjusted opponent repeatability' (Wilson et al. 2009).

Multivariate mixed-effects models

We estimated patterns of trait covariance at each estimable hierarchical level using a multivariate mixed-effects model. We fitted activity, exploration, and weight as response variables as well as approach as a proxy for aggressiveness. Though we had measured various proxies of aggressiveness (detailed above), we used here only approach because multivariate models including other proxies of aggression did not converge. However, we were able to confirm with sets of simpler models that all proxies for aggressiveness were highly correlated (Results not shown), implying that our decision to use approach as a measure of aggressiveness was justified (for a further discussion on rationale of this approach, see Araya-Ajoy & Dingemanse 2014).

To avoid over-parameterization, we only included fixed (sequence) and random effects (identity of the focal individual) that explained significant variation in the univariate analyses

(see Results). Modelling random intercepts for the focal individual's identity enabled decomposition of phenotypic covariances (and hence correlations derived from them) into among-individual and residual within-individual components (Wilson et al. 2009). Among-individual correlations occur when an individual's average phenotype over all repeated measures is correlated across traits, called a 'behavioural syndrome' in the context of behaviour (Dingemanse et al. 2012b); within-individual correlations occur when two phenotypic traits show correlated changes in expression within the same individual due to the combined effects of integration of within-individual plasticity and/or correlated measurement error (Dingemanse and Dochtermann 2013). We further included random intercepts for the opponent's identity for the response variable approach during the aggression test, which enabled us to assess whether individuals were repeatable in the aggressiveness elicited in conspecifics (we will sometimes present this indirect effect as a trait, 'eliciting aggressiveness', for clarity). Because the same individual repeatedly played focal in some and opponent in other trials (see above), we also estimated (i) the correlation between the focal and opponent identity effect on aggressiveness (see above for biological interpretation) as well as (ii) the correlation between the opponent identity effect on aggressiveness and the focal identity effect on each of the other traits (activity, exploration, body weight). This latter type of correlation tests whether individuals that elicit more aggressiveness in others are themselves more (or less) active, explorative, or heavier than individuals eliciting less aggressiveness in others.

Significance testing in mixed-effects models

We tested statistical significance of fixed effects using numerator and denominator degrees of freedom (df) estimated from the algebraic algorithm in ASReml 3.0 (Gilmour et al. 2009). We used likelihood ratio tests (LRTs) to evaluate the statistical significance of

random effects. This χ^2-distributed test statistic is calculated as twice the difference in log-likelihood between a model where a target random effect was fitted versus not fitted (Shaw 1991). Variances are bound to be positive, therefore probability (P) of a LRT applied to a variance was calculated assuming an equal mixture of P (χ^2, df=0) and P (χ^2, df=1), i.e. df=0.5 (Self and Liang 1987; Pinheiro and Bates 2000; Visscher 2006). Covariances (correlations) are not bound to be positive, and their probability was therefore calculated assuming P (χ^2, df=1). LRTs involving one variance and one covariance were tested assuming an equal mixture of P (χ^2, df=1) and P (χ^2, df=2), i.e. df=1.5. We applied this latter test, for example, to evaluate support for opponent identity effects in aggressiveness, which requires fitting both a variance (i.e. the variance attributable to the opponent's identity) and a covariance (i.e. the covariance between focal and opponent identity effects). All models were implemented in ASReml 3.0 (Gilmour et al. 2009).

Structural equation modelling

To test our *a priori* hypotheses on behavioural syndrome structure, we applied structural equation modelling to nine *a priori* conceived scenarios based on the behavioural syndrome literature (described in Figure 1). We analysed the among-individual correlation matrix estimated from the multivariate mixed-effects model using the package 'SEM' in the software R v. 3.1.0. (Team R Core 2012). We then statistically compared the models using the Akaike information criterion (AIC) (Akaike 1973; Burnham and Anderson 2002), and evaluated the relative support for each based on AIC differences relative to the model with the lowest AIC (ΔAIC), which represents the best fitting model; we also calculated each model's weight and relative likelihood (Anderson 2008).

RESULTS

Sources of variation in single traits

Exploration, approach, and weight changed significantly within individuals as a function of test sequence (Supplementary Table S1). On average individuals became leaner, less explorative, and less aggressive over the 12-day course of the experiment. None of the traits were significantly affected by tag color, time of day, testing shelf or within-shelf test location (Results not shown); neither did the traits vary between groups or days (Supplementary Table S1).

All traits except singing were significantly repeatable (Table 2); adjusted behavioural repeatabilities were highest for exploration (0.40, SE 0.10) and activity (0.33, SE 0.09), and substantially lower (though significant) for approaching (0.19 SE 0.06) and chasing (0.09 SE 0.05) during the aggression test. Approaching and chasing were significantly affected by the identity of the opponent, though singing was not (Table 2). For approaching and singing, the proportion of variance explained by opponent identity (i.e. adjusted opponent repeatability) was of a similar magnitude (0.16, 0.06 SE for approaching; 0.12, 0.05 SE for chasing) when compared to adjusted individual repeatability (0.19 and 0.09, respectively). Opponent identity effects imply that unidentified individual-level characteristics of opponents affect the behaviour of focal individuals. These effects did not appear to be due to repeatable differences in approaching, chasing or singing among opponents, since the correlation between focal and opponent identity effects was rather weak and non-significant for all three behaviours expressed in the aggression test (Table 2).

Body weight was, as expected also repeatable (Table 1). Interestingly, repeatability was relatively low (0.46, SE 0.11) compared to what might typically be expected. This

indicates that body weight strongly depended on day-to-day changes in environmental conditions experienced by our crickets.

Among-trait correlations

Among-individual correlations

The multivariate mixed-effects model provided strong evidence for the existence of among-individual correlations between most traits (Table 3; Supplementary Table S2, Supplementary Table S4). Individuals that were on average relatively aggressive towards conspecifics were also relatively active in a novel environment ('explorative') and relatively active in a familiar environment, providing strong evidence for the existence of an aggressiveness-activity-exploration syndrome. Furthermore, aggressive individuals were relatively heavy compared to less aggressive individuals.

Our univariate analysis implied that aggressiveness elicited in conspecifics also harbored among-individual variation (Table 2). Aggressiveness elicited in conspecifics was not (tightly) related to an individual's average level of aggressiveness, owing to a non-significant correlation between focal and opponent identity effects (Table 2, see also Table 3). Our multivariate analysis revealed that individuals that were on average relatively explorative in novel environments also elicited approach by opponents in the aggression test (Table 3). This among-individual correlation was relatively strong (0.45, SE 0.17), implying that personality variation with respect to exploratory tendency constitutes a major factor explaining why individuals are repeatable in the level of aggressiveness that they elicit in others.

Residual within-individual correlations

Various traits were also correlated within individuals, implying the existence of integration of within-individual plasticity across multiple traits and/or correlated measurement error. Specifically, when individuals increased their body weight across trials, they also became significantly more explorative in the novel environment (though not more active in familiar environments) (Table 3, Supplementary Table S4). In contrast to patterns observed at the among-individual level, most traits were only weakly and non-significantly associated at the within-individual level.

Behavioural syndrome structure

Based on literature, we constructed and compared nine different *a priori* conceived models of behavioural syndrome structure (Figure 1). The domain-general syndrome, which included correlations between activity, exploration, aggression, and aggression elicited (model 3; Figure 4), best explained the observed data, as inferred from it having the lowest AIC score (Table S3). The second-best model, hypothesizing a domain-general structure with only exploration eliciting aggressiveness (model 9) also had a relatively good fit to the data (model 9: ΔAIC=2.35; Table S3). The domain general model nevertheless fitted the data 3.26 times better compared (Akaike weight of model 3 (0.75) divided by the Akaike weight of model 9 (0.23); Table S3), and is therefore treated as the sole best-fitting model throughout the remaining text.

The four observed behaviours were, notably, also partially independent from each other, as the syndrome structure implied in our best-supported model explained relatively little variation in aggressiveness (Figure 4). Exploration behaviour represented an extreme case, where all the among-individual variance was captured by the hypothesized syndrome

structure. We verified that this was not an artefact caused by the tight among-individual correlation between exploration and activity, as re-analyses of the data after excluding activity yielded the same results (not shown). These findings therefore provide strong support for the presence of a domain-general syndrome that includes indirect effects.

DISCUSSION

This study investigated the occurrence of indirect effects on aggressiveness in male European field crickets, and asked whether such effects could be driven by personality. As expected, we found evidence for indirect effects as individuals differed consistently in the level of aggressiveness that they elicited in others. Direct and indirect effects on focal behaviour were integrated within an overarching behavioural syndrome, existing largely because more explorative individuals were also more active and aggressive, and tended to elicit more aggressiveness in conspecifics. This finding constitutes unambiguous evidence for the notion that an individual's aggressiveness-activity-exploration type influences aggressiveness of conspecifics. Our study thus implies that repeatable differences in behaviour represent an important component of the social environment affecting social behaviours of conspecifics. If representing heritable patterns of variation, these findings imply that personality variation in the environment will have consequences for evolutionary trajectories of socially expressed behaviours that would only be appropriately predicted by quantitative genetics theory developed to model indirect genetic effects (Montiglio et al. 2013; Dingemanse and Araya-Ajoy 2015).

Individual and opponent repeatabilities

All behaviours (except singing during the aggression test) were repeatable and their estimates were within the range reported for behavioural traits (meta-analysis: Bell et al. 2009). Values of repeatabilities of non-social behavioural traits (activity, exploration) were also similar to those documented previously in our and other cricket species (Wilson et al. 2010; Niemelä et al. 2012a,b; Dochtermann and Nelson 2014; Fisher et al. 2015a,b). In contrast, behaviours expressed in a social context (approaching, singing, chasing) had relatively low repeatabilities (0.09-0.19) compared to those expressed in a non-social context (0.33-0.40; Table 2). This was not unexpected since the social environment experienced varied substantially within-individuals across trials, whereas the non-social trials represented a more stable environment. As advocated in recent reviews (Briffa et al. 2015; Dingemanse and Araya-Ajoy 2015), our setup allowed us to assess repeatability of social behaviour as it would typically be expressed in the wild (i.e., across an ecologically relevant sample of partners) rather than artificially minimizing social environment effects by using dummies or video playback stimulation. Few other studies of aggression have explicitly modelled such indirect effects by considering identity effects of interaction partners; all such studies have focused on vertebrates. Importantly, the repeatability estimates in these few studies were in the same range of ours (0.11 to 0.27; Wilson et al. 2009, 2011, 2013). These estimates of repeatability are lower compared to the ones reported by meta-analyses (Bell et al. 2009), where aggression was one of the most repeatable classes of behaviours (roughly R=0.50). Our findings therefore imply that published estimates might often be substantially biased upwards due to the usage of unnatural standardization standardized experimental conditions (e.g. size matching) that may lack ecological relevance.

Of course, the magnitude of indirect effects that we detected might be less substantial under field conditions. In the wild, other environmental effects (kept stable under laboratory conditions), such as temperature, availability of mates, or predators might affect the amount of among-individual variation in aggressiveness and aggressiveness elicited in conspecifics. Importantly, studies based on dyadic interactions might typically overestimate the magnitude of IGEs for species that naturally interact in larger groups (Hadfield and Wilson 2007; Bijma 2014). This critic may not be applicable in our case because male-male interactions over ownership of burrows or mates in our study species are typically dyadic under field conditions too (personal observation NJD and FS, Fisher et al. 2016). Therefore, our staged dyadic interactions accurately reflect the natural social environment in which aggressive interactions take place.

The opponent effect provides clear evidence for the notion that individuals plastically adjusted their aggression level in response to the opponent's phenotypic traits that vary at the individual level. Among-individual variation in opponents therefore explains part of the within-individual variation in aggression, which can be interpreted as a social form of phenotypic plasticity, or 'social responsiveness' (Webster and Ward 2011; Taborsky and Oliveira 2012; Wolf and McNamara 2013; Wolf and Krause 2014; Dingemanse and Araya-Ajoy 2015). By modelling the opponent identity as a random effect, we integrated the influence of all the aspects of an opponent's individual-level phenotype. However, by also measuring phenotypic traits of the opponent (including its personality), we were able to tease apart which traits were driving the opponent effect as we describe below.

Individual-level correlations of behavioural traits across contexts

Among-individual correlations between focal aggression, exploration and activity were positive, confirming our predictions and revealing a syndrome structure including behaviours expressed in social and non-social contexts. Positive correlations among activity and exploration behaviour are well-documented in the behavioural syndrome literature (Sih et al. 2004b; Réale et al. 2007; Garamszegi et al. 2012), and are generally supported in crickets (e.g. Wilson et al. 2010, but see Dochtermann and Nelson 2014) The coping style literature predicts the presence of bold, exploratory, and aggressive individuals called 'proactive' types (Koolhaas et al. 1999; Coppens et al. 2010), which we indeed found in our study. We note however, that the relationship between aggressive behaviour and other personality traits is often population-specific (Bell 2005; Dingemanse et al. 2007). Our findings therefore confirm the notion that behaviours from different functional contexts covary and that domain-general syndromes may be common among organisms (Sih et al. 2004a,b; Bell 2007; Dochtermann and Jenkins 2007). Such a structure may be important from an evolutionary perspective because trait correlations are known to affect evolutionary trajectories (Lande and Arnold 1983; Sih et al. 2004b; Sprenger et al. 2012; Dochtermann and Dingemanse 2013).

The repeatable part of an individual's weight was correlated with an individual's average level of exploration and aggressive behaviour, implying that repeatable variation in body condition might have proximately underpinned the observed behavioural syndrome (see e.g. Royauté et al. 2015b). However, post hoc analyses showed this not to be the case: we S3conducted a set of SEMs expanding upon our best-supported (domain-general) model. We then compared the relative fit of models where body weight constituted an additional expression of the latent variable rather than representing the latent variable itself (versus a

null model where body weight was not associated with behaviour) (Figure S1, Table S5). The domain-general model, where body weight also constituted an expression of the latent variable, was best supported; this finding implies that weight indeed did not 'drive' the syndrome structure. Interestingly, body size has often been hypothesized to represent an important mediator of agonistic behaviour in the animal contest literature (Briffa et al. 2015). Our study implies that social partners responded to personality rather than other characteristics of conspecifics, such as their body weight. It is of course possible that crickets instead responded to *relative* differences in body weight. However, this scenario is not supported by our data because it should result in effects of the unique combination of focal and partner identity effects (so-called 'pair identity' effects), which were not present when modelled in our data (Results not shown).

Because behaviours are plastic, feedbacks between traits may occur during interactions and can either increase or decrease the amount of among-individual variation (Moore et al. 1997; Wolf et al. 1998, 1999; Dingemanse and Araya-Ajoy 2015; Sih et al. 2015). The level of aggression in one individual is often affected by the aggression displayed by the interacting individual in escalated fights (Huntingford and Turner 1987), and positive correlations between the focal and opponent effects on this trait would indicate this type of reciprocal feedback. By contrast, if aggression in part reflects dominance (e.g., with dominant individuals only displaying aggression towards subordinates), negative correlations can arise (Wilson et al. 2011). Interestingly, the lack of a significant correlation between being aggressive and eliciting aggression in others may suggest the absence of feedback loops (either positive or negative) in this trait. However, our SEM-analysis uncovered a relationship between being aggressive and eliciting aggressiveness

underpinned by the behavioural syndrome (see below), indicating that positive feedbacks may have been present instead.

In contrast to patterns found at the among-individual level, most traits were only weakly and non-significantly associated at the within-individual level. This suggests that trait correlations were caused by different proximate mechanisms within versus among individuals (van Noordwijk and de Jong 1986; Hadfield et al. 2007). This finding contrasts recent studies reporting within- and among-individual correlations of similar magnitude (Araya-Ajoy and Dingemanse 2014; Brommer et al. 2014; Royauté et al. 2015a). The partial difference in correlation structure at the within vs. among individual level highlights the importance of separating the two in order to make unbiased inferences about behavioural syndrome structure (Dingemanse et al. 2012b; Dingemanse and Dochtermann 2013; Brommer et al. 2014).

Integration of indirect effects as part of a multivariate behavioural phenotype

All behavioural traits were linked with aggressiveness elicited in conspecifics as part of a behavioural syndrome, highlighting the key importance of considering the opponent's personality in the expression of social behaviours. These findings are supported both by the multivariate mixed-effect model and the SEM. Because exploration, aggression and activity were highly correlated, we expected that they would jointly affect the aggression of conspecifics, implying that the syndrome as a whole would explain the indirect effects on aggression (model 3). This was indeed the case: despite the 'penalization' for a higher number of parameters, the best-supported model was the one with a domain-general behavioural syndrome (Table S3, Figure 4). A mechanism for the overarching indirect effect could be that individuals who explore a new environment are likely to also be involved in

more social interactions and this may result in more attacks received by others within an aggression trial. This interpretation is warranted by the strong bivariate correlation between indirect effects on aggression and exploration in the among-individual correlation matrix. Indeed, exploration was the factor loading most strongly with the latent variable in our structural equation model. The overarching importance of exploration behaviour was implied by the second-best fitting model, which attracted some support (Table S3). Indeed, movement variables may represent obvious cues of an individual's aggressiveness. Nevertheless, other components of the behavioural syndrome definitely played an important role in eliciting aggression because a full domain-general model was over three times better supported. Because indirect effects were integrated as part of the behavioural syndrome, our findings imply that studies of the evolution of behaviour should explicitly recognize that behavioural phenotypes are multivariate and include responses of the social environment.

Evolutionary implications

Adaptive explanations in the field of behavioural ecology are often based solely on phenotypic observations, with evolutionary inference relying on the 'phenotypic gambit', assuming correspondence of phenotypic and genetic patterns of (co)variation (Grafen 1984). Support for this position has been provided by a behavioural meta-analysis in which phenotypic correlations explained 75% of the variation in genetic correlations (Dochtermann 2011), although this correspondence should not readily be assumed for individual cases. Despite this important caveat in our knowledge, the presence of indirect effects documented here suggests a strong potential for IGEs to contribute to evolutionary trajectories of aggression and/or traits genetically correlated with it, which in our case

include exploration and activity. Our study thus implies that behavioural types certainly have the potential to alter the evolutionary trajectories of behavioural traits by inducing indirect effects in a social context. Further partitioning the repeatable among individual behavioural variance into additive genetic and environmental effects would enable us to move from studying causes to revealing the consequences of personalities. This would ultimately enable us to predict the evolutionary consequences of the very existence of personality types in natural populations.

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TABLES

Table 1. Timetable of the daily experimental procedure (see the Methods for further details). Each individual was subjected to this procedure for 12 consecutive days.

Order within day	Action/behavioural test	Location	Duration
1	Food and water removal + acclimation	Home container	30 min
2	Activity	Home container	1 hr
3	Transfer to exploration arena		1 min
4	Exploration	Exploration arena	1 hr
5	Shelter removal + acclimation	Exploration arena	10 min
6	Aggression	Exploration arena w/o divider	10 min

Table 2. The proportion of total phenotypic variation not attributable to fixed effects explained by focal identity (adjusted individual repeatability; direct effects) and opponent identity (adjusted opponent repeatability; indirect effects), and the correlation between focal and opponent identity effects. Positive values for this correlation indicate patterns where individuals that on average express a high value for the given behaviour also elicit an increased expression of this behaviour in opponents. Estimates (with SE) are derived from univariate mixed-effect models with random intercepts for focal and opponent identity as appropriate, and associated χ^2-values, degrees of freedom (df), and values of P derived from likelihood ratio tests where the full model is compared to one where the random effect of interested was excluded.

Trait	**Individual repeatability (SE)**	χ^2_{df}	***P***	**Opponent repeatability (SE)**	χ^2_{df}	***P***	**Focal - opponent correlation (SE)**	χ^2_{df}	***P***
Activity	**0.33** (0.09)	$65.60_{0.5}$	**<0.01**	-	-	-	-	-	-
Exploration	**0.40** (0.10)	$92.56_{0.5}$	**<0.01**	-	-	-	-	-	-
Weight	**0.46** (0.11)	$253.37_{0.5}$	**<0.01**	-	-	-	-	-	-
Approach	**0.19** (0.06)	$17.65_{1.5}$	**<0.01**	**0.16** (0.06)	$14.74_{1.5}$	**<0.01**	0.22 (0.26)	0.70_{1}	0.40
Chase	**0.09** (0.05)	$6.49_{1.5}$	**<0.05**	**0.12** (0.05)	$9.72_{1.5}$	**<0.05**	-0.34 (0.39)	0.71_{1}	0.40
Sing	0.07 (0.05)	$3.60_{1.5}$	0.11	0.05 (0.05)	$1.86_{1.5}$	0.28	-0.21 (0.56)	0.15_{1}	0.69

Table 3. Estimated among- and residual within-individual correlations (with associated SE) for two non-social behaviours (activity and exploration), two social behaviours (aggressiveness and aggressiveness elicited in opponents), and weight. We present among-individual correlations on the upper off-diagonals and within-individual correlations on the lower off-diagonals. Correlations printed in bold-face are significant based on likelihood ratio tests derived from the multivariate model detailed in the main text. Note that the experimental setup solely allowed for estimating of among-individual correlations with aggressiveness elicited in other individuals.

	Aggressiveness	**Activity**	**Exploration**	**Weight**	**Aggressiveness elicited**
Aggressiveness	-	**0.39** (0.18)	**0.39** (0.17)	**0.43** (0.16)	0.09 (0.22)
Activity	-0.02 (0.07)	-	**0.81** (0.08)	**0.17** (0.15)	0.16 (0.20)
Exploration	0.03 (0.07)	**0.17** (0.06)	-	0.22 (0.14)	**0.45** (0.17)
Weight	0.05 (0.06)	0.06 (0.06)	**0.13** (0.06)	-	-0.31 (0.18)

FIGURES

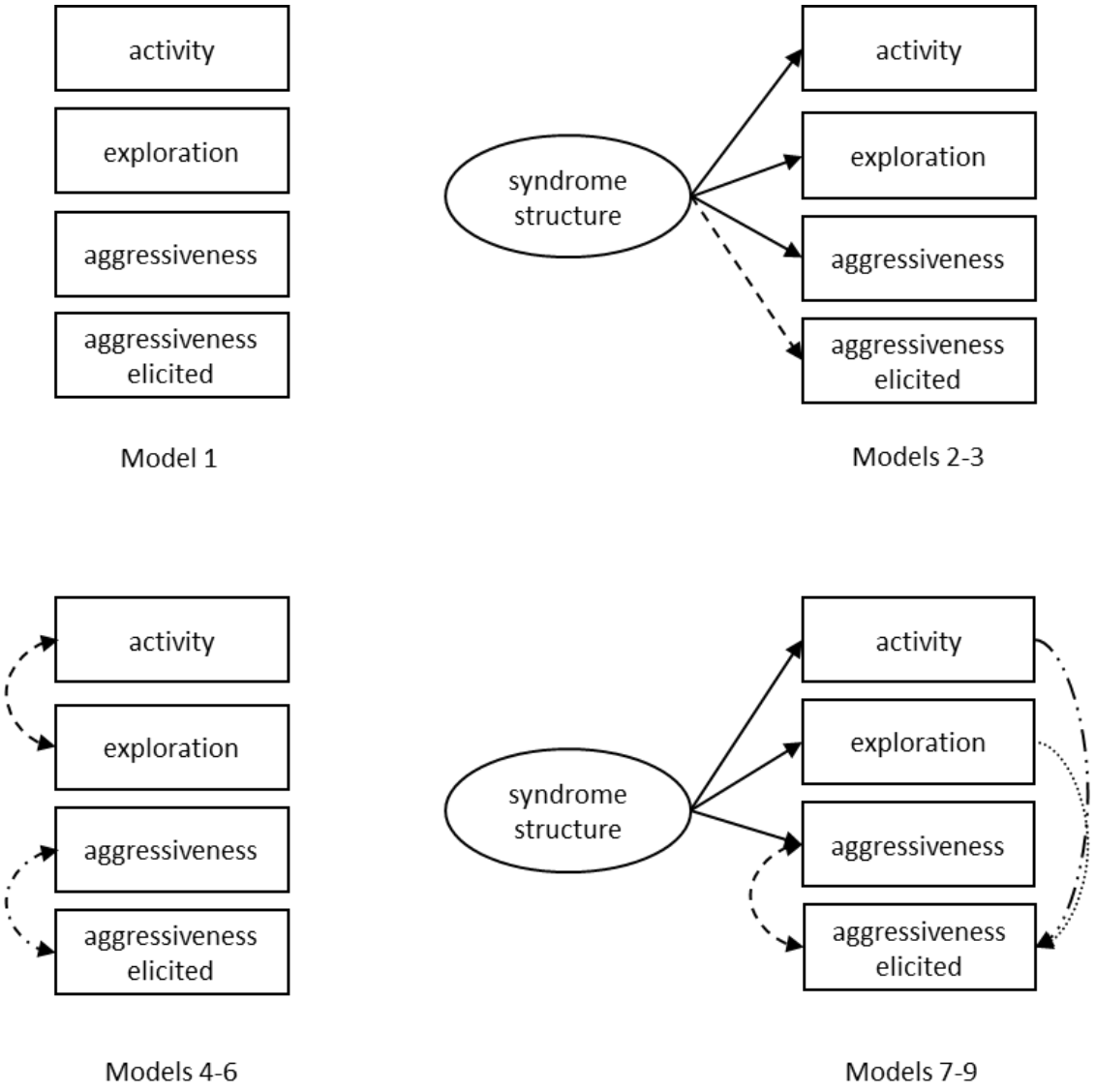

Figure 1. Models (1–9) of hypothesized relationships between behaviours (syndrome structure). Unidirectional arrows represent causal relationships between variables, bidirectional arrows represent correlations. Solid lines represent relationships present across the whole set; dashed, dotted, and mixed lines represent relationships expressed in specific syndrome structures. Model 1: null model of behavioural independency (Coleman and Wilson 1998). Model 2: coping styles with 'proactive' and 'reactive' types (correlations among exploration, activity and aggression) (Koolhaas et al. 1999; Coppens et al. 2010). Model 3: The dashed arrow is active; a modification of model 2, where all behaviours

including the indirect effects are expression of a general-domain syndrome. Models 4-6: the dashed arrow alone is active in model 4, the mixed arrow alone in model 5, both in model 6. Semi-independency of modules (Sih et al. 2004b) for different contests (social and non-social behaviours). Model 7: the dashed arrow is active; combination of model 2 with reciprocal feedbacks on aggressiveness (covariance between aggression and eliciting aggressiveness) (Moore et al. 1997; Wilson et al. 2009). Models 8-9: the mixed arrow is active only in model 8, the dotted arrow is active only in model 9; combination of model 2 with ideas from (Verbeek et al. 1996). Exploration, aggression, and activity are part of syndrome and either exploration (8) or activity (9) has a direct of effect on aggressiveness of others.

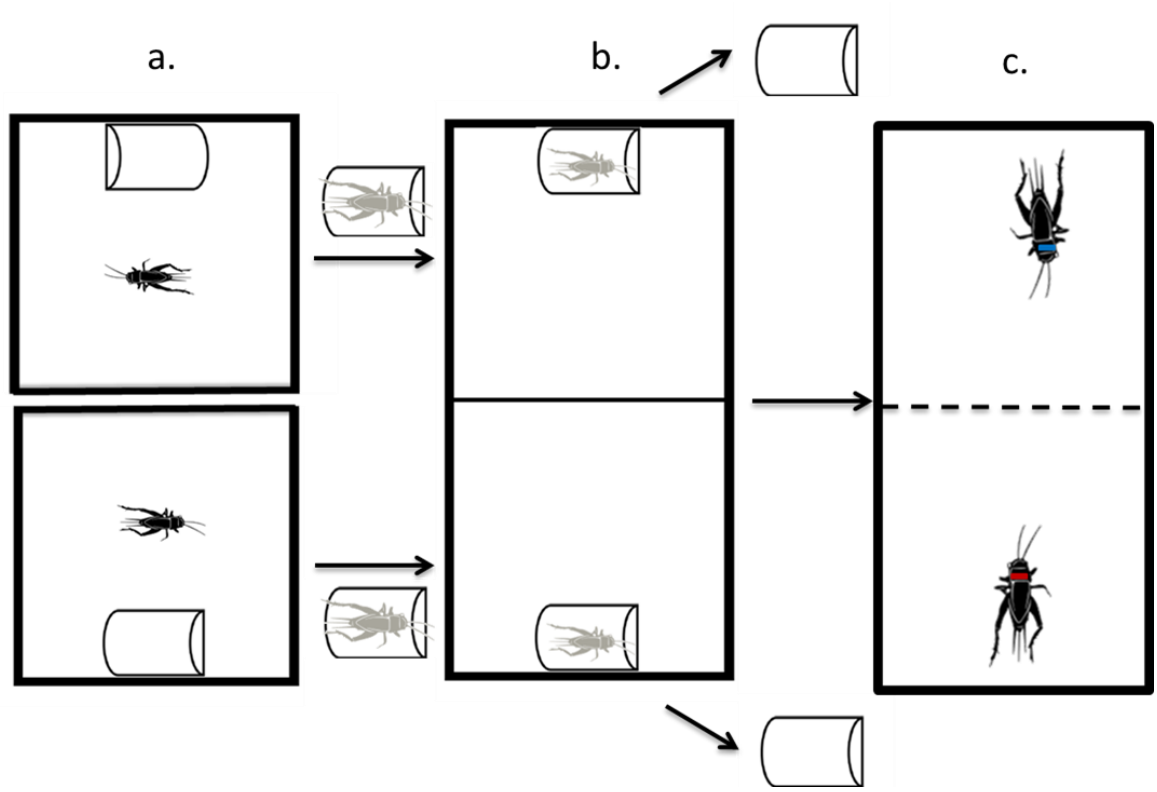

Figure 2. Daily experimental timeline, showing a top view of the experimental setups used to measure a) activity b) exploration and c) aggression.

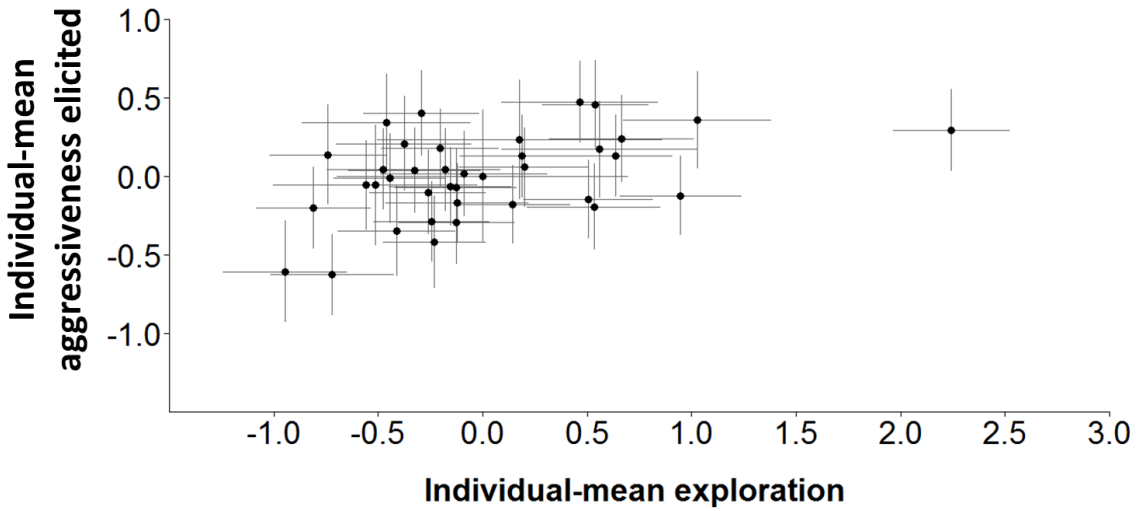

Figure 3. Graphical illustration of the indirect effect of exploration behaviour on aggression. We present here correlations between BLUPs derived from the multivariate mixed-effects model: individual-mean levels of exploration (x axis) and aggression elicited in the opponents (y axis). Each point represents the BLUP of an individual (in standard deviation units) with its associated standard error.

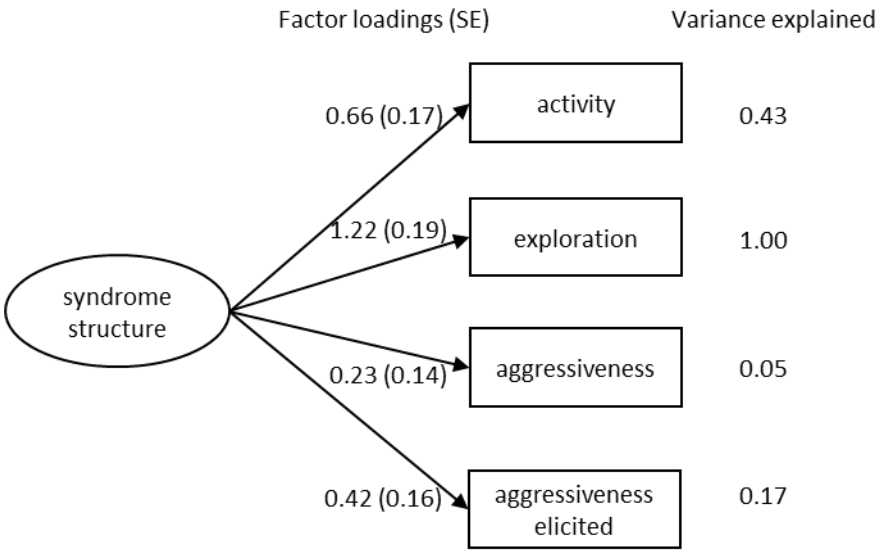

Figure 4. Parameter estimates of the structural equation model that best fitted our data. For each variable, we report the variance explained by the SEM structure (R^2) and factor loadings with the corresponding SE in parentheses. These represent how behavioural responses are predicted to change based on changes to the underlying syndrome structure.

SUPPLEMENTARY TEXT

As detailed in the main text, analyses of aggressiveness that estimate focal and opponent identity effects typically focus on variation in the behaviour expressed by the (arbitrarily assigned) focal individual alone. Here we detail how we incorporated information on the same behaviour measured on the opponent in the statistical model while avoiding pseudo-replication. We started with the following data structure, where each line consisted of information regarding the identity of both individuals, one arbitrarily called 'Individual A' and the other 'individual B', with associated information regarding their aggressiveness:

trial ID	Individual A	Individual B	Aggressiveness A	Aggressiveness B
1	14	12	3	6

We then rearranged the data in the following way:

trial ID	Focal	Opponent	Dataset	Aggressiveness 1	Aggressiveness 2
1	14	12	1	3	NA
1	12	14	2	NA	6

In this re-ordered dataset, the data is printed over two lines, once viewing individual A as the 'focal' individual in trial 1 (Dataset 1) and once viewing individual B as the 'focal' individual in trial 1 (Dataset 2). Importantly, the behaviour of the individual dubbed 'focal' in Dataset 1 was printed in another column (Aggressiveness 1) than the behaviour of the individual dubbed 'focal' in Dataset 2 (column Aggressiveness 2). We then proceeded to

estimate model parameters under the imposed assumption that all model parameters (fixed effect coefficients and (co)variance components) are equal for the homologous traits as defined in the two non-overlapping data sets (i.e., Aggressiveness 1, Aggressiveness 2). Practically this can be achieved for a pair of homologous traits by fitting a bivariate mixed effect model with the following code in ASReml, which we have annotated in footnotes below:

```
agg1 agg2  ~mu !r !{Trait.foc Trait.opp !}

1 2 1
0
Trait 0 US !GPZP !=a0a !S2==1   #A
0.5
0     0.5
Trait.foc 2
4 0 US     !GPZPUZPZUZP  !=a0ab0c0b0c    #B
0.5
0     0.5
0.1  0         0.5
0     0.1      0         0.5
foc
```

The !{Trait.tag Trait.opp !} command enables joining the focal and opponent variance-covariance matrix into a single matrix such that covariances between focal and opponent identity effects can be estimated.

A – Residual covariance structure. Residual variances are constrained to be positive and equal for the two traits. A starting value of 0.5 is supplied. Since no line of data is informative for both traits the residual covariance is not estimable and is fixed (arbitrarily) to zero.

B – Among-individual covariance structure. There are four random effects in the model (focal and opponent effects on two homologous traits) so a 4x4 covariance matrix is specified. Variances are constrained to be positive (starting value of 0.5 supplied for each), while covariance terms are identifiable between focal and opponent effects with each trait (starting value of 0.1 supplied). Covariance parameters for Aggression 1 are constrained to equal those of Aggression 2. All other cross-trait covariance terms are fixed to zero.

SUPPLEMENTARY TABLES

Table S1. Parameter estimates (with standard errors) of fixed and random effects derived from our univariate models fitted to analyse variation in activity, exploration, weight, and aggressiveness (approach, chasing, singing). Values printed in bold face represent significant effects based either on Wald F tests (fixed effects) or likelihood ratio tests (random effects) detailed in the main text. Values of adjusted individual and opponent repeatability reported in Table 2 (main text) are derived from the univariate models printed here.

Table S1 continued

	Activity	**Exploration**	**Weight**	**Approach**	**Chase**	**Sing**
Fixed effects	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)
Intercept	-0.01 (0.20)	0.16 (0.22)	-0.21 (0.23)	0.33 (0.12)	0.07 (0.10)	0.06 (0.01)
Sequence	-0.03 (0.02)	**-0.04** (0.01)	**-0.04** (0.01)	**-0.07** (0.01)	-0.02 (0.02)	-0.01 (0.02)
Random effects	σ^2 (SE)	σ^2 (SE)	σ^2 (SE)	σ^2 (SE)	σ^2 (SE)	σ^2 (SE)
Focal	**0.32** (0.11)	**0.40** (0.10)	**0.46** (0.11)	**0.19** (0.07)	**0.09** (0.05)	0.08 (0.05)
Opponent	-	-	-	**0.16** (0.07)	**0.12** (0.06)	0.05 (0.05)
Group	0.01 (0.07)	0.06 (0.10)	0.15 (0.15)	0.03 (0.09)	0.00 (0.00)	0.00 (0.00)
Date	0.00 (0.02)	0.00 (0.01)	**0.18** (0.06)	0.00 (0.03)	0.00 (0.00)	0.04 (0.09)
Residual	**0.66** (0.07)	**0.55** (0.08)	**0.22** (0.04)	**0.63** (0.07)	**0.78** (0.08)	**0.85** (0.09)
Covariance				Cov (SE)	Cov (SE)	Cov (SE)
Focal - Opponent	-	-	-	0.04 (0.05)	-0.03 (0.04)	-0.01 (0.03)

Table S2. Estimated bivariate among-individual correlations for all traits included in our multivariate model (Table 3): activity, exploration, aggression, aggression elicited in others, and body weight. We print here the χ^2-value (associated degrees of freedom = 1 in all cases) and values of *P* associated with likelihood ratio tests (LRTs) detailed in the main text (Methods).

Traits	r (SE)	X^2_1	*P*
activity - exploration	0.81 (0.08)	18.2	**<0.001**
activity - aggression	0.39 (0.18)	3.88	**<0.05**
activity - aggression elicited	0.16 (0.20)	0.63	0.426
exploration - aggression	0.39 (0.17)	230.5	**<0.001**
exploration - aggression elicited	0.45 (0.17)	5.54	**<0.05**
aggression - aggression elicited	0.09 (0.22)	0.14	0.700
weight - activity	0.17 (0.15)	225.4	**<0.001**
weight - exploration	0.22 (0.14)	2.27	0.132
weight - aggression	0.43 (0.16)	5.66	**<0.05**
weight - aggression elicited	-0.31 (0.18)	2.58	0.110

Table S3. Relative fit of nine candidate models (detailed in the main text) based on the Akaike's information criterion (AIC). We present each model's AIC-value relative to the model with the lowest AIC-value (ΔAIC), its weight, and relative likelihood.

Model	ΔAIC	Akaike Weight	Relative LL
3 - Full domain behavioural syndrome	0	0.75	1
9 – Behavioural syndrome with aggressiveness elicited only affected by exploration	2.35	0.23	0.31
2 - Behavioural syndrome with aggressiveness elicited independent	8.91	0	0.01
8 – Behavioural syndrome with aggressiveness elicited only affected by activity	9.90	0	0
7 – Behavioural syndrome with aggressiveness elicited linked only to aggressiveness	10.78	0	0
4 – General activity syndrome with social behaviours independent	11.62	0	0
6 – Independent social behaviours and general activity syndromes	13.33	0	0
1 – Behavioural independence	48.94	0	0
5 – Social behaviours syndrome with general activity behaviours independent	50.65	0	0

Table S4. Estimated among-individual (a) and residual within-individual (b) variances and covariances/correlations (with SE) for two non-social behaviours (activity and exploration), two social behaviours (aggressiveness and aggressiveness elicited in opponents), and weight. We present variances on the diagonal, and covariances (correlations) on the lower (upper) off-diagonals, respectively. Covariances/correlations printed in bold-face are significant based on likelihood ratio tests derived from the multivariate model detailed in the main text.

a.	Aggressiveness	Activity	Exploration	Weight	Aggressiveness elicited
Aggressiveness	**0.21** (0.07)	**0.39** (0.18)	**0.39** (0.17)	**0.43** (0.16)	0.09 (0.22)
Activity	**0.11** (0.06)	**0.35** (0.06)	**0.81** (0.08)	**0.17** (0.15)	0.16 (0.20)
Exploration	**0.13** (0.06)	**0.33** (0.08)	**0.49** (0.10)	0.22 (0.14)	**0.45** (0.17)
Weight	**0.17** (0.07)	**0.08** (0.08)	0.13 (0.09)	**0.70** (0.13)	-0.31 (0.18)
Aggressiveness elicited	0.02 (0.04)	0.04 (0.05)	**0.13** (0.06)	-0.11 (0.07)	**0.18** (0.06)

Table S4 continued

b.	Aggressiveness	Activity	Exploration	Weight
Aggressiveness	**0.61** (0.06)	-0.02 (0.07)	0.03 (0.07)	0.05 (0.06)
Activity	-0.01 (0.04)	**0.65** (0.06)	**0.17** (0.06)	0.06 (0.06)
Exploration	0.02 (0.04)	**0.10** (0.04)	**0.52** (0.04)	**0.13** (0.06)
Weight	0.03 (0.03)	0.03 (0.03)	**0.06** (0.03)	**0.35** (0.03)

Table S5. Relative fit of three a posteriori considered models (detailed in the main text) based on the Akaike's information criterion (AIC). We present each model's AIC-value relative to the model with the lowest AIC-value (ΔAIC), its weight, and relative likelihood. Here we investigate how body weight is best incorporated into the best-fitting SEM presented in Table S3. For a visualization of each model's structure, see Fig. S1.

Model	ΔAIC	Akaike Weight	Relative LL
B – Weight as part of the full syndrome	0	0.73	1
A - Weight independent from full domain behavioural syndrome	1.98	0.27	0.37
C – Weight driving the behavioural syndrome	44.93	0	0

SUPPLEMENTARY FIGURES

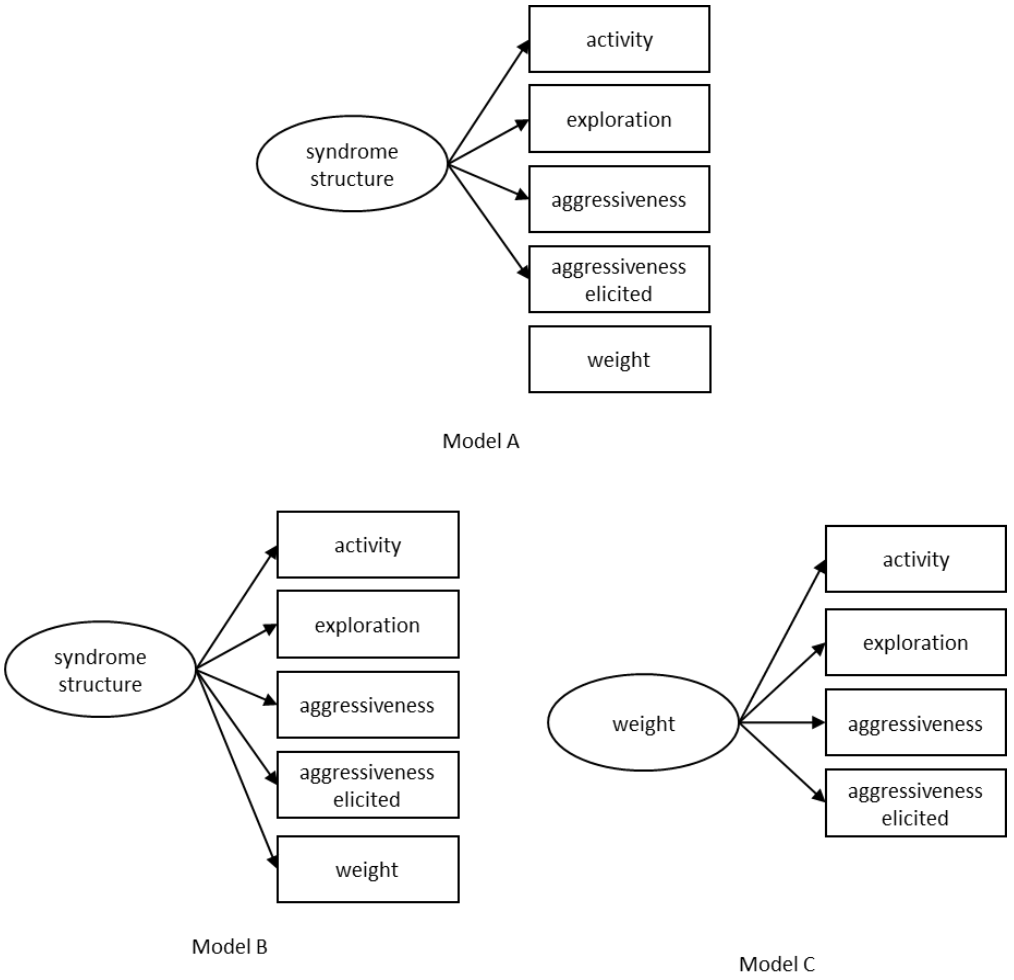

Figure S1. Three a posteriori considered models. The relative fit of these models (Table S5) was considered to investigate how body weight is best incorporated into the best-fitting SEM presented in Table S3. Model A: weight is independent from the behavioural syndrome; model B: weight is part of the syndrome; model C: weight causes the behavioural syndrome.

CHAPTER 2.

Indirect genetic effects: a new explanation for the maintenance of personality variation

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Manuscript

ABSTRACT

Recently, the social environment has been implicated as an important factor generating and maintaining among-individual behavioural variation ('personality'). Quantitative genetics theory implies that social environments can have major evolutionary repercussions when heritable phenotypes affect the phenotypes of other conspecifics: these are known as 'indirect genetic effects' (IGEs). This is true particularly when IGEs are correlated with direct genetic effects (DGEs) of an individual's genes on its own phenotype, as this is predicted to either constrain (negative correlation) or speed up (positive correlation) the response of a trait to directional selection. Here, we use a pedigreed lab population of Mediterranean field crickets (*Gryllus bimaculatus*) descending from wild-caught individuals to ask whether IGEs can maintain genetic variation in 'personality' expressed in both social (aggression) and non-social (exploration) contexts. Aggression depended both on the genotype of the focal individual (DGEs), and on the genotype of the opponent (IGEs). Furthermore, these effects were genetically tightly negatively correlated. When we extended analyses of IGEs to include effects on other behaviours, we found some evidence that even the evolution of a non-social trait such as exploration may get 'anchored', given that its DGEs were correlated with the IGEs acting on aggression. With this approach, we could identify in IGEs a largely overlooked mechanism to explain the maintenance of personality despite directional selection.

INTRODUCTION

Behavioural ecologists increasingly focus on studying the adaptive processes maintaining individual differences in behaviour within animal populations. Behaviours can be expressed multiple times throughout the lifetime of an individual, and thus vary both within ('plasticity') and among individuals ('personality')(Dingemanse et al. 2010). Several adaptive explanations have been proposed for why selection might maintain among-individual variation rather than erode it (reviewed by Dall et al. 2004; Dingemanse and Wolf 2010; Dingemanse and Réale 2013). For example, frequency dependent selection (Dall et al. 2004), temporal and spatial heterogeneity (Dingemanse et al. 2004, Réale and Dingemanse 2010) or life-history trade-offs (Wolf et al. 2007, Biro and Stamps 2008, Réale et al. 2010) have all been implied to explain the stable coexistence of different behavioural 'types' within populations. Recently, the social environment has also been implicated as an important factor generating and maintaining behavioural variation (Dingemanse and Araya-Ajoy 2015, Niemelä and Santostefano 2015, Santostefano et al. 2016). A key overlooked mechanism that may explain the maintenance of standing individual variation in behaviour is the existence of 'indirect genetic effects' (IGEs) caused by social interactions (Moore et al. 1997, Wolf et al. 1998, 1999, Wilson et al. 2009, McGlothlin et al. 2010).

Quantitative genetic theory implies that social environments can have major evolutionary repercussions when heritable phenotypes affect the phenotypes of other conspecifics. When the genes of an individual influence the expression of a trait in an interacting individual, these effects are called IGEs (Wolf et al. 1998, 1999, McGlothlin et al. 2010). IGEs can greatly influence evolutionary processes particularly when they are correlated with direct genetic

effects (DGEs) of an individual's genes on its own phenotype. For example, in mussel cultures, individuals genetically predisposed to grow quickly in competitive situations, are also genetically predisposed to reduce growth in other individuals by depriving them of feeding opportunities (Brichette et al. 2001). The resulting negative genetic correlations between DGEs and IGEs can impose major evolutionary constraints, as they effectively reduce the amount of heritable variation that is available to selection (Bijma 2011, 2014). IGEs may thereby explain why (genetic) variation can be maintained even in cases where there is strong directional selection acting on the focal trait. By contrast, positive genetic correlations between DGEs and IGEs on a trait are instead predicted to greatly speed up the response to directional selection compared to expectations derived from classic evolutionary theory (e.g. McGlothlin et al. 2010). For example, for a social behaviour such as aggression, a positive covariance between a genotype's aggression and the aggressiveness that it elicits in others is predicted to lead to rapid evolution of the trait, because selection for increased aggression also alters the social environment in which it is expressed (Wilson et al. 2009). Therefore, IGEs arising from social interactions can both provide a major source of heritable variation on which selection can act, as well as a represent a major source of evolutionary constraint (Wilson et al. 2011), and may thus explain why 'personality' variation might be maintained in natural animal populations.

IGEs are expected to exist particularly for traits such as aggression or dominance (Moore et al. 1997), i.e., traits that are expressed explicitly as part of social interactions. Interestingly, the effects of IGEs can also extend to other behaviours, such as those not expressed as part of social interactions (Niemelä and Santostefano 2015), as long as those are genetically correlated with traits that do harbour IGEs. For example, the literature on 'behavioural syndromes' often

finds that traits expressed in social interactions (e.g., aggressiveness, sociability) are correlated with other risky behaviours expressed in non-social contests, such as exploratory tendency, or anti-predator boldness (meta-analysis, Garamszegi et al. 2012). Of course, such effects will be important for the evolution of traits only if those associations are underpinned by genetic correlations. Thus, if IGEs are present in a social behaviour such as aggression, the evolution of any trait genetically correlated either with the social behaviour or its IGEs will be affected.

Here, we asked whether IGEs characterize the genetic architecture of heritable behavioural variation expressed in social and non-social contexts. We repeatedly measured behavioural traits (exploration, non-social, and aggression, social) in a pedigreed laboratory population of Mediterranean field crickets (*Gryllus bimaculatus*) descending from wild-caught grandparents. We quantified (i) whether exploratory behaviour and aggressiveness were heritable, (ii) whether they were genetically correlated, (iii) whether IGEs characterized the genetic architecture of aggressiveness, (iv) whether DGEs in aggressiveness were correlated with its IGEs (i.e., whether an individual's genetic merit for aggressiveness was correlated to the genetic merit for aggressiveness that it elicits in conspecifics), and (v) whether IGEs on aggression were also correlated to exploration, a trait not directly involved in social interactions. We did so using the 'animal model' (Kruuk 2004, Wilson et al. 2010) to estimate genetic correlations amongst the DGEs and IGEs within and across these behavioural traits. This approach suggests that drawing evolutionary predictions ignoring IGEs not only on the focal trait, but also on other seemingly independent traits, can be misleading. Because traits correlated with aggression cannot evolve if IGEs are present, we identify in IGEs a new mechanism to explain the maintenance of personality.

METHODS

This study was part of a large scale experiment fully described in Chapter 3. For details on cricket collection, breeding, rearing, as well as behavioural testing protocols, please see the Methods section of Chapter 3.

Statistical analyses

We conducted two sets of statistical analyses. First we partitioned the total phenotypic variance (V_P) for each measured trait into its underlying components: residual within-individual variance (V_R) and among-individual variance (V_I), the latter was then further partitioned into additive genetic (V_A) and permanent environmental (V_{PE}) effects. For aggression, we also estimated the variance explained by the opponent identity (V_{IIE}), which was, in turn, also split into its environmental (V_{IPE}) and genetic (V_{IGE}) components. This was achieved by utilizing a univariate mixed-effects modelling framework. The relatedness matrix calculated from the genetic pedigree was implemented in this 'animal model' to estimate the additive genetic variance (Kruuk 2004). As a second step, we used a multivariate extension of this framework to estimate patterns of trait covariance at the among-individual (**I**) level, further partitioned into the permanent environmental and genetic levels by respectively estimating the **PE** and **G** matrices. All models were fitted using restricted maximum likelihood; dependent variables were mean-centered and variance standardized to facilitate comparison of variance components across traits. Throughout, we assumed a Gaussian error distribution, which was confirmed for all response variables after visual inspection of model residuals.

Univariate mixed-effects models

Sources of variation in aggression and exploration were estimated by fitting the focal trait as the response variable into a univariate mixed-effect model. To statistically control for potential sources of variation in behaviours, we included the following fixed effects identified as important predictors for this dataset (described in Chapter 3): test sequence (covariate, range 1-6, mean centered), generation (F2 or F3) and clutch number (first or second, see Methods) (both coded as -0.5 and 0.5, following Gelman 2008).

We fitted random intercepts for focal individual (455 levels) and opponent identity (455 levels; fitted for aggression only). We structured the dataset in a way that it enabled us to simultaneously estimate focal and opponent identity effects (and their correlation). For details on how we assigned focal and opponent identities, as well as what types of the constraints were implemented in our models to avoid pseudo-replication, see the Supplementary material of Santostefano et al. 2016 (Chapter 1).

Adjusted individual repeatability (Nakagawa and Schielzeth 2010) was estimated for each behavioural trait by calculating the proportion of the total phenotypic variance not attributable to fixed effects that was explained by among-individual variance (i.e., where $V_I = V_{PE} + V_G$). For aggression, we also estimated opponent repeatability (i.e., the proportion of phenotypic variation in behaviour not attributable to fixed-effects that was explained by opponent identity) with the same approach. Heritability (h^2), indirect genetic effects (IGEs), and the proportional contribution of V_{PE} (pe^2) relative to the total phenotypic variance were estimated as the focal variance component divided by total phenotypic variance not attributable to fixed effects.

From this latter model, we further calculated the total breeding value (TBV) for aggression. TBV allows estimating the total heritable variation for this trait available to selection, taking

into account DGEs, IGEs, and their genetic correlation. TBV was calculated following Bijma et al. 2007) (eqn. 6, for a group size of two interacting individuals, n =2) as TBV = $V_G + V_{IGE} + 2COV_{G,IGE}$. We calculated the total heritable variation for aggression as h^2_{TOT} =TBV/V_{TOT} (Bijma et al. 2007).

Multivariate mixed-effects models

Using a multivariate mixed-effects model, we estimated patterns of trait covariance at the additive genetic (**G** matrix), and permanent environmental (**PE** matrix) level, by expanding upon the same structure of the univariate models. The multivariate model allowed us to estimate the correlation between the opponent identity effect on aggressiveness and the focal identity effect on exploration (**I** matrix), and enabled us to partition it into its genetic and environmental components. We fitted exploration and aggression as response variables and included only fixed effects that explained significant variation in univariate analyses (detailed above).

Significance testing in mixed-effects models

We tested the statistical significance of fixed effects using numerator and denominator degrees of freedom (df) estimated from the algebraic algorithm in ASReml 4.1 (Gilmour et al. 2015). We used likelihood ratio tests (LRTs) to evaluate the statistical significance of random effects. This χ^2-distributed test statistic is calculated as twice the difference in log-likelihood between a model where a target random effect was fitted versus not fitted (Shaw 1991). Variances are bound to be positive, therefore probability (P) of a LRT applied to a variance was calculated assuming an equal mixture of P (χ^2, df=0) and P (χ^2, df=1), i.e. df=0.5 (Self and Liang 1987, Pinheiro and Bates 2000, Visscher 2006). For multivariate models, we compared the fit of a model where all covariances at a specific focal level were estimated with one where those

covariances were instead all constrained to zero as an overall significance test of nonzero covariance structure. We further tested the significance of each covariance separately applying a LRT (df=1) as described above. To ask which of the alternative models fitted the data best, we also statistically compared the fit of the alternative models (both for univariate and multivariate, separately) using the AIC values (Akaike information criterion) (Akaike 1973, Burnham and Anderson 2002) associated with each model estimated in ASReml. AIC values were converted to numbers relative to the model with the lowest AIC (i.e., ΔAIC), where this latter model represented the best fitting model. We calculated the Akaike weight and model likelihood for each model (Anderson 2008) using the package 'qpcR' (Ritz and Spiess 2008) in R 3.1.0 (R Development Core Team 2013).

RESULTS

Sources of variation in single traits

An analysis described in Chapter 3 showed that all traits were repeatable and heritable (Table 1, Chapter 3, page 159). Our previous analyses showed that aggression was also significantly affected by the opponent identity (Table 1, Chapter 3, page 159), indicating that individuals differed consistently in how they affected another individual's aggressiveness. Here we expanded upon these analyses by estimating (genetic correlations with) IGEs. Doing so, demonstrated that this opponent effect harboured significant genetic variation ($V_{IGE} = 0.026$, SE 0.017) (Model 6, Table 1). In other words, aggressiveness harboured IGEs as there was heritable variation in the aggressiveness that individuals elicited in opponents. Furthermore, the genetic correlation between DGEs and IGEs for aggression was strong and negative (r_G -0.83, SE 0.37)

(Model 7, Table 1). AIC model comparison also provided strongest support for this final model (Model 7, Table S1). In other words, individuals genetically predisposed for higher levels of aggression also were genetically predisposed to suppress aggressiveness in opponents. As a consequence of this tight negative genetic correlation, the total heritable variation in aggression (h^2_{TOT} =TBV/V_{TOT} = 0.016, SE 0.030; where TBV = 0.051 + 0.026 – 0.030 = 0.016; V_{TOT} = 0.99) was considerably smaller (namely, 3.19 times) than what 'traditional' estimates of heritability based on DGEs would (inappropriately) conclude (h^2 = 0.051, SE 0.024).

Among-trait correlations

Our AIC model comparison also provided some evidence for the existence of genetic correlations between IGEs on aggression and DGEs expressed in another, non-social, behavioural trait (exploration; Table 3, Figure 1). That is, a model where both the correlation between DGEs on exploration and IGEs on aggression, as well as the correlation between DGEs and IGEs on aggression were estimated (model 3) fitted the data best. However, a genetic correlation between aggression and exploration was not present (see also Chapter 3). These findings were also supported by analyses of bivariate correlations in the multivariate mixed-effects model (Table 2). If IGE and DGE for aggression are highly correlated, then if one is correlated with another trait (exploration) than so should the other. As this was not the case, we suggest that the IGEs and DGEs for aggression were not as tightly correlated as implied by the point estimate. This interpretation is in line with the broad confidence interval around this genetic correlation between DGEs and IGEs, which implies that it could in fact be weaker in magnitude. In short, individuals with a high genetic merit for explorative tendency in novel environments also had a high genetic merit for eliciting aggression in opponents in the

aggression test (Table 2, Figure 1). This genetic correlation was relatively strong, although marginally non-significant when tested individually (0.59, SE 0.28), and together with the strong genetic correlation between DGEs and IGEs on aggression already detected in the univariate model (See above, Table 1; Table 2), implies that the social environment may indeed impose a strong genetic constrain on the evolution of personality traits, including those expressed outside the social context.

DISCUSSION

In this study we investigated a largely overlooked mechanism explaining the maintenance of genetic variation in behaviour despite selection. We asked whether IGEs might represent a mechanism maintaining genetic variation in 'personality' expressed in both social and non-social contexts. Our study on male Mediterranean field crickets confirmed that the phenotypic expression of aggression does indeed depend on the genotype of the focal individual (DGEs), as well as on the genotype of the opponent, as IGEs were indeed present. Furthermore, these direct and indirect effects on aggression were genetically tightly, and negatively, correlated. This finding can thus explain why genetic variation in aggressive personalities is not eroded by selection. As aggressiveness represents an important component of an often-documented "aggression-boldness syndrome", those constraining effects may thus also extend to other associated traits. When we thus extended analyses of IGEs to included effects on other behaviours, we found some evidence that even the evolution of a non-social trait such as exploration may get 'anchored', given that its DGEs were correlated with the IGEs acting on aggression. Our study therefore identifies IGEs as an important overlooked mechanism that

may represent a potent explanation for the maintenance of personality in various contexts. Our results also generally imply that genetic variation represented by indirect effects caused by social interactions can have consequences for the evolutionary trajectories of a wide range of traits, including those not expressed as part of social interactions (e.g., exploratory tendency, body size, etc.).

Direct and indirect genetic effects on aggression and their correlation

Both the focal and the opponent identity effect on aggression were repeatable (Chapter 3), and part of this among-individual variation was underpinned by additive genetic variation and thus heritable (h^2 focal = 0.051; IGEs = 0.026). The magnitude of IGEs on aggression, albeit small, was similar to that documented in other species (e.g. Wilson et al. 2009; Alemu et al. 2014). This result provides clear evidence for the notion that the phenotypic expression of aggression can potentially depend on the behavioural genotype of other individuals (i.e., because those "personality" traits were heritable).

We found a strong negative correlation between an individual's genetic merit for being aggressive (DGEs) and its genetic merit for eliciting aggression in conspecifics (IGEs). This negative correlation was expected for traits linked to competition such as dominance, because genetic predisposition to winning a contest in the focal will also make the opponent more prone to lose (Wolf 2003, Bijma et al. 2007, Wilson et al. 2011, Sartori and Mantovani 2012). If aggression in part reflects dominance (e.g., with dominant individuals only displaying aggression towards subordinates), negative correlations can be expected in this trait too (Wilson et al. 2011, Weiß and Foerster 2013). As a consequence, the potential for phenotypic response to selection in this behaviour is reduced (Bijma 2011, 2014), thus aggressiveness (like

dominance) cannot evolve in this species despite being a heritable trait (Moore et al. 2002, Wilson et al. 2011). This is highlighted in our results by the discrepancies between heritability estimates (h^2 aggression = 0.051), and the total heritable variation for aggression including IGEs and their covariance with DGEs (h^2_{TOT}=TBV/$V_{TOT,}$ = 0.016). Both positive (Wilson et al. 2009) and negative (Alemu et al. 2014) genetic correlations between direct and indirect effects on aggression have been documented in other species. The direction of this correlation is likely due to the difference between species where fights escalate with positive feedbacks (Huntingford and Turner 1987) and traits reflecting dominance, thus showing negative feedback. Therefore, the biology of aggressive interactions in each species may have implications for the maintenance of personality through IGEs, as only negative correlations between IGEs and DGEs will prevent erosion of genetic variation due to selection. The sign of the correlation of behaviours with IGEs, as well as the biological mechanism behind it, should receive more attention in future studies to be able to draw general conclusions on when personality is expected to be maintained/constrained by IGEs.

Cross-trait genetic correlations between direct and indirect genetic effects

Genetic merit for higher levels of exploratory tendency was linked with genetic merit for inducing higher levels of aggressiveness in conspecifics (Table2a, Figure 1). This same relationship between direct and indirect effects on two different behaviours exists at the among-individual level in a closely related species (r= 0.45, SE 0.17, Santostefano et al. 2016)(the among-individual correlation in the current study was similar: r= 0.37, SE 0.09; Table S2). A possible reason for this positive correlation could be that individuals genetically predisposed for higher tendency to explore a new environment are likely to also be involved in

more social interactions, and this may result in provoking more attacks in others. An alternative explanation could be that exploration is correlated with another trait important in social interactions, such as size or weapon morphology, and this is the trait directly correlated with the IGEs. Regardless of the mechanism driving this relationship, IGEs can cause heritable variation on the focal phenotype to become associated with heritable variation in a different trait of the interacting partners. Our example highlights that the evolution of behaviours expressed in a non-social context (e.g. exploration) can become 'anchored' to social traits (e.g. aggression) when IGEs are present on the latter. Specifically, this is true because the strong negative genetic correlation between DGEs and IGEs on aggression will likely fully constrain the microevolution of the social behaviour itself, and by consequence prevent selection from eroding genetic variation of any traits genetically correlated with social behaviours. For example, a genetic correlation with traits such as aggression and dominance means that the addictive genetic variance for traits that are causally intermediate between contest outcome/dominance and fitness (e.g. weaponry, resource-dependent traits) will also not all be available to facilitate a selection response (Wilson 2014). Importantly, this constraint would be present even when the DGEs of both traits are uncorrelated, as was the case in our study. In other words, had we not considered IGE, we would inappropriately have concluded that exploratory behaviour and aggressiveness were evolutionarily independent (Sih et al. 2004, Dochtermann and Dingemanse 2013).

Conclusions

In conclusion, an overlooked but crucial consequence of social interactions not considered in 'animal personality' research is that its associated IGEs can act as an 'anchor' on the

evolution of behaviour, preventing selection from eroding standing genetic variation. The 'genetic makeup' of the social environment may thus play a major role in maintaining genetic variation in animal personality, and other phenotypic traits. The merit of our approach is that by including IGEs into behavioural ecology's existing ecological frameworks to study 'personality', we may finally start fully integrating distinct areas of evolutionary biology such as quantitative genetics and behavioural ecology, and address outstanding questions regarding the maintenance of genetic variability and evolution of behaviour. Importantly, this heuristic framework may be broadly applied to any trait associated with traits involved in social interactions, and thus explain the maintenance of genetic variation in labile traits, such as behaviour, physiology, or metabolism. Indeed, traits such as coloration, ornaments, badge of status, are often correlated with aggression or dominance (Moore et al. 2002). More generally, our study demonstrates the importance of viewing the phenotype (or genotype) from a multivariate perspective. That is, predictions of how 'personality' traits respond to selection can be profoundly misleading if effects of social interaction mediated by IGEs are not considered when predicting their evolutionary trajectories.

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TABLES

Table 1. Results of the univariate mixed 'animal model' fitted to partition variation in aggressive behaviour with random intercepts for focal and opponent identity. Estimates of variance components and their correlations are given with the associated standard error. Random effects are expressed as the proportion of total phenotypic variation not attributable to fixed effects explained by each effect. Focal and opponent variances, as well as their covariance, are partitioned into environmental (PE) and genetic (G) components. For each model, variance terms are provided with a likelihood ratio test (LRT) between the given model and the previous model, with associated df and p value. The most parsimonious model (model 7) is denoted in bold face.

Model	Variance σ^2 (SE)							Correlations r (SE)			Test			
	Among-individual			Within-individual				Among-individual			LogL	X^2	df	p
	Focal			Opponent			Residual							
		PE	G		PE	G			PE	G				
1	-	-	-	-	-	-	0.98 (0.03)	-	-	-	-1168.01	-	-	-
2	0.17 (0.02)	-	-	-	-	-	0.83 (0.02)	-	-	-	-1131.75	72.52	0.5	<0.0001
3	0.17 (0.02)	-	-	0.11 (0.02)	-	-	0.71 (0.03)	-	-	-	-1116.89	29,27	0.5	<0.0001
4	0.17 (0.02)	-	-	0.11 (0.02)	-	-	0.71 (0.03)	-0.21 (0.11)	-	-	-1115.34	3.1	1	0.0782
5	-	0.12 (0.03)	0.05 (0.02)	0.11 (0.02)	-	-	0.71 (0.03)	-0.19 (0.14)	-	-	-1110.99	8.7	0.5	<0.05
6	-	0.12 (0.03)	0.05 (0.02)	-	0.08 (0.03)	0.03 (0.02)	0.71 (0.03)	-0.20 (0.15)	-	-	-1109.15	3.68	0.5	<0.05
7	-	**0.12** (0.03)	**0.05** (0.02)	-	**0.08** (0.03)	**0.03** (0.02)	0.71 (0.03)	-	0.01 (0.18)	**-0.83** (0.37)	-1107.05	4.2	1*	<0.05

*tested in addition over 1.5 df (test of variance and covariance together, against model 11), $X^2 = 7.88$, $p<0.05$

Table 2. Estimated (a) additive genetic (**G**) and (b) permanent environmental (**PE**) covariances and correlations (with SE) between two behaviours (aggression and exploration), and IGEs on aggression. We present covariances (lower-off diagonals) and correlations (upper-off diagonals) for each set of traits. Correlations printed in bold-face are significant (P<0.05) based on likelihood ratio tests derived from the multivariate model detailed in the main text.

a. G	Aggressiveness (DGE)	Exploration (DGE)	Aggressiveness elicited (IGE)
Aggressiveness (DGE)	-	-0.044 (0.243)	**-1.024** (0.404)
Exploration (DGE)	-0.005 (0.028)	-	0.591 (0.277)
Aggressiveness elicited (IGE)	-0.035 (0.015)	0.047 (0.025)	-

b. PE	Aggressiveness (DGE)	Exploration (DGE)	Aggressiveness elicited (IGE)
Aggressiveness (DGE)	-	0.341 (0.185)	0.029 (0.178)
Exploration (DGE)	0.045 (0.025)	-	0.302 (0.186)
Aggressiveness elicited (IGE)	0.003 (0.018)	0.035 (0.023)	-

Table 3. Relative fit of five multivariate models (detailed in the Methods) differing in architecture of genetic correlations between direct genetic (DGE) and indirect genetic (IGE) effects based on the Akaike's information criterion (AIC). We present each model's AIC-value relative to the model with the lowest AIC-value (ΔAIC), its weight, and relative likelihood. Model denominations refer to Figure 1: A is the correlation between DGEs and IGEs on aggressiveness; B is the correlation between DGEs on exploration and DGEs on aggressiveness; C is the correlation between DGEs on exploration and IGEs on aggressiveness. Model 5 (the complete model) is presented in Table 2a.

Model	ΔAIC	Akaike Weight	Relative LL
3. B = 0	0	0.78	1.00
4. C = 0	3.62	0.13	0.16
5. A, B, C estimated	5.49	0.05	0.06
1. A, B, C = 0	6.06	0.04	0.05
2. A = 0	8.64	0.01	0.01

FIGURES

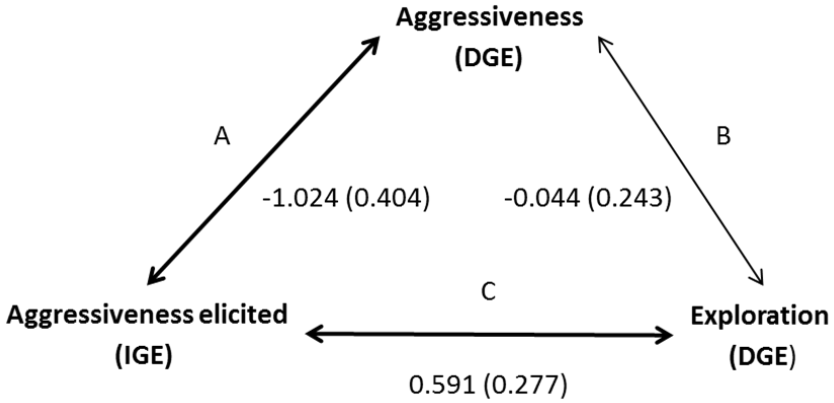

Figure 1. Correlation structure of the five hypothesized multivariate model structures presented in Table 3 (detailed in the Methods). A is the correlation between DGEs and IGEs on aggressiveness; B is the correlation between DGEs on exploration and DGEs on aggressiveness; C is the correlation between DGEs on exploration and IGEs on aggressiveness. Estimated correlations with correspondent SEs derived from the full model (Model 5, presented in Table 2a) are shown with each arrow; bolded arrows represent paths with statistical support from the LRT and AIC.

SUPPLEMENTARY TABLES

Table S1. Relative fit based on the Akaike's information criterion (AIC) of the seven univariate mixed models presented in Table 1. These models partition variation in aggressive behaviour and differ in random effects structure. We present each model's AIC-value relative to the model with the lowest AIC-value (ΔAIC), its weight, and relative likelihood.

Model	ΔAIC	Akaike Weight	Relative LL
7	0	0.82	1
6	3.64	0.13	0.16
5	5.71	0.05	0.06
3	13.39	0	0
4	14.37	0	0
2	41.64	0	0
1	102.36	0	0

Table S2. Estimated among-individual (**I**) covariances and correlations (with SE) between two behaviours (aggression and exploration), and IGEs on aggression. We present covariances (lower-off diagonals) and correlations (upper-off diagonals) for each set of traits. Correlations printed in bold-face are significant (P<0.05) based on likelihood ratio tests derived from the multivariate model detailed in the main text.

I	**Aggressiveness (DGE)**	**Exploration (DGE)**	**Aggressiveness elicited (IGE)**
Aggressiveness (DGE)	-	0.142 0.075	**-0.220** 0.115
Exploration (DGE)	0.037 0.020	-	**0.366** 0.087
Aggressiveness elicited (IGE)	**-0.030** 0.016	**0.077** 0.016	-

CHAPTER 3.

Behavioural mediators of genetic life-history trade-offs in field crickets

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Evolution, under review

ABSTRACT

The pace-of-life (POL) hypothesis predicts associations between life-history traits and 'risky' behaviours (i.e., behaviours facilitating resource acquisition at the cost of reduced longevity). Individuals with a 'fast' lifestyle should develop faster, reproduce at an earlier age, behave more risk-prone, and live shorter than those with a 'slow' lifestyle. Empirical tests, focusing on individual-level patterns, have provided equivocal support for POL theory. As environmental effects likely obscure the hypothesized genetic trade-offs, empirical tests on POL should focus on the genetic level. In this paper, we estimated genetic correlations between life-history (development, lifespan, size) and risky behaviours (exploration, aggression), using a pedigreed population of Mediterranean field crickets (*Gryllus bimaculatus*). We first estimated genetic trade-offs between life-history traits, which indeed existed. We then investigated whether genetic relationships between life-history traits were mediated by behaviours as expected by POL theory using path analyses. The best-supported model implied that risky behaviours indeed mediated certain genetic relationships between life-history traits (namely, between size and lifespan), though not those involved in genetic trade-offs. Predictions of POL theory were thus not supported. The genetic integration of behaviour and life-history nevertheless implied a major role of behaviour in life-history evolution and suggests that both aspects co-evolve in natural populations.

INTRODUCTION

Individuals of the same population often show tremendous variation in behaviour, physiology, metabolism, and morphology (Sih et al. 2004; Roff and Fairbairn 2007; Biro and Stamps 2008; Williams 2008; Réale et al. 2010; Careau and Garland 2012). Trade-offs in the allocation of resources among multiple costly traits are often implied as a mechanism maintaining among-individual variation in such phenotypic traits. Variation can be maintained because alternative solutions to resolve trade-offs may have similar fitness outcomes (Williams 1966; Roff and Fairbairn 2007). Trade-offs occur when a change (yielding positive fitness consequences) in one trait is linked to a change (yielding negative fitness consequences) in another trait (Stearns 1989), and may exist whenever individuals have access to limited amounts of resources to allocate to multiple costly traits. Examples of important life-history trade-offs are those between current versus future reproduction (Stearns 1989), between age and size at maturity (Stearns and Koella 1986), and between quantity and quality of produced offspring (Charnov and Krebs 1974).

Behavioural traits are often implicated as mediators of life-history trade-offs (Duckworth 2009). For example, aggressive individuals can be successful in competition and acquire more resources to be invested in current reproduction. However, aggressiveness can also increase risk of injury and thus shorten lifespan (Smith and Blumstein 2008). This putative integration of behaviour and life-history has recently come to the foreground in behavioural ecology, particularly in the context of research on ‘pace-of-life’ syndromes (POLSs) (Réale et al. 2010). The POL hypothesis predicts the integration between behavioural, life-history, and physiological traits along a ‘slow’ to ‘fast’ pace-of-life continuum. Specifically, Individuals with a

'fast' lifestyle should develop faster, reproduce at an earlier age but live less long (Stearns 1989) compared to those with a 'slow' lifestyle. Fast individuals are also expected to show increased expression of behaviours that facilitate resource acquisition at the cost of reduced longevity ('risky' behaviours) such as aggressiveness, boldness, exploratory tendency, or foraging activity (Stamps 2007; Wolf et al. 2007; Réale et al. 2010). The POL hypothesis has primarily been proposed to explain the maintenance of individual variation in behaviour (also called 'animal personality' in the behavioural ecology literature) by suggesting that their associated alternative life-history strategies may yield equal fitness (see above). Interestingly, despite repeated attempts to test the predicted correlations, there is considerable ambiguity about the direction, causality, and mechanistic bases of these presumed links between behaviour and life-history (e.g., Réale et al. 2010; Roff and Fairbairn 2011; Niemelä et al. 2013; Nicolaus et al. 2016).

This ambiguity in support of POL theory might stem from the fact that predicted relationships among traits are often tested solely at the individual level, while the hypothesized POL structure should logically exist at the genetic level (Sgrò and Hoffmann 2004; Réale et al. 2010). Individual-level tests explicitly assume that patterns of among-individual correlations reflect underlying genetic correlations (e.g., Brommer 2013), called the 'phenotypic gambit' in behavioural ecology (Grafen 1984) or 'Cheverud's conjecture' in evolutionary biology (Cheverud 1988). Theory and empirical examples convincingly demonstrate that this key assumption may often not be valid (Hadfield et al. 2007). For example, negative genetic correlations between life-history traits due to trade-offs can often be masked because individuals differ in resource availability (van Noordwijk and de Jong 1986; Stearns 1992; Reznick et al. 2000). This implies

that forceful tests of POL theory should involve the partitioning of patterns of phenotypic (or individual-level) correlations between behaviour and life-history to estimate the underlying POL structure at the genetic level. Surprisingly few studies have attempted to do so (Boersma et al. 1998; Niemelä et al. 2013).

Importantly, natural selection is expected to act on traits that have sequential or structured causal relationships with one another, and many biological processes have multiple pathways affecting fitness (Scheiner et al. 2000; Morrissey 2014). A key outstanding question is therefore whether life-histories vary as a function of behaviour (implying that each behavioural type might have its own optimal life-history; Wolf et al. 2007), or whether certain life-histories instead select for certain optimal behaviours. For instance, exploratory behaviour might facilitate fast growth by increasing resource acquisition (Biro and Stamps 2008; Biro and Stamps 2010; Wilson 2014), but fast growth might, alternatively, also affect the expression of risky behaviours (Wolf et al. 2007; Luttbeg and Sih 2010). Importantly, both scenarios might predict the same trait correlations while they differ in the direction of implied causality. Differentiating between such alternative scenarios requires comparison of the fit of statistical models that differ in their causal pathways, with tools such as path analyses (Wright 1934).

This paper aimed to test whether behaviours do indeed represent mediators of genetic life-history trade-offs as predicted by POL theory. We measured life-history (development, lifespan, size at maturation) and behavioural traits (exploration, aggression) in adult males of a pedigreed laboratory population of Mediterranean field crickets (*Gryllus bimaculatus*) descended from wild-caught grandparents. We first partitioned the phenotypic (**P**) into permanent environmental (**PE**) and genetic (**G**) variance-covariance matrices between life-

history traits using the 'animal model' (Kruuk 2004; Wilson et al. 2011). We then applied path analysis to each of these estimated matrices to test Cheverud's (1988) conjecture. Having empirically rejected Cheverud's conjecture, we then focused on life-history trade-offs at the genetic level, where we predicted a trade-off between development time and lifespan (Stearns 1989) and tested whether it was, as often predicted, mediated by size at maturity (Stearns and Koella 1986; Roff and Fairbairn 2007). As a next step, we included 'risky' behaviours (exploratory tendency and aggressiveness) in our models to estimate the genetic covariances among life-history and behavioural traits. We then compared the relative fit of different *a priori* considered path models as explanations for the estimated **G** matrix structure. These models differed in where in the pathway behaviour mediated genetic correlations between life-history traits, if at all (Figure 2). This study therefore forcefully evaluates whether risky behaviours indeed mediate genetic life-history trade-offs as predicted by POL theory, and thereby tests whether life-history trade-offs can indeed be invoked as a mechanism maintaining individual differences in risky behaviours in wild animal populations.

METHODS

Cricket collection and housing

The parental generation of crickets was collected from a tomato field of approximately 2500 m^2 near Capalbio, Italy (42°42'46.7' N 11°33'99.3' E) in July 2013. Collected individuals were part of a large meta-population as singing crickets were present in a 10×10 km^2 visited area surrounding the study site. We collected a total of 100 individuals: 34 adult males, 33 adult females, 12 near-final instar males, and 21 near-final instar females. Following capture, crickets

were placed in individual containers with food and water and transported to a (L×W×H: 4.5×3.6×2.7 m^3) climate chamber at the Ludwig Maximilians University of Munich (Planegg-Martinsried, Germany), where they were housed at 26°C (±0.5) and 65% (±0.5) humidity, under a 14:10 light:dark photoperiod (h) that wild crickets experienced at the time of capture.

Breeding and rearing

Sexually mature wild-caught individuals from the parental generation were randomly paired 4 days after arrival in the laboratory (August 1st 2013). A total of 35 males and 35 females produced a total of 34 clutches from which offspring hatched. We raised 40 offspring (F1) per parental pair (1360 offspring in total; see below for rearing protocol), from which we randomly selected breeders once reaching adulthood. We adopted a full-sib/half-sib breeding design (Lynch and Walsch 1998) for the F1 and F2 generations by having each male fertilize the clutches of two females. We used a total of 35 males and 70 females from the F1 generation, and 15 males and 30 females from the F2 generation. This resulted in 47 F2 and 21 F3 viable full-sib families.

Each adult male ('sire') was mated twice with each of two unrelated females ('dams') to ensure offspring production with each female in case the first clutch failed. Mating took place inside a plastic box (10×8×14 cm^3) equipped with a cardboard shelter, *ad libitum* food and water, and a plastic cup (diameter × height: 7×4.5 cm^2) filled with moist humus for oviposition. The male was moved after 3 days to the mating box of the second female; at the same time, the oviposition cup of the first female was moved to a plastic box (6×9×9 cm^3), where the eggs hatched on average after 13.04 (SD 2.63) days. Provided that ≥50 offspring hatched from the

first clutch, we discarded the second egg batch. If not, we used offspring from the second egg batch for our experiments. 5-6 days following hatching, we counted the nymphs in each box and placed 20 randomly chosen offspring in each of two new plastic rearing boxes (13×15×22 cm^3). In other words, 40 offspring per full-sib family were taken forward. Each rearing box contained a carton shelter, water and food *ad libitum,* and a substrate of fine pebbles and sand. After 5 weeks, containers were checked daily for final instars nymphs, which were subsequently removed and housed individually awaiting sexual maturation. Each adult individual was held in a plastic container (10×10×9 cm^3) with a sand-covered floor and a flow-through plastic netted lid that prevented escape but allowed air circulation. Each container included an artificial, half-cylindrical shelter (6×3.5×2 cm^3), a petri dish (with a diameter of 3.5 cm) with food, and another petri dish with water held within a cotton-plugged vial. Individuals were fed with a mix of dry bird food (Aleckwa Delikat, Germany) and fresh slices of apples *ad libitum*. Food and water were replaced every 3-4 days. Individuals were kept in these same conditions until natural death (F2 generation) or until they were euthanized at the end of the experiment by placing them in a -20°C freezer (F3 generation).

Adult males of the F2 and F3 generation were subjected to repeated behavioural assays, and measured for life-history (described in detail below). The study focused on males only because aggression through escalated stereotyped fights is largely male-limited, thus more difficult to measure in females. The final sample size of individuals that both reached the adult stage and were available for testing was partly a function of natural mortality. The number of available adult offspring (of both sexes) per female was n = 622 for the F2 and n = 281 for the F3 (per female mean ± SD: 8.64 ± 2.46 for the F2 and 5.51 ± 2.44 for the F3). Of these, a total of

455 males were selected and screened for behavioural and morphological phenotypes (335 from the F2 and 120 from the F3).

Life-history traits

We recorded femur length as a proxy for body size at maturity (Simmons 1986). We also recorded two key life-history traits, developmental time and lifespan. The right hind femur was measured with vernier calipers to the nearest 0.05 mm on the day after the final molt to maturity, and again after the individual was found dead. Because insects do not grow after the final metamorphosis, we averaged these two measurements (which were strongly positively correlated, r = 0.90) to reduce measurement error and we used this mean as measure of structural adult size in our statistical analyses. We calculated developmental time for each individual as the difference between the individual's date of final molt and the date of hatching for the egg clutch from which it emerged. We calculated an individual's adult lifespan as the difference between the death date and the date of final molt (for the F2 only because the F3 was euthanized prematurely).

Experimental protocol

Behavioural trials were conducted between January and June 2014. Each individual was repeatedly assayed for each of 2 behaviours on the same day (exploration and aggression, described in detail below) following Santostefano et al. (2016); the same individual was assayed for each behaviour 6 times, with measurements taken approximately one week apart (range 7-

9 days). Because individual identification is required for the aggression test (detailed below), subjects were marked with colored tape on the pronotum (red or blue, randomly assigned each time) the day before a focal trial (see also Santostefano et al. 2016). The two tests were always done sequentially and in the same order; carry-over effects could therefore not be modelled. We chose this set-up because it ensured that all individuals were given the exact same treatment since this greatly facilitates comparison between individuals (Dingemanse et al. 2007; Dochtermann 2010).

The 455 males were divided into 7 groups of 40 individuals (F2), one group of 55 individuals (F2), and 3 groups of 40 individuals (F3). 15 individuals of the F2 were only tested twice, because they were subsequently used for other purposes. Individuals were divided into groups according to their estimated age (days post-molting) to ensure that all the individuals of the same group were approximately the same age; this enabled us to avoid any possible age-related effects on aggression (see also Santostefano et al. 2016). It has been shown that adult crickets do not show fully developed aggressive behaviour within one week post-molting (Hofmann and Schildberger 2001); the behavioural assays were therefore conducted when individuals were at least 7 days post-molting. Groups were on average 13.3 days (SD 3.95) post-molting at the onset of their behavioural trials. All individuals within a group were tested on the same day (8 individuals simultaneously), randomized for time of the day and test location. Dyads of males put together for the aggression tests were randomly assigned amongst the non-related individuals within the same group to produce social environments that were homogenous with respect to relatedness.

All trials were performed on a rack fitted with two shelves, each equipped with a camera, in the same climate room where the individuals were housed (detailed in Santostefano et al. 2016). All trials were recorded using high-resolution digital video cameras (Basler GenICam, Germany) fitted 43 cm above each testing arena. The cameras were connected to a computer outside of the climate room and managed using the software MediaRecorder (Noldus, Netherlands). Videos were recorded at 27.81 frames per second and 1600×1200 pixels resolution.

A small number of trials were excluded from the final dataset: 31 of 1888 (F2) and 3 of 608 (F3) for exploration trials (respectively 1.64% and 0.49%), and 27 of 944 (F2) and 5 of 304 (F3) for aggression trials (respectively 2.86% and 1.64%) due to technical problems with data recording or video-tracking. Note that the total number of aggression trials is approximately half of that of other trials since two individuals are involved in each aggression test. The final sample size (behavioural tests) was therefore 2462 for exploration (mean number per individual: 5.27, SD 1.23) and 1195 for aggression (mean number per individual: 5.16, SD 1.28) tests.

Behavioural trials and scoring

Exploration: Prior to the onset of the behavioural trials, we gently moved the selected subjects (inside their home container) to the side of the exploration arenas. These arenas (29.5×15.5×9 cm^3), two of which were placed under each camera, had floors covered with white sand and an opaque removable divider in the middle (for an illustration of the setup, see Figure 2 in Santostefano et al. 2016). With the divider in place, the two halves (each

14.5×15.5×9 cm^3) of a single arena could be used to simultaneously assay exploration behaviour of the two individuals that would subsequently meet in an aggression test. At the onset of the exploration test, each individual was moved (inside its own shelter) from its home container to a (randomly allocated) half of the arena. Exploration activity was then recorded automatically for 30 minutes.

Aggression: Following the exploration test, the shelters were removed and the individuals given a further 10 minutes to acclimatize to their half of the arena. The divider was then lifted, after which we filmed each dyad engaging in social interactions for a period of 10 minutes. We then changed each individual's color tag according to the next trial's schedule, and returned it to the home container in the allotted housing slots within the climate room.

Exploration and aggression videos were analysed using Ethovision version 11.0 (Noldus, the Netherlands). This software package enables tracking of isolated individuals and extracts the spatial coordinates for each video frame. We summed up all distances to calculate the total distance moved in the novel environment (exploration test), viewed as proxy for 'exploration behaviour' (following Réale et al. 2007). For the aggression test, we instead calculated the total time each individual spent moving towards the opponent ('relative movement' for simplicity), by summing up only the consecutive samples (frames) where the relative distance between subjects decreased (see User manual of Ethovision v11.0, Noldus Information Technology 2014, for details). We set a maximum interaction distance between the two subjects of 8 cm based on pilot trials to define a range in which the directional movement towards the other cricket would be meaningful. The choice and validation of relative movement as a measure for aggression is detailed in the Supplementary Material.

Statistical analyses

We conducted four sets of statistical analyses. As a first step, we partitioned the total phenotypic variance (V_P) for each measured trait into its underlying components: residual within-individual variance (V_R) and among-individual variance (V_I), the latter was then further partitioned into additive genetic (V_A), permanent environmental (V_{PE}) and common environment (i.e. container) (V_C) effects. This was achieved by utilizing a univariate mixed-effects modelling framework. The relatedness matrix calculated from the genetic pedigree was implemented in this 'animal model' to estimate the additive genetic variance (Kruuk 2004). As a second step, we used a multivariate extension of this framework to estimate patterns of trait covariance across the same levels detailed for the univariate models above; common environment effects were not modelled here as univariate models indicated that there was no variation among containers for most of the traits. During this step, we implemented (i) a model fitting life-history traits only, and (ii) a model fitting all behavioural and life-history traits together. All models were fitted using restricted maximum likelihood; dependent variables were mean-centered and variance standardized to facilitate comparison of variance components across traits. Throughout, we assumed a Gaussian error distribution, which was confirmed for all response variables after visual inspection of model residuals. As a third step, we applied path analyses to the estimated **I**, **G**, and **PE** matrices calculated for the life-history traits to test Cheverud's conjecture. As a final step, we fitted sets of path models to the **G** matrix that included the genetic correlations between all behavioural and life-history traits, to quantify the relative amount of support for models differing in presumed causal relationships between life-history and behaviour.

Univariate mixed-effects models

Sources of variation in the repeatedly measured behavioural traits (aggression, exploration), as well as in life-history (developmental time, lifespan, adult size) were estimated by fitting the focal trait as the response variable into a univariate mixed-effect model. To statistically control for potential sources of variation in behaviours not relevant to our biological hypotheses, we included the following fixed effects: test sequence (covariate, range 1-6), time of the day (minutes from midnight, covariate), shelf (categorical variable with two levels), and location of the arena within a shelf (categorical variable with four levels). Test sequence and time of day were mean-centered, such that the fixed-effect intercept of the model was estimated for the phenotype expressed on the average testing day and time (following Dingemanse and Dochtermann 2013). Effects of variables fitted to control for variation induced by the experimental design (time of day, shelf, location) were unimportant (results not shown) and are not discussed further, except for the variable test sequence which explained significant variation. We fitted two further fixed effects of biological relevance as covariates for all traits: generation (F2 or F3) and clutch number (1^{st} or 2^{nd}, see Methods) (both coded as -0.5 and 0.5, following Gelman 2008).

For repeatedly expressed traits, we fitted random intercepts for date (64 levels; aggression and exploration only) and opponent identity (455 levels; aggression only). For details on rules applied to assign focal and opponent identities, as well as the constraints implemented in our models to avoid pseudo-replication when estimating sources of variation in aggressiveness, see the Supplementary Material provided by Santostefano et al. (2016). As this paper is not concerned with within-individual variation, effects of day or opponent identity are

not discussed further. For all traits, we fitted individual (455 levels) and group rearing container identity (120 levels) as additional random effects, while the residual variance was constrained to zero for traits that were not repeatedly measured (life-history). This is because with repeated measures, V_R is interpretable within-individual variance but with a single observation per trait, V_R and V_I are not separately identifiable. Following Wilson et al. (2010), we proceeded to partition the among-individual variance (V_I) into its permanent environmental (V_{PE}) and additive genetic (V_A) effects. It is important to note that because traits such as morphology and life history were measured only once, their V_{PE} thus includes all sources of phenotypic variance that were not modelled (such as plasticity and measurement error). Adjusted individual repeatability (Nakagawa and Schielzeth 2010) was estimated for each behavioural trait by calculating the proportion of the total phenotypic variance not attributable to fixed effects that was explained by among-individual variance (i.e., where $V_I = V_{PE} + V_C + V_G$). Heritability (h^2) and the proportional contribution of V_{PE} (pe^2) and V_C (c^2) relative to the total phenotypic variance were estimated for all traits as the focal variance component divided by total phenotypic variance not attributable to fixed effects.

Multivariate mixed-effects models

Using multivariate mixed-effects models, we estimated patterns of trait covariance at the phenotypic (**P** matrix), among-individual (**I** matrix), additive genetic (**G** matrix), and permanent environmental (**PE** matrix) level, by expanding upon the same structure of the univariate models. We fitted two multivariate models, one including only the three life-history traits, and another including all behavioural and life-history traits together. Following Wilson et al. (2010), we only included fixed (sequence) and random effects that explained significant variation in the

univariate analyses (see Results). Residual variances for (and covariance between) the two repeatedly observed behaviours were modelled but residual (co)variance terms for other traits were not identifiable (as detailed above) thus fixed to zero. Note that environmental sources of covariance among all traits were thus modelled in the **PE** matrix.

Significance testing in mixed-effects models

We tested the statistical significance of fixed effects using numerator and denominator degrees of freedom (df) estimated from the algebraic algorithm in ASReml 3.0 (Gilmour et al. 2009). We used likelihood ratio tests (LRTs) to evaluate the statistical significance of random effects. This χ^2-distributed test statistic is calculated as twice the difference in log-likelihood between a model where a target random effect was fitted versus not fitted (Shaw 1991). Variances are bound to be positive, therefore probability (P) of a LRT applied to a variance was calculated assuming an equal mixture of P (χ^2, df=0) and P (χ^2, df=1), i.e. df=0.5 (Self and Liang 1987; Pinheiro and Bates 2000; Visscher 2006).

Path analyses

We applied a single type of path model to the standardized correlation matrix estimated for each hierarchical level to investigate the presence of (level-specific) relationships between the life-history traits. This path model estimated the effect of development time on longevity via size (i.e., an indirect pathway) as well as any residual effect of development time on longevity (i.e., due to the mediating effects of any unmeasured, size-unrelated, variable) (Stearns and Koella 1986; Roff and Fairbairn 2007). These analyses confirmed the presence of significant paths at the additive genetic level (see Results). As a next step, we therefore investigated the relative fit of five alternative causal models to explain whether and how

behaviours mediated these genetic relationships between life-history traits (Figure 2). The genetic correlation matrix estimated among all behaviours and life-history traits was used as input for this analysis. These models broadly described the following scenarios: behaviours are independent from life-history traits (Figure 2, model 1), behaviours drive variation in life-history traits (Figure 2, model 2), or behaviours mediate specific relationships between specific life-history traits (Figure 2, models 3, 4, 5). The correlation matrices on which these path analyses were based are provided in the Supplementary Material (Table S1, Table S2).

The path analyses were performed using the structural equation modeling package 'SEM' in R 3.1.0 (Team R Core 2012). This package estimates a path coefficient and associated standard error (SE) for each specified path as well as the Akaike information criterion (AIC) value associated with a focal model. We statistically compared the fit of our five models using AIC (Akaike 1973; Burnham and Anderson 2002). AIC values were converted to numbers relative to the model with the lowest AIC (i.e., ΔAIC); this latter model represented the best fitting model.

RESULTS

Univariate analyses

Exploration and aggression showed significant among-individual variation (adjusted repeatabilities from univariate models: 0.46, SE 0.03 and 0.17 SE 0.02, respectively; Table 1). All traits harbored significant additive genetic variance, with heritability estimates ranging between 0.04 and 0.33 (Table 1). Behaviours also harbored significant among-individual variation not attributable to additive genetic effects (range: 0.11-0.16). Common environment

effects (variance attributable to rearing container) explained significant variation in size and developmental time, but not in behaviour or lifespan (Table 1).

A number of fixed effects on trait means were also important. Exploration and aggression changed significantly within individuals as a function of test sequence: individuals became less explorative and less aggressive over the course of the experiment (Table 1). All traits were significantly different between generations: individuals from the later generation (F3) were on average more explorative, aggressive, bigger, and developed slower (Table 1) compared to the earlier generation (F2). Exploration, size, and developmental time also differed between clutches: crickets that had hatched from second clutches were less explorative, bigger and developed faster compared to those from first clutches (Table 1). Neither of the behavioural traits were significantly affected by our experimental protocol (i.e., tag color, time of day, testing shelf or within-shelf test location were not significant; Results not shown). There was little (exploration) or no (aggression) evidence for day effects for the behavioural traits (Table 1). Aggression was significantly affected by opponent identity, which thus explained part of the within-individual variance (0.11, SE 0.02).

Path analyses

The path model applied to life history traits provided evidence for the expected genetic trade-off between development time and lifespan: genotypes associated with slow development were also predisposed to living longer (Figure 1c). This trade-off was not mediated by size: larger individuals did live longer as expected but development time did not positively affect size. Importantly, the residual (size-independent) relationship indicative of a

life-history trade-off between development time and lifespan was opposite at the permanent environmental level (Figure 1b), and we therefore rejected Cheverud's conjecture for this dataset. The phenotypic (i.e., individual-level) patterns therefore did not appropriately reflect those characterizing the genetic level (compare Figure 1a with 1c).

As a next step, we compared the relative fit of the five path structures to investigate the role of behaviour in mediating genetic relationships (Figure 2). The model where the behaviours mediated the relationship between size at maturity and lifespan (model 4), was unequivocally best supported (Figure 2). The best supported model described a structure where size influenced the behaviours, which, in turn affected longevity (Figure 2, Model 4). Specifically, individuals genetically predisposed for a larger size were also genetically predisposed for higher levels of aggression, which had a positive effect on lifespan. On the contrary, these individuals were also genetically predisposed for lower levels of exploratory tendencies, which in turn negatively affected lifespan. The two behaviours were, importantly genetically linked only because both were affected by size, as there was no residual covariance between them. Importantly, the presence of a direct (size-unrelated) genetic influence of development time on lifespan was also supported in this model. This trade-off was, notably, not mediated by the behaviours. These findings therefore do support a role of behaviour in mediating relationships between life-history traits but do not support the notion that they mediated trade-offs between life-history traits (POL hypothesis).

DISCUSSION

Our study combined path analysis with quantitative genetics to assess the role of behaviour in mediating key life-history trade-offs in Mediterranean Field crickets. A first analysis implied that there were indeed genetic trade-offs between development time and lifespan (Stearns 1989). However, when we included behaviours in the analysis, the best supported path model indicated that this trade-off was not mediated by 'risky' behaviours (i.e., *contra* POL theory). Behaviours instead mediated the size-lifespan relationship, which was not part of the trade-off between development time and longevity (as size affected longevity but development time did not affect size). In other words, an individual's 'personality type' did not determine how it resolved this focal life-history trade-off, and therefore we did not find support for the existence of a POL syndrome at the genetic level. Importantly, as expected, we did find some relevant differences between patterns of covariance between life history traits at the genetic and individual level (the latter presenting the normal target of behavioural ecology studies of POL), which were mediated by permanent environmental effects. This suggests that extreme caution is required in predicting evolutionary consequences of POL structures without information on its additive genetic architecture.

Sources of variation and heritabilities of traits

Both behaviours (exploration and aggression) were repeatable and their estimates were within the normal range reported for behavioural traits (meta-analysis: Bell et al. 2009) and similar to those documented previously in this and other cricket species (Wilson et al. 2010a; Niemelä et al. 2012a; Niemelä et al. 2012b; Dochtermann and Nelson 2014; Fisher et al. 2015a;

Fisher et al. 2015b; Santostefano et al. 2016). Aggression harbored a relatively low repeatability (0.17), as expected for a socially plastic trait expressed in the presence of conspecifics (Santostefano et al. 2016). All traits (behaviours and life-history) were significantly heritable (range 0.04 to 0.33); again, heritability of aggression (0.04) was substantially lower than the other four traits as expected. For all behaviours, the relative magnitude of environmental compared to genetic effects confirms that environmental effects play an important role in shaping the phenotype, which is typical even under standardized laboratory conditions (reviews: van Oers and Sinn 2011; Dingemanse and Dochtermann 2014; Dochtermann et al. 2015). Overall, we found evidence that the quantified traits varied across multiple hierarchical levels (i.e., individual, genetic, and environmental). This finding gives rise to the possibility of level-specific patterns of covariance, which we indeed detected. Caution is therefore needed in interpreting individual-level relationships between components of POL syndromes when testing POL theory.

Life-history trade-offs

Our results confirm the overall existence of an allocation trade-off (Stearns 1989) as development time directly and positively affected lifespan at the genetic level when controlling for size. This indicates that individuals do seem to pay a cost for fast development, perhaps due to a 'lower quality' soma, immune function, or repair mechanisms (reviewed by Mangel and Stamps 2001). Importantly, this trade-off was not mediated by size, as the predicted trade-off between age and size at maturity (Stearns and Koella 1986; Roff 2000; Roff and Fairbairn 2007) was not supported by our analyses. Interestingly, we did find evidence for a trade-off between

age and size and maturity but only at the permanent environmental level. Furthermore, at this level the size-independent effect of development time on lifespan was negative. This level-specific residual relationship was expected as individuals with abundant resources might both develop fast and live long, thereby masking the trade-off between those two life-history traits at the genetic level (van Noordwijk and de Jong 1986). This finding is consistent with the famous 'big cars, big houses' scenario predicting that environmental heterogeneity masks genetic trade-offs.

Do behaviours mediate life-history trade-offs?

The best supported model (model 4) indicated that risky behaviours did not mediate life-history trade-offs (*contra* POL theory). Rather, the genetic trade-off between developmental time and lifespan was independent from risky behaviours and caused by other intrinsic factors not measured in our study (see below). Instead, behaviours mediated the positive relationship between size and lifespan. Individuals with high residual reproductive value should invest in improving survival and thus be less (instead of more) willing to take risks (Wolf et al. 2007; Dingemanse and Wolf 2010; Réale et al. 2010; for an experimental test, see Nicolaus et al. 2012). We therefore expected that bigger individuals (having a 'slower' pace-of-life), would be less aggressive and explorative than smaller ones. The effect of size on exploration was negative as predicted, however, size affected aggression positively. This relationship is not consistent with the POL hypothesis but might instead be explained by ideas in the contest behaviour literature, where size is assumed to be an important determinant for competitive ability, and larger size is often thought to mediate aggressiveness and dominance. Various

studies on crickets confirm the predictions of this alternative explanation (e.g. Simmons 1986; Brown et al. 2006; Santostefano et al. 2016).

Because both aggression and exploration are assumed to represent 'risky' behaviours, we expected both of them to influence lifespan in a qualitatively similar way. Interestingly, aggression and exploration instead affected lifespan in opposite directions: individuals genetically predisposed for higher levels of aggression live longer, but those genetically predisposed for higher exploratory tendencies live shorter. This finding highlights the strength of our experimental design, which allowed us to disentangle 'intrinsic' from 'extrinsic' causes of mortality (*sensu* Medawar 1952; Williams 1957). The effect of exploration on lifespan in our study represents mostly the intrinsic effect of behaviour on mortality, because no external factors such as predation, resource (food, territory) limitation, adverse weather, etc., were present. Thus, permanent environmental effects should not substantially divert the phenotype from the direction of the genetic correlations. Both the antagonistic pleiotropy (Williams 1957) and the disposable soma (Kirkwood 1977) theories of aging address intrinsic mortality. These theories lead to similar predictions in terms of life-history trade-offs between reproduction during early life and allocation to mechanisms for somatic maintenance. The negative effect of exploration on lifespan may thus be explained in this context. The case for aggression is different, because individuals do meet opponents and therefore more aggressive individuals may be involved in more agonistic interactions and may thus accumulate more damage, which may in turn shorten their lifespan; for aggressiveness there is thus also the potential for extrinsic mortality to act within this laboratory set-up. This reasoning is consistent with patterns observed at the permanent environmental level, where the relationship between the two traits

tended to be negative (though not significantly so) (Figure S1). Surprisingly, aggressiveness instead positively affected lifespan at the genetic level, indicating that the intrinsic costs for this behaviour may not follow the same mechanism as for exploration. A possible explanation for this outcome is provided by the 'coping style' literature (Koolhaas et al. 1999; Coppens et al. 2010), where research on behavioural stress physiology implies that less aggressive individuals are more reactive to their environment and therefore need a more 'expensive' fine-tuned sensory machinery to respond appropriately to external cues. An interesting idea for future research would thus be to test whether aggressive animals have a lower intrinsic mortality but a higher extrinsic mortality compared to less aggressive conspecifics.

Conclusions

The POLS framework has been proposed to explain patterns of among-individual correlations, however, the underlying implicit assumption is that life-history trade-offs exist at the genetic level. The importance of our study lies in that it explicitly tests the POL predictions at the genetic level. Our results point out that presumed genetic trade-offs predicted by POL theory might be masked by environmental effects, and therefore the popular 'phenotypic gambit' may not hold. Furthermore, in this paper we applied path analyses as a useful tool to uncover causal relationships between traits that may otherwise be undetected by addressing simple correlations among traits. For example, as it appears from our path analysis of **G**, aggressiveness and exploratory tendency strongly depend on size, and both mediated its effects on lifespan, and thus their independent evolution may still be constrained despite them not showing any size-unrelated genetic correlation. This example highlights the importance of

(multilevel) path analysis to better understand the biological causal pathways underlying genetic correlations (Roff and Fairbairn 2011).

In conclusion, the application of the quantitative genetics approach allowed us to infer whether individual-level relationships represented those on which selection is ultimately acting. We achieved this by explicitly focusing on the genetic architecture of POL syndromes. Furthermore, path analysis enabled us to test the fit of alternative hypotheses predicting cause-effect relationships between behaviour and life-history. By combining these two approaches, we were able to draw novel biological inferences concerning POL research that would have remained hidden if variance partitioning and path analyses would not have been applied.

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TABLES

Table 1. Parameter estimates (with SE) of fixed and random effects derived from univariate models fitted to partition variation in exploration, aggression, size, lifespan, and developmental time. Random effects are expressed as the proportion of total phenotypic variation not attributable to fixed effects explained by each effect. Among-individual and additive genetic variances therefore represent repeatability and heritability, respectively. Values printed in bold face represent significant effects based either on Wald F tests (for fixed effects) or likelihood ratio tests (for random effects).

	Exploration	**Aggression**	**Size**	**Lifespan**	**Development time**
Fixed effects	**β (SE)**	**β (SE)**	**β (SE)**	**β (SE)**	**β (SE)**
Intercept	0.046 0.105	0.053 0.041	0.270 0.114	0.011 0.124	0.312 0.083
Sequence	**-0.083** 0.013	**-0.072** 0.012	-	-	-
Generation[a]	**0.566** 0.128	**0.155** 0.078	**0.599** 0.183	-	**1.810** 0.132
Clutch[b]	**-0.337** 0.109	0.027 0.069	**0.397** 0.169	-0.111 0.206	**-0.589** 0.123
Random effects	**σ^2 (SE)**	**σ^2 (SE)**	**σ^2 (SE)**	**σ^2 (SE)**	**σ^2 (SE)**
Individual	**0.459** 0.030	**0.173** 0.024	1.0*	1.0*	1.0*
- Additive genetic	**0.281** 0.085	**0.039** 0.027	**0.320** 0.155	**0.332** 0.117	**0.256** 0.143
- Permanent environment	**0.162** 0.062	**0.112** 0.029	**0.513** 0.122	**0.668** 0.117	**0.551** 0.113
- Common environment	0.016 0.024	0.023 0.017	**0.167** 0.071	0.000 0.000	**0.193** 0.069
Within-individual	0.537 0.018	0.816 0.030	0.0*	0.0*	0.0*
- Opponent	-	**0.111** 0.022	-	-	-
- Date	**0.015** 0.006	0.008 0.007	-	-	-
- Residual	0.526 0.029	0.708 0.031	-	-	-

*This trait did not vary within individuals; therefore all variance is among-individuals.
[a]Reference is 2nd generation
[b]Reference is 1st clutch

FIGURES

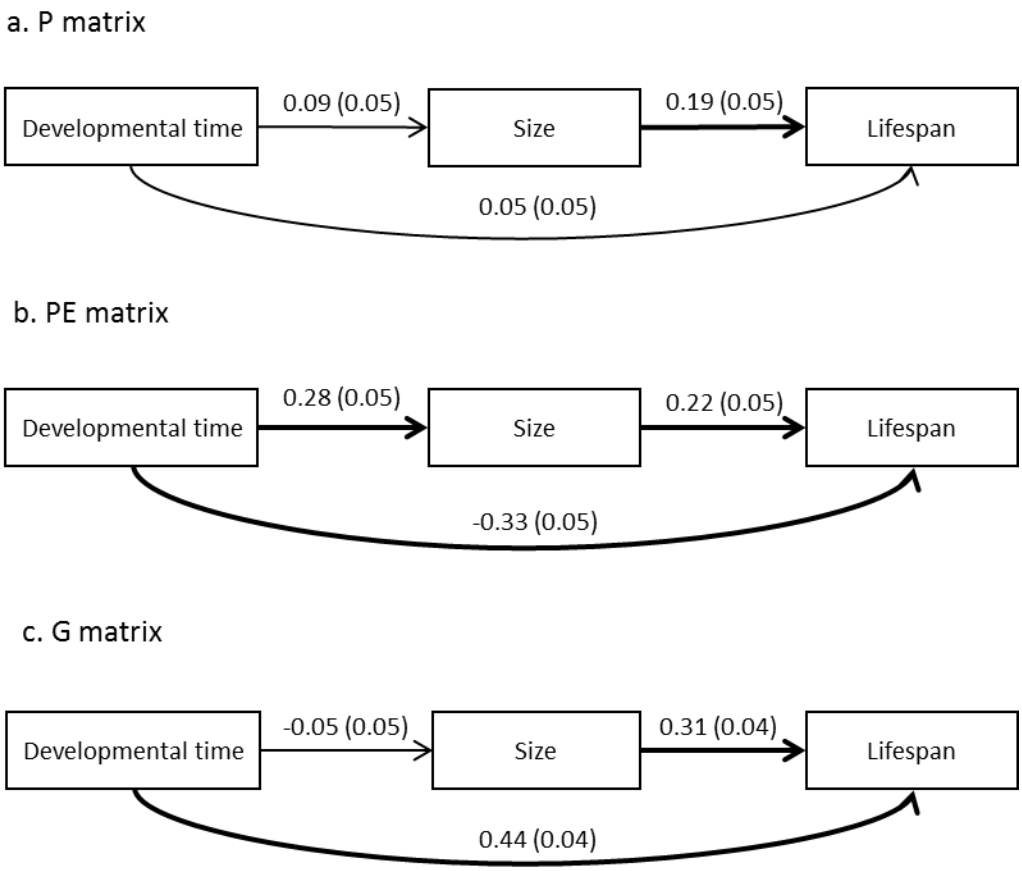
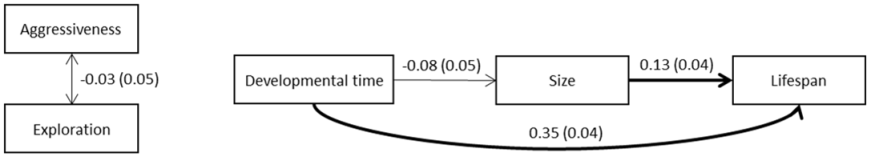
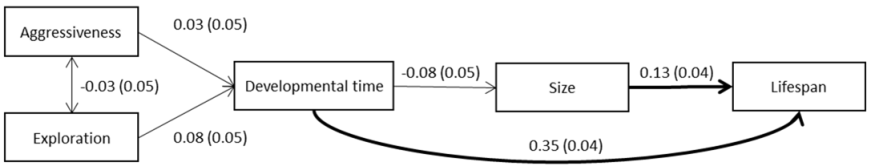


Figure 1. Path models based on estimated correlations between life-history traits (development, size, lifespan) at the (a) phenotypic, (b) permanent environmental, and (c) additive genetic levels. One-headed arrows indicate the direction of hypothesized causal links. Estimated partial regression coefficients with correspondent SEs are shown with each arrow; bolded arrows represent paths with statistical support ($p<0.05$).

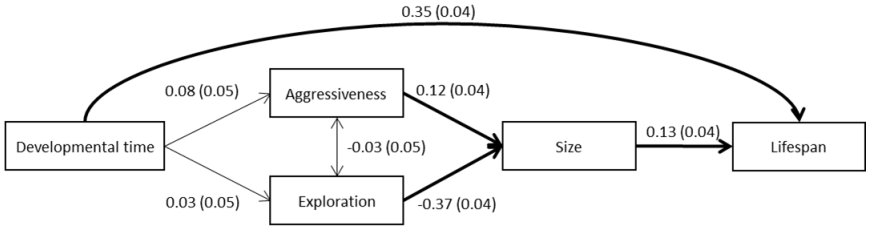
Model 1 **AIC = 138.07**



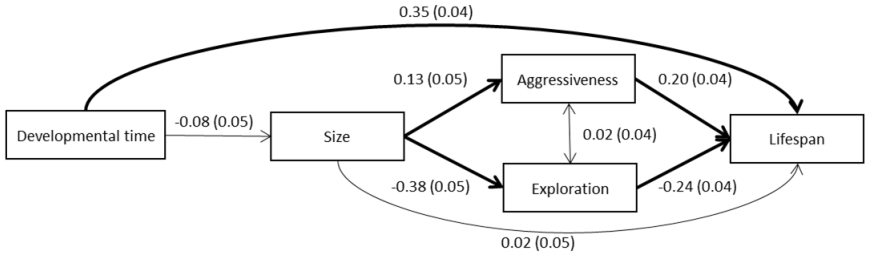
Model 2 **AIC = 138.74**



Model 3 **AIC = 69.40**



Model 4 **AIC = 28.04**



Model 5 **AIC = 98.69**

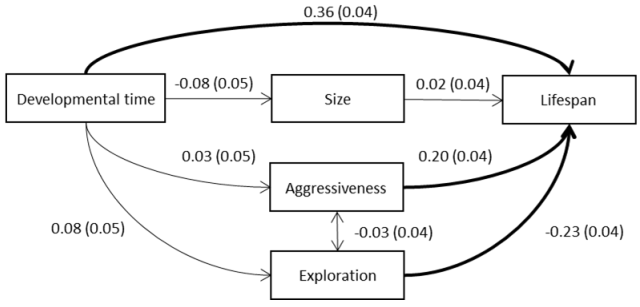


Figure 2. Path models integrating behaviour when modelling relationships between life-history traits applied to the genetic level. One-headed arrows indicate the direction of hypothesized causal links. Double-headed arrows indicate hypothesized correlations without a hypothesized cause-effect relationship. Estimated partial regression coefficients with correspondent SEs are shown with each arrow; bolded arrows represent paths with statistical support ($p<0.05$). Each model is presented with its associated AIC value. The model with the lowest AIC value (Model 4) is best supported by the data.

SUPPLEMENTARY MATERIAL

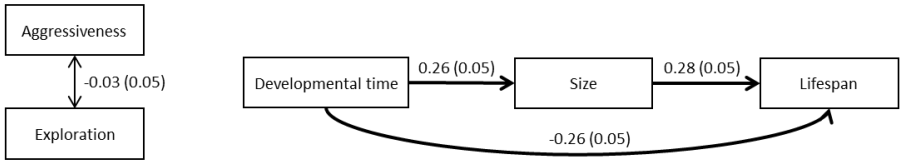
SUPPLEMENTARY TEXT

Validation of aggression measurements

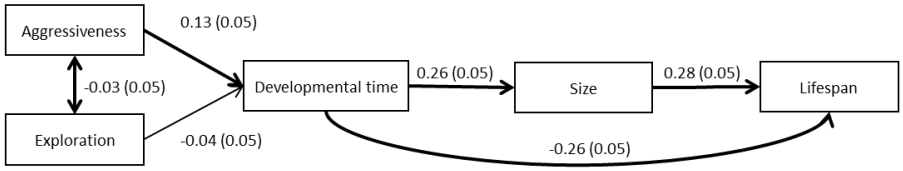
The choice of relative movement as a measure for aggression was taken in two steps. First, we explored how various candidate metrics (automatically derived from our tracking software) predicted aggression, scored manually as 'approach' towards the opponent in another dataset obtained on a related species (*G. campestris*) (detailed in Santostefano et al. 2016). We scored an individual as 'approaching' during an interaction when it moved towards the other individual from any angle until they came into contact. When only one individual was actively approaching the other (i.e. the other cricket sat still), we assigned the behaviour to that individual alone. In cases where both contestants approached each other at the same time, we assigned the behaviour to both. Amongst the automatically-derived candidate metrics, 'relative movement' provided the highest correlation with this manually scored measure of aggression (r = 0.85, 0.03 SE). We therefore selected this metric and validated its correlation with aggression (i.e., approach) in a randomly chosen subsample of the current dataset, where the correlation was indeed satisfactory (r= 0.80, 0.06 SE, n = 30 videos). This independent confirmation therefore supported the notion that 'relative movement' represented a reliable measure of aggression, and we therefore relied on this automatically-tracked measure of aggression for the full dataset.

SUPPLEMENTARY FIGURES

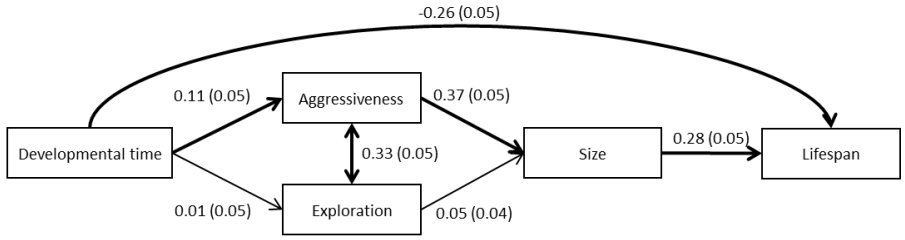
Model 1 AIC = 128.42



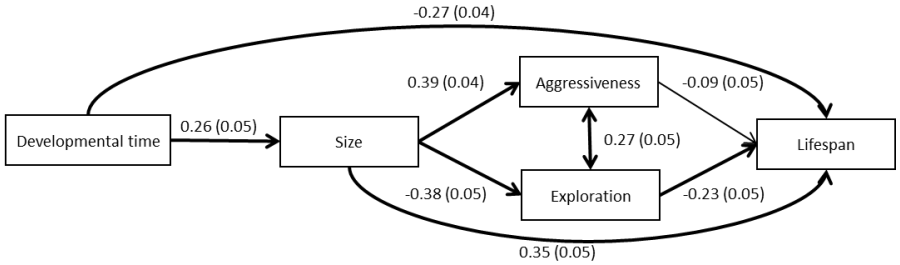
Model 2 AIC = 126.26



Model 3 AIC = 85.22



Model 4 AIC = 27.07



Model 5 AIC = 94.61

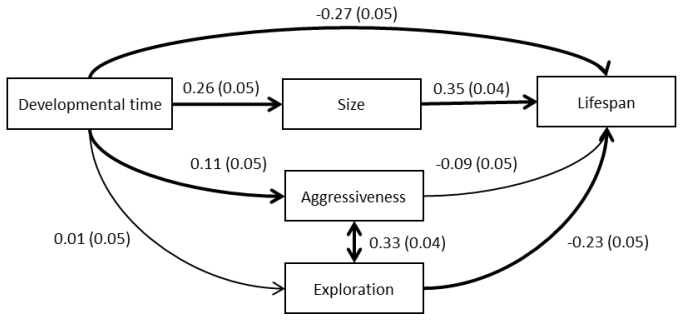


Figure S1. Path models estimating paths mediating non-genetic relationships between risky behaviours and life-history traits (analysis based on the **PE** correlation matrix; Table S2c). One-headed arrows indicate the direction of hypothesized causal links. Double-headed arrows indicate hypothesized correlations without a hypothesized cause-effect relationship. Estimated partial regression coefficients with correspondent SE are shown with each arrow; bolded arrows represent paths with statistical support ($p<0.05$). Each model is presented with its associated AIC value.

SUPPLEMENTARY TABLES

Table S1. Estimated (a) phenotypic (**P**), (b) permanent environmental (**PE**), and (c) additive genetic (**G**) covariances and correlations (with SEs) between life-history traits (size, development time, and lifespan). We present covariances (lower-off diagonals) and correlations (upper-off diagonals) for each set of traits.

a. P	**Size**	**Lifespan**	**Developmental time**
Size	-	0.20 (0.06)	0.07 (0.06)
Lifespan	0.18 (0.06)	-	0.09 (0.06)
Developmental time	0.06 (0.04)	0.05 (0.05)	-

b. PE	**Size**	**Lifespan**	**Developmental time**
Size	-	0.13 (0.14)	0.28 (0.17)
Lifespan	0.07 (0.08)	-	-0.27 (0.15)
Developmental time	0.10 (0.06)	-0.12 (0.06)	-

c. G	**Size**	**Lifespan**	**Developmental time**
Size	-	0.29 (0.24)	-0.05 (0.23)
Lifespan	0.13 (0.11)	-	0.43 (0.24)
Developmental time	-0.02 (0.09)	0.14 (0.09)	-

Table S2. Estimated (a) phenotypic (**P**), (b) among-individual (**I**), (c) permanent environmental (**PE**), and (d) additive genetic (**G**) covariances and correlations (with SE) between two behaviours (aggression and exploration), two life-history traits (development time and lifespan), and size. We present covariances (lower-off diagonals) and correlations (upper-off diagonals) for each set of traits.

a. P	Exploration	Aggression	Size	Lifespan	Developmental time
Exploration	-	0.11 (0.02)	-0.15 (0.05)	-0.07 (0.05)	0.04 (0.05)
Aggression	0.11 (0.02)	-	0.16 (0.05)	-0.10 (0.05)	0.02 (0.05)
Size	-0.14 (0.05)	0.15 (0.05)	-	0.19 (0.06)	0.08 (0.06)
Lifespan	-0.07 (0.05)	-0.10 (0.05)	0.02 (0.06)	-	0.08 (0.06)
Development time	0.03 (0.03)	0.02 (0.03)	0.05 (0.04)	0.06 (0.05)	-

b. I	Exploration	Aggression	Size	Lifespan	Developmental time
Exploration	-	0.14 (0.08)	-0.13 (0.04)	-0.20 (0.04)	0.04 (0.04)
Aggression	0.04 (0.02)	-	0.30 (0.06)	0.03 (0.06)	0.07 (0.06)
Size	-0.08 (0.02)	0.11 (0.02)	-	0.18 (0.06)	0.08 (0.06)
Lifespan	-0.13 (0.03)	0.01 (0.03)	0.16 (0.05)	-	0.07 (0.06)
Development time	0.02 (0.02)	0.02 (0.02)	0.05 (0.04)	0.05 (0.05)	-

c. PE	Exploration	Aggression	Size	Lifespan	Developmental time
Exploration	-	0.33 (0.19)	0.17 (0.15)	-0.20 (0.11)	0.01 (0.12)
Aggression	0.04 (0.03)	-	0.39 (0.12)	-0.06 (0.11)	0.11 (0.12)
Size	0.05 (0.03)	0.09 (0.03)	-	0.21 (0.13)	0.26 (0.15)
Lifespan	-0.06 (0.04)	-0.02 (0.03)	0.11 (0.07)	-	-0.19 (0.15)
Development time	0.00 (0.03)	0.02 (0.02)	0.10 (0.05)	-0.09 (0.06)	-

d. G	Exploration	Aggression	Size	Lifespan	Developmental time
Exploration	-	-0.03 (0.23)	-0.38 (0.12)	-0.21 (0.15)	0.08 (0.14)
Aggression	-0.00 (0.03)	-	0.13 (0.19)	0.22 (0.21)	0.03 (0.21)
Size	-0.13 (0.05)	0.02 (0.03)	-	0.11 (0.25)	-0.08 (0.24)
Lifespan	-0.06 (0.05)	0.03 (0.03)	0.04 (0.10)	-	0.34 (0.25)
Development time	0.02 (0.04)	0.00 (0.03)	-0.03 (0.08)	0.10 (0.08)	-

CHAPTER 4.

Do social partners affect same-sex sexual behaviour in male water striders?

Chang S. Han, Francesca Santostefano and Niels J. Dingemanse

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ABSTRACT

Same-sex sexual behaviour (SSB) represents an emergent trait of two interacting same-sex individuals. Though empirical studies have investigated how social environments can influence SSB, little is known about the effect of the interacting partner and its associated phenotype on SSB. In species where females are larger than males or males express male-specific behaviour, a male's morphology or behaviour can serve as a way for other males to recognize its sex and express SSBs. Here we used both a trait-based and variance-partitioning approach to test for the effect of the interacting male's identity and his multivariate phenotype on SSB, using water striders *Gerris lacustris* as a model. We repeatedly subjected males to dyadic interactions with the same and different partners and measured their SSBs. We used the variance-partitioning approach to estimate the partner's identity effect, and the trait-based approach to assess which trait of the partner explains changes in SSB. We found that the partner's SSB reduced the tendency of males to show SSB. SSB was affected by their interacting partner's identity, but the partner effect was not due to the repeatable components ('personality') of the partner's SSB. Males also did not differ in their responses to variation in the partner's SSB at different levels (among-partner, or partner 'personality', and within-partner levels, or partner 'plasticity'). Taken together, these findings provide the first empirical evidence that SSB can be plastically expressed in response to traits in social partners. We also highlight the usefulness of combining the trait-based and variance-partitioning approach to test whether partners represent a component of the social environment affecting the expression of labile traits.

Key words: Interaction coefficient, Same-sex sexual behaviour, Trait-based approach, Variance-partitioning approach, Water strider

INTRODUCTION

Many animals show sexual actions (e.g. courtship, mating attempts) towards members of their own sex. Such so-called same-sex sexual behaviour (SSB) is relatively widespread in nature (Bailey & Zuk 2009; Burgevin et al. 2013; Scharf & Martin 2013). Although SSB can be non-adaptively expressed as a product of weak sex discrimination (Scharf & Martin, 2013), SSB can also play a role in increasing mating opportunity (Han & Brooks 2015; Engel et al 2015) and confer fitness benefits (Han & Brooks 2015) despite its inherent cost (Bailey & Zuk, 2009; Gavrilets & Rice, 2006). Furthermore, SSB represents an emergent trait that requires social interactions between individuals (Kim & Ehrman 1998; Field & Waite 2004; Svetec & Ferveur 2005; Dukas 2006; Bailey et al. 2013; Han & Brooks 2014, 2015a, b) and thus varies as a function of the social environment. For example, deprivation of potential mates (Kim & Ehrman 1998; Svetec & Ferveur 2005) or restriction to mating with a single partner (Han & Brooks 2014) increases the level of SSB in various insects. Furthermore, density experienced during juvenile and adult life-stages can also interactively affect the expression of SSB (Han & Brooks 2015a).

SSB is, importantly, predicted to be primarily dependent on the phenotypes of the interacting individuals (e.g. Bailey et al. 2013). In the same way as the individual adjusts its sexual behaviour (e.g. courtship, mate choice) towards phenotypes expressed by members of the opposite sex, sexual behaviour towards the same-sex individuals may also be affected as a 'spill-over' effect (Sih et al. 2004). For example, male insects may adjust SSB plastically in response to morphological or physiological characters (e.g. body size or pheromones) of social partners. In species where females are considerably larger than males, males may particularly exhibit SSB when confronted with males that are relatively larger than themselves (Harari et al.

2000). Males may also increase the expression of SSB when conspecific males fail to express typical male-specific (e.g. courtship) behaviours, causing them to be mistakenly identified as females. This occurs in sexually monomorphic species with limited cues for sex recognition (e.g. Henry & Wells 2009). In contrast, male-specific courtship signalling may help to avoid mating attempts with same-sex partners. As a result, courtship behaviour does not only function to attract females but also to advertise sex and avoid mistakenly being viewed as a female. Therefore the occurrence of SSB is generally predicted to be affected by the phenotype of same-sex conspecifics, which further highlight the emergent property of SSB. However, there has been little empirical study of such effects to date.

Water striders are well suited for testing how SSB is influenced by the phenotype of interacting same-sex partners. SSB is prevalent in water striders (Hemiptera: Gerridae), semi-aquatic insects that live on the water surface (Sih & Watters 2005; Eldakar et al. 2009b; Chang & Sih 2013; Han & Brooks 2013a, b, 2014; Sih et al. 2014; Han & Brooks 2015a, b; Wey et al. 2015a; Wey et al. 2015b). Water strider mating is characterised by coercive mating attempts of males (i.e. forceful mounting), coerced copulation, and mating with multiple partners (Arnqvist 1997). Under a strong male-male scramble competition and male-biased sex ratio condition, it is important for males to find unmated females before other males do (Han & Brooks 2015b). As a result, the best male reproductive strategy may be to increase the rate of mating attempts even if they might therefore also mistakenly attempt to mate with males (Han & Brooks 2015b). Indeed, in many water strider species, males attempt to mount and mate not only with single unmated females, but also single males and mating pairs (Han & Brooks 2013b). In addition, adult male water striders housed in high densities, or female-biased sex ratio environments,

show less frequent SSB than males housed in low densities, or male-biased sex ratio environments (Han & Brooks 2014, 2015a). Given these strong social environment effects, we predict that a focal male's SSB is a function of the phenotype of the partner with whom he is interacting. Specifically, the partner's body size should affect the expression of the focal male's SSB. This is because in water striders females are larger than males (Fairbairn & Preziosi 1994; Fairbairn 2005), and males may therefore misidentify large males as females (see above). Furthermore, as mounting behaviour represents a male-specific behaviour, it should function as an advertisement of gender to other males. SSB in male partners should therefore down-regulate the expression of a focal male's SSB.

Two approaches have been developed in quantitative genetics to study social evolution (the "trait-based" approach and the "variance-partitioning" approach; McGlothlin & Brodie 2009; Bijma 2014), which can be used to empirically measure the effect of the interacting partner's phenotype on the focal SSB. The trait-based approach is commonly used to estimate the effect of the social partner's phenotype (here, SSB or body size) on the phenotype (here, SSB) of a focal individual. This effect is captured by the 'interaction coefficient' psi (ψ) (Moore et al. 1997; McGlothlin & Brodie 2009) which represents a standardized regression coefficient measuring the extent to which the phenotype of one individual affects that of another (Bijma 2014). This approach can only be applied when researchers have measured the phenotypes of social partners that are hypothesized to affect a focal individual's phenotype. The alternative variance-partitioning approach may be applied when individual-level characteristics of partners were hypothesized but not measured, and instead quantifies how much of the variation in a focal individual's phenotype (here, SSB or body size) was due to the identity of the interacting

partner. Such partner identity effects are caused by the combined effects of all individual-level phenotypic characteristics of social partners affecting the focal phenotype. In its simplest form, the variance partitioning approach partitions phenotypic variance in a focal individual's phenotype into variance attributable to the identity of the focal individual, the identity of the social partner, and within-pair residual variance. Using both approaches in conjunction therefore enables one to evaluate the extent to which unmeasured individual-level phenotypes of partners affect a focal individual's SSB, and whether measured phenotypic traits of social partners (SSB or body size) fully explain this social environment effect.

The trait-based approach is typically applied to situations where the phenotype of the social partner is either measured once or represents a stable individual characteristic (e.g. body size). However, individuals may also respond to 'labile' phenotypic characters of social partners, i.e. characters that vary both among and within individuals (Araya-Ajoy & Dingemanse 2014). This means that individuals may therefore modify their behaviour either with respect to the stable (repeatable, or 'personality') or labile (plastic) part of the partner's phenotype. Adaptive theory developed in behavioural ecology implies that ecological conditions should often dictate whether individuals modify their behaviour responding specifically to the repeatable versus plastic part of the partner's phenotype (e.g. Dall et al. 2004; McNamara et al. 2009; Dingemanse & Wolf 2013). This also suggests that the interaction coefficient $\boldsymbol{\psi}$ may thus be usefully split into an among-partner ($\boldsymbol{\psi_A}$) and within-partner ($\boldsymbol{\psi_W}$) component (Dingemanse & Araya-Ajoy 2015). In the context of SSB, a labile phenotypic character, water strider males may thus respond either to the predictable part of their partner's SSB (i.e. partner 'personality'; $\boldsymbol{\psi_A} \neq 0$) or to its changeable part (i.e. partner plasticity $\boldsymbol{\psi_W} \neq 0$). Previous research in this system

implies that both mechanisms might act: SSB in the water strider *Aquarius remigis* reduces the activity of conspecifics at the population level (Eldakar et al. 2009a; Chang & Sih 2013; Sih et al. 2014); a key outstanding question is therefore whether such effects are due to partner personality (Fig. 1a), partner plasticity (Fig. 1b) or whether individuals instead simply respond to the partner's 'raw' phenotype (Fig. 1c).

In this study, we repeatedly subjected pairs of males to dyadic interactions and measured SSB of both the individual assigned as 'focal' and the individual assigned as 'partner', using the water strider *G. lacustris* as a model. We used a design where each male interacted repeatedly with the same partner and repeatedly with different partners. This enabled us to 1) quantify how much of the variation in SSB of the focal individual was due to the focal's identity, partner's identity, pair's identity, and within-pair residual (variance partitioning approach), 2) quantify the hypothesized effects of partner phenotypes, body size and SSB (trait-based approach), and 3) quantify whether focal individuals differed in how they responded to stable and labile parts of partner phenotype.

METHODS

Study species and rearing conditions

Adult water striders of the species *Gerris lacustris* were collected at the pond near the Max Planck Institute of Biochemistry, Planneg-Martinsried, Germany (48°06'N 11°27'E). Individuals were marked on the pronotum with enamel paints, kept individually in small containers (10 x 15 cm, water depth) under a 14h:10h light/dark cycle at 24±2 °C, 30% humidity, and fed *ad*

libitum with surplus frozen crickets (*Acheta domestica*) every day. Pieces of floating Styrofoam were provided as rest sites for the water striders.

SSB assay and body size measurement

SSB data were collected from a total of 276 male-male dyadic trials. In each dyadic trial, we randomly assigned one male as the 'focal' and the other male as the 'partner'. Forty-eight males were divided into 12 experimental groups of 4 males within which all combinations of males were used (Fig. 2). Males were subjected to 12 days of dyadic trials in a scheme shown in Fig. 2. During the dyadic trials, each male was assayed 12 times, 6 times as a 'focal' and 6 times as a 'partner'. In these dyadic interactions, each male interacted with the same male 4 times. This allowed us to measure the effect of the pair's identity (i.e. effect of the unique combination of two individuals, i.e., the interaction term between focal identity and partner identity effects).

The experimental tank (10×15cm, water depth 1cm) for SSB assays was divided by an opaque partition (7×10 cm; height × length) which could be lifted by the experimenter to allow the water striders to interact. In each SSB test, one focal and one partner male were separated by the partition for 1 min as an acclimation period in the experimental tank. After the acclimation period, the partition was lifted and the focal male interacted with the partner male in the experimental tank. We then recorded, for a period of 10 mins, the number SSBs of both males separately (defined as the number of times that a male mounted another male).

Each male was subject to a SSB assay once every day for a twelve-day period (Fig. 2). Because limited access to females is known to affect the expression of male SSB (Han & Brooks

2015a), regular mating experience with females was required for males to maintain their mating motivation during the SSB assays. Thus, after the SSB assay, the males were returned to their individual rearing containers (15×20cm, water depth 1cm) with one female randomly selected from a stock population. This allowed males to experience mating with different females for 2 hrs every day. After 2 hours, the female was removed from the rearing container, and the male provided with *ad libitum* food (crickets, *Acheta domestica*).

After the 12 days of SSB assays, we measured each male's body size by placing it in a ventral position at a fixed distance from the lens of a digital camera and taking a photograph. From these photographs, we then determined the dimensions of the body size using Image J software (National Institutes of Health, Bethesda, MD) to the nearest 0.01 mm.

Statistical analyses

We applied both trait-based and variance partitioning approaches (McGlothlin & Brodie 2009; Bijma 2014) using mixed-effect models solved by restricted maximum likelihood implemented in ASReml 3.0. Prior to analysis, focal SSB values were standardised (mean=0, SD=1) to ease interpretation. In order to estimate effects of focal identity, partner identity and pair identity on the expression of the focal individual's SSB, we fitted a univariate model with the focal individual's, the partner's, the pair's, and the experimental group's identity as random effects (variance partitioning approach, model 1, Table 1). We fitted testing day (1-12) as a fixed-effect covariate to account for continuous testing order effects. We assessed the significance of fixed effects using Wald F-tests, and the significance of random effects using likelihood ratio tests (LRTs). The test statistic associated with the LRT was calculated as twice

the difference in log likelihood between a model with vs. without a random effect of interest, where the value of P was calculated using a mixture of $P(\chi^2, df=0)$ and $P(\chi^2, df=1)$ (Self & Liang 1987; Pinheiro & Bates 2006; Visscher 2006). Visual inspection of the residuals of our models (detailed below) also confirmed that the number of SSBs followed a Gaussian distribution.

To test whether 1) body size and 2) SSB frequency of partners affected the expression of SSB in focal males, we used a combination of both variance-partitioning and trait-based approaches. We fitted the partner's phenotypes (size and SSB) as standardized fixed-effect covariates into the most parsimonious model of the set fitted as part of the variance partitioning approach described above (see Results; model 2, Table 2). For the reciprocal phenotype SSB, we followed Bijma (2014) to calculate $\boldsymbol{\psi}$ from the ordinary least-squares regression coefficient. In addition, using this model, we assessed whether the partner identity effect disappeared when the hypothetical partner phenotype was fitted as a covariate in the model (Dingemanse & Araya-Ajoy 2015). Thus, using model 1 and 2, we tested 1) whether repeatable partner traits affected SSB (i.e. effect of partner identity) and 2) whether the partner effect was due to hypothesized partner traits (e.g. size and SSB).

Furthermore, to estimate the separate effect of repeatable versus plastic components of the partner's SSB, we followed procedures detailed by Dingemanse & Araya-Ajoy (2015), and included 1) the mean SSB of the partner over all its observations (i.e., representing the repeatable component of the partner's SSB) and 2) the deviation from this mean during a focal instance (i.e., representing the plastic component of the partner's SSB) as two separate covariates instead of the untransformed ('raw') value into our statistical model (model 3). In

addition, to assess whether the effects of the repeatable and plastic component differed significantly, we reformulated model 3 as detailed in equation (3) in (van de Pol & Wright 2009).

Ethical note

Experiments on insects do not require approval from the ethics committee of Ludwig-Maximilians University of Munich, Germany. However, our design did seek to minimize the number of individuals used and our protocols were designed to minimize disturbance of the animals.

RESULTS

A substantial part of the variation in SSB was explained by the focal individual's identity (Table 1, individual repeatability (r) = 0.27, SE = 0.07). The effect of the partner's identity was much smaller but nonetheless significant (Table 1, partner repeatability = 0.07, SE = 0.05). This implied that individual-level traits of the partner did, as expected, also affect the focal individual's SSB. Pair and group identity did not explain significant variation (Table 1) and neither did testing day (all values of $P > 0.5$).

As a next step, we applied a trait-based approach to investigate which traits of the partner affected the focal individual's phenotype. The baseline model used for this analysis (model 1) was the same model used for the variance partitioning approach (see Table 1) but without the random effects of group and pair's identity because those did not explain significant variation (see above). To this base model (Table 2), we first added the 'unpartitioned' raw values of the two partner traits hypothesized to affect the focal individual's

SSB: the partner's body size and its SSB. In contrast to predictions, the partner's body size did not affect the focal individual's SSB (Table 2; β = 0.001, SE = 0.007, F = 0.0, df = 1, *P* = 0.99). As expected, focal individuals did significantly decrease their SSB with increasing SSB in social partners (β = -0.13, SE = 0.05, F = 4.86, df = 1, *P* = 0.03, model 2, Table 2). Following Bijma (2014) for calculating **ψ** for reciprocal phenotypes, this negative regression coefficient translated into a value of **ψ** = -0.07. Interestingly, adding the partner's SSB to the model did not lead to a notable decrease in the variance attributable to partner's identity (compare Model 1 and 2 in Table 2), implying that focal individuals did not respond to the repeatable part of the partner's SSB, or else that the partner's SSB was not of key importance. When we decomposed the effect of the partner's SSB into the effects of its repeatable and plastic parts, the effect of the repeatable part did not differ from the effect of the plastic part (β=-0.12, SE = 0.09, F = 1.55, df = 1, *P*=0.22).

In addition to the approaches detailed above, we also ran a bivariate mixed-effects model (Appendix 1) to verify that our method to calculate the among-individual correlation was not affected by bias that is sometimes associated with within-subject centering approaches (Lüdtke et al. 2008). This alternative bivariate modelling approach (Appendix 1) also confirmed the lack of a partner 'personality' effect as the among-individual correlation between an individual's SSB was not correlated to the SSB elicited in other individuals (r = -0.03, SE = 0.24, χ^2 = 0.018, df = 1, *P* = 0.89, Table A1).

DISCUSSION

Water strider males were individually repeatable in their SSB (Table 1). This finding implies that males differed in their tendency to attempt to mount other individuals, which supports results from a previous study showing moderate heritability of SSB in another water strider species (*Tenagogerris euphrosyne*, Han & Brooks 2015a). Moreover, we showed that SSB was also affected by the partner's identity, implying that individual-level traits of partners modify SSB in male water striders. The partner's SSB reduced the tendency of focal individuals to show SSB. However, the partner effect was not due to the repeatable part ('personality') of the partner SSB. Males also did not differ in how they responded to variation in the partner SSB at different levels (among-partner and within-partner levels). These findings imply that water strider males responded to the raw SSB of the partner males during the focal interaction rather than the partner's personality (SSB) or plasticity (i.e. scenario in Fig. 1c).

Negative effects of the partner's SSB on the focal's SSB

Water strider males differed in their expression of SSB in response to the raw phenotypic value of the partner male's SSB during the interaction (Table 2). The negative effect implies that SSB of water strider males has a role in suppressing SSB of male partners. In the water strider species *Aquarius remigis,* male-male mountings have been documented as aggressive behaviour towards other males (Sih & Watters 2005; Eldakar et al. 2009a; Chang & Sih 2013; Sih et al. 2014; Wey et al. 2015a; Wey et al. 2015b). Aggressive males of the species *A. remigis* reduced the activity of other males and females (Eldakar et al. 2009a; Chang & Sih 2013; Sih et al. 2014). However, males of our current study species (*G. lacustris*) are not territorial and do

not defend or resist other male's mounting attempts (CS Han, personal observation). When a mounting male of this species realizes, when attempting to copulate, that he has mounted a male, he quickly dismounts (Han & Brooks 2013b, 2015b; Han et al. 2016). Thus aggression unlikely constitutes the sole explanation for the negative value of SSB (e.g. Al-Wahaibi et al. 2005).

Instead, the negative sign of the partner's SSB on SSB of the focal individual may have a function in advertising the sex of the mounting male. The mounting attempt is male-specific behaviour in water striders. If the male is sure that the mounting individual is a male, he does not need to waste his time in distinguishing the sex of the individual and attempting to mate with him. Therefore SSB can reduce another male's tendency to express SSB by advertising the sex. In addition, the negative effect of the partner's SSB in this study was not due to autocorrelation in the data. Autocorrelation would occur when a social partner mounts and thereby prevents the focal male from mounting itself. In our data, however, the duration of each SSB is very brief (mean ± SE = 2.8 ± 0.6 s, N=184; see also Han & Brooks 2013b), and the experimental tank was not so large that males could easily locate each other. Because water striders can recognize the presence of other individuals with ripples created by the movement on the surface of water, two males in the experimental tank were also recognizing and locating each other during assays even when they did not physically contact. Thus males have ample opportunity to express SSBs independently from the duration of the partner's SSB (duration mounted by the partner) given the duration of the assay. We therefore do not view autocorrelation as a viable explanation for this effect.

Against expectations, the body size of the partner did not affect the expression of the focal's SSB. The lack of pair identity effect, furthermore, implied that the interaction between individual-specific phenotypes (e.g. 'relative' size) of the focal and the partner also did not affect the focal's SSB. In a water strider *G. lacustris*, although females were larger than males (Andersen 1994), the size difference did not cause males to wrongly classify larger males as females. We therefore conclude that our water strider males probably simply do not use the body size as a cue to recognize sex.

Partner effect

This paper provides the first evidence in animals that the identity of a male partner can affect the expression of SSB in a focal individual (Table 1). Individually repeatable differences in phenotypic characteristics thus affect SSB in interacting individuals. Importantly, the application of trait-based approaches implied that these partner effects were not caused by repeatable differences in SSB or body size of social partners (Table 2). Based on this finding, we conclude that other repeatable phenotypic traits of males must have affected SSB in conspecific males. Those traits could, for example, be caused by individual differences in activity levels or chemical signals. As females are less active on the water surface than males (CS Han, unpublished data), less active males may receive more mating attempts (SSB) from other males. If males can recognize the sex of the partner with chemical signals (Tsoukatou et al. 2001), individual variation in the amount of sex-specific chemical signals may also explain the partner effect on the focal's SSB. Those traits might therefore represent suitable targets as for explaining social partner effects on SSB in future research.

Variation in ψ across hierarchical levels

When partner identity explains variation in the focal individual's phenotype, and a labile partner phenotype (e.g. behaviour, physiology) affects the expression of the focal's phenotype, it becomes necessary to consider that different hierarchical levels may shape the effect of interaction coefficient $\boldsymbol{\psi}$ (here, the among-partner and within-partner levels, $\boldsymbol{\psi_A}$ and $\boldsymbol{\psi_W}$, Dingemanse & Araya-Ajoy 2015). Indeed, personality and plasticity of partners could have very different effects on the focal's phenotype. For example, when fights are costly, individuals might be selected to become more predictable in their aggression (i.e., personality), which may in turn further increase the amount of among-individual differentiation in aggression in a population (Dall et al. 2004). Focal males may therefore decrease their aggression when they face partners that, based on previous interactions, are known to be relatively aggressive, and instead increase their aggression when they face partners that are on average less aggressive (i.e. $\boldsymbol{\psi_A} \neq 0$,). However, when the focal male repeatedly interacts with the same partner male, the focal might not flexibly express its aggression in response to changes in the partner's aggression (i.e. $\boldsymbol{\psi_W} = 0$). That is, at the within-partner level, a subtle decrease in the partner aggression may not elicit an increase in the focal aggression when their dominance hierarchy is already established. Thus, in this scenario, the focal aggression is predicted to respond solely to the repeatable parts of the partner aggression (Fig. 1a).

Our data nevertheless did not confirm the notion that individuals differently respond to personality and plasticity of partners. When male water striders expressed SSB, they did not respond (significantly) to the repeatable part of the partner SSB but rather to the phenotype (raw values of SSB, Fig. 1c). It is possible that we lacked statistical power to detect a significant

difference between the within- and among-partner effect in the partner's SSB. Nevertheless, we feel that it is important to consider whether the interaction coefficient **ψ** varies across different levels in fully understanding how the expression of labile traits is affected by the interaction between individuals.

Conclusion

Our study showed evidence for a social partner effect on SSB, and a negative effect of SSB on the other individual's SSB (i.e. negative **ψ)**. This is the first empirical evidence that SSB can be plastically expressed in response to the partner's trait. In addition, we decomposed the partner effect into different levels (e.g. among-partner, within-partner) and tested the differences between them. We thus highlight that both trait-based and variance-partitioning approaches can be used to assess the partner effect on the expression of traits and which trait of the partner explains the effect (Dingemanse & Araya-Ajoy 2015). Our work also highlights the importance of considering the separate effect of personality and plasticity of partners in research examining the partner effect. Together, our approach and experimental design may be applied more commonly by researchers interested in studying effect of partner phenotypes that are themselves labile in nature, such as aggression (Wilson et al. 2009; Wilson et al. 2011; Santostefano et al. 2016) and sexual traits (Chenoweth et al. 2010; Bailey & Zuk 2012).

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TABLES

Table 1. Results of Linear Mixed Model to assess the contribution of social components to variation in expression of same-sex sexual behaviour

Random effects	Variance (SE)	$X^2_{0.5}$	*P*
Focal ID	0.27 (0.09)	26.4	<0.001
Partner ID	0.07 (0.05)	3.0	0.03
Pair ID	0.007 (0.05)	0.02	1.0
Group ID	0.03×10^{-6} (0.003×10^{-6})	0	1.0
Residual	0.66 (0.07)		

Table 2. Linear mixed-effects model structures to assess the effect of partner phenotypes on the same-sex sexual behaviour. Variances of random terms and estimates of fixed terms are given with their standard errors in parentheses. Significant terms are indicated in bold.

	Model 1	Model 2
Fixed effects		
Partner SSB	-	**-0.13 (0.06)**
Partner body size	-	0.001 (0.07)
Random effects		
Focal ID	**0.27 (0.09)**	**0.28 (0.09)**
Partner ID	**0.07 (0.05)**	**0.07 (0.05)**

FIGURES

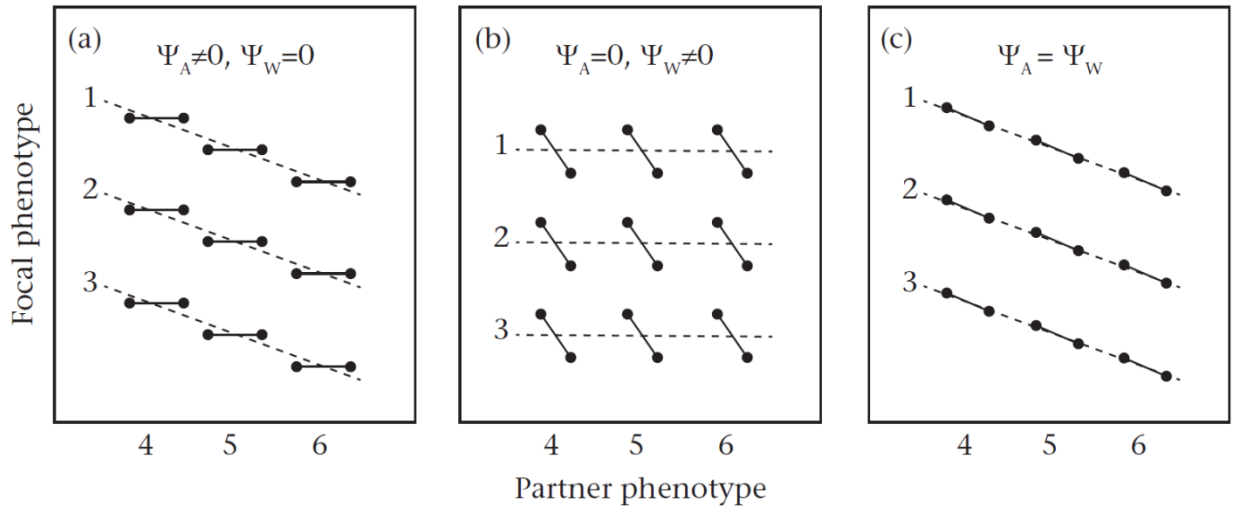

Figure 1. Different levels at which the interaction coefficient psi (ψ) might vary. Diagrams illustrate a situation where phenotypes of three different focal individuals (numbered 1-3) are measured in repeated interactions with different partners (numbered 4-6, plotted on the x-axis). (a) The focal individual's phenotype can respond to the partner's personality (dashed line, $\psi_A \neq 0$) but not the plastic parts of the partner's phenotype (solid line, $\psi_W = 0$; two black dots linked with the solid line represent repeated measurements with the same partner). (b) The focal's phenotype can also respond to the plastic parts of its partner's phenotype ($\psi_W \neq 0$) but not the partner's personality ($\psi_A = 0$). Finally, (c) the effect of the partner's personality is the same with as the effect of the partner's plastic part. This latter scenario implies that the focal individual does not differ in its response to any level, but instead responds to the raw phenotype of the partner. This lack of level-specific variation in ψ is the common assumption made in quantitative genetics theory (Moore et al. 1997).

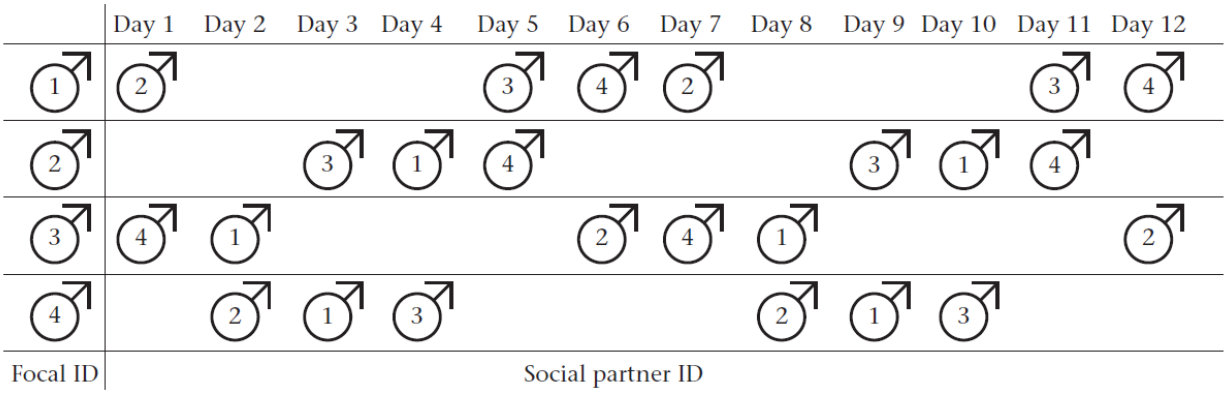

Figure 2. The experimental setup. Groups of four males were subjected to 12 days of dyadic trials, where each male was assayed 6 times (three times as a 'focal' and three times as a 'partner' individual).

APPENDIX 1.

In the bivariate mixed-effect model, focal SSB and partner SSB were fitted as the two response variables. Thus the phenotypic variance of the focal male (V_{focal}) can be partitioned into the focal's identity effect on the focal's SSB ($V_{focal(focalSSB)}$ in $\boldsymbol{\Omega}_{(focalSSB)}$), the focal's identity effect on the partner's SSB ($V_{focal(partnerSSB)}$ in $\boldsymbol{\Omega}_{(partnerSSB)}$) and a residual component ($V_{residual(focal)}$) (i.e., $V_{focal} = V_{focal(focalSSB)} + V_{focal(partnerSSB)} + V_{residual(focal)}$). In the same way, phenotypic variance of the partner male ($V_{partner}$) can be partitioned into the partner's identity effect on the focal's SSB ($V_{partner(focalSSB)}$), the partner's identity effect on the partner's SSB ($V_{partner(partnerSSB)}$) and a residual component ($V_{residual(partner)}$) (i.e., $V_{partner} = V_{partner(focalSSB)} + V_{partner(partnerSSB)} + V_{residual(partner)}$). Because the specifics of the experimental design (see Methods), the covariance between the focal's and the partner's SSB could also be estimated at the individual level ($COV_{(focalSSB,partnerSSB)}$, $COV_{(partnerSSB,focalSSB)}$) and the residual level ($COV_{residual}$). Here, $COV_{(focalSSB,partnerSSB)}$ and $COV_{(partnerSSB,focalSSB)}$ indicated the covariance between the repeatable components of focal's SSB and partner's SSB, whereas $COV_{residual}$ indicated the covariance between the within-individual plastic components of the focal's SSB and the partner's SSB (Dingemanse & Araya-Ajoy 2015).

Furthermore, since we measured the same behaviour (SSB) on the focal and the partner, and each male played both the focal and social partner, there are some variance components that should be the same in the variance-covariance matrix of bivariate mixed-effect model (detailed in Santostefano et al. 2016). Logically, the variance attributable to the focal individual's identity effect on the focal's SSB ($V_{focal(focalSSB)}$) in the variance-covariance matrix $\boldsymbol{\Omega}_{(focalSSB)}$ should be the same as the variance attributable to the partner individual's identity effect on the partner's SSB ($V_{partner(partnerSSB)}$) in the variance-covariance matrix

$\boldsymbol{\Omega}_{(partnerSSB)}$ (i.e. $V_{focal(focalSSB)} = V_{partner(partnerSSB)}$). In the same way, the variance component on the partner identity effect on the focal's SSB ($V_{partner(focalSSB)}$) in the variance-covariance matrix $\boldsymbol{\Omega}_{(focalSSB)}$ should be the same with the variance component on the focal individual's identity effect on the partner's SSB ($V_{focal(partnerSSB)}$) in the variance-covariance matrix $\boldsymbol{\Omega}_{(partnerSSB)}$ (i.e. $V_{partner(focalSSB)} = V_{focal(partnerSSB)}$). Covariances between the focal's SSB and partner's SSB in the matrices $_{(focalSSB)}$ and $_{(partnerSSB)}$ should also be the same (i.e. $COV_{(focalSSB,partnerSSB)} = COV_{(partnerSSB,focalSSB)}$). We thus created a new bivariate mixed-effect model we were constrained such variance components to be the same (Table A1). We then calculated the SSB repeatability, the repeatability of the partner's SSB, the among-pair correlation between the repeatable components of the focal's SSB and the partner's SSB and the residual correlation. This residual correlation in the constrained bivariate mixed-effect model may imply a within-pair correlation between plastic parts of the focal and the partner's SSBs (Dingemanse & Araya-Ajoy 2015). That is, a negative within-pair correlation in the bivariate mixed-effect model might indicate that the focal upregulates its SSB when its partner downregulates SSB. However, the analysis of the residual correlation in this bivariate mixed-effect model is still under debate and therefore not presented in the main text.

We assessed the significance of random factors using likelihood ratio tests (LRTs). The test statistic is twice the difference in log likelihood between a model run with and without the random factor. The probability of the LRT of a variance was against a mixture of $P(\chi^2, df=0)$ and $P(\chi^2, df=1)$ because the variance must be positive definite (Self & Liang 1987; Pinheiro & Bates 2006; Visscher 2006). However, for the probability of the LRT of a covariance, we used $P(\chi^2,$

df=1) because the covariance can have negative values. Thus, LRTs involving one variance and one covariance were tested assuming a mixture of $P(\chi^2, df=1)$ and $P(\chi^2, df=2)$, i.e. df=1.5.

Table A1. Variance-covariance components obtained from constrained bivariate mixed-effect models for same-sex sexual behaviours of the focal and partner

	σ^2 (SE)
$\boldsymbol{\Omega}$ **(*focalSSB*)**	
$V_{focal(focalSSB)}$: the focal effect on the focal SSB	0.34 (0.05)
$V_{partner(focalSSB)}$: the partner effect on the focal SSB	0.05 (0.03)
$COV_{(focalSSB,partnerSSB)}$: among-pair covariance between the focal and the partner SSB	-0.004 (0.03)
$COR_{(focalSSB,partnerSSB)}$: among-pair correlation between the focal and the partner SSB	-0.03 (0.24)
$\boldsymbol{\Omega}$ **(*partnerSSB*)**	
$V_{focal(partnerSSB)}$: the focal effect on the partner SSB	0.05 (0.03)
$V_{partner(partnerSSB)}$: the partner effect on the partner SSB	0.34 (0.05)
$COV_{(partnerSSB,focalSSB)}$: among-pair covariance between the focal and the partner SSB	-0.004 (0.03)
$COR_{(partnerSSB,focalSSB)}$: among-pair correlation between the focal and the partner SSB	-0.03 (0.24)
$\boldsymbol{\Omega}_{residual}$	
$V_{residual(focal)}$	0.62 (0.05)
$V_{residual(partner)}$	0.62 (0.05)
$COV_{residual}$: within-pair covariance between the focal and the partner SSB	-0.10 (0.04)
$COR_{residual}$: within-pair correlation between the focal and the partner SSB	-0.17 (0.07)

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GENERAL DISCUSSION

Evolutionary biologists are generally interested in understanding the diversity of life on Earth, particularly the evolutionary emergence and maintenance of phenotypic variation. Genetic diversity in a trait plays an important role in the survival and adaptability of species faced with changing environmental circumstances. Quantifying the available phenotypic variation in a population, as well its heritability, is important for predicting how a trait will respond to selection. Labile traits such as behaviours can be expressed multiple times throughout the lifetime of an individual, and thus phenotypic variation can exist both within and among individuals (Dingemanse et al. 2010). Within individual variation ('plasticity') refers to the efforts of an individual to match the current environmental conditions within its lifetime. Among-individual (genetic) variation instead refers to changes in phenotypic (genotypic) frequencies from one generation to the other and thus trait evolution. Because behaviour is considered very plastic, a major question is why individuals often consistently differ from each other in these labile traits, or in other words, why among-individual behavioural variation is maintained in a population.

Recent research in behavioural ecology has centered on the causes generating and maintaining among-individual variation in behaviour ('personality'), as well the evolutionary consequences of such variation (Dall et al. 2004, 2012, Sih et al. 2004a, 2004b, Réale et al. 2007, 2010a, Wolf and Weissing 2012, Dingemanse and Araya-Ajoy 2015). The social environment has been implied as a largely overlooked mechanism shaping behavioural variation at both levels (Bergmüller and Taborsky 2010, Montiglio et al. 2013, Dingemanse and Araya-Ajoy 2015, Niemelä and Santostefano 2015). The social environment is unique because it is composed of

genotypes of other individuals, and thus can itself evolve. When the genes of an individual influence the expression of a trait in an interacting individual, these effects are called indirect genetic effects (IGEs) (Wolf et al. 1998, 1999). Quantitative geneticists, interested in predicting the evolutionary trajectories of traits, have long integrated the social environment in their studies. IGEs are known to have profound effects on both the magnitude and the direction of response to selection (Moore et al. 1997, Wolf et al. 1998, 1999, McGlothlin et al. 2010, Bijma 2014). The framework developed by quantitative geneticists to study the consequences of social interactions between different 'types' of individuals should thus be useful for behavioural ecologists interested in understanding why individuals are repeatable in their behaviour.

My dissertation considered the social environment as an important and often overlooked evolutionary force affecting the evolution of individual variation in behaviour. I focused on the role of the social environment in causing and maintaining (genetic) variation in behaviour, and conversely, the evolutionary consequences of the existence of (genetically underpinned) personalities for behavioural variation. Specifically, my dissertation bridges two previously distinct fields by using quantitative genetics methods to address behavioural ecological questions. By incorporating the social environment, this dissertation therefore offers overlooked explanations for the maintenance and consequences of animal personality variation in nature.

Social environment effects: interplay between levels of phenotypic variation

In social behaviours, phenotypes of conspecifics represent the environment of an individual. Recently, personality of conspecifics has been suggested as an important trait to which

individuals respond by plastically changing their behavioural phenotypes (Briffa et al. 2015, Dingemanse and Araya-Ajoy 2015). To date, it has been difficult to empirically quantify such social environment effects. To address this important shortcoming, I adopted the variance partitioning approach traditionally used in the field of quantitative genetics (Lynch and Walsch 1998) to estimate the effects of repeatable phenotypes on social behaviours. This framework allowed me to split the total phenotypic variance of a social trait (e.g., aggression) present in a population, into among-individual variance (variance explained by differences in average behaviour among repeated observations, i.e. 'personality'), and residual within-individual variance, which includes phenotypic plasticity (as well as measurement error). When I further included the social partner identity (i.e., the indirect effects) in my analyses, I was able to identify a biologically relevant source of variation that was previously unexplained residual variance. Individuals indeed responded differently (i.e. adjusted their aggressive behaviour) when confronted with different opponents.

We found that the social partner explained a large portion of phenotypic variance in all of our studies. For example, the opponent identity explained 16% of the total phenotypic variance in aggression in *G. campestris* (Chapter 1), 11% in aggression in *G. bimaculatus* (Chapter 2), and 7% of the total phenotypic variance in same sex sexual behaviour (SSB) in water striders (Chapter 4). These estimates were in the same range of the few other behavioural studies that estimated such indirect effects on aggression (11%–27%; Wilson et al. 2009, 2011a, 2013). Importantly, the variance attributable to indirect effects was of comparable magnitude to that explained by the focal individual's identity effects, indicating that the observed behaviour depended equally strongly on the identity of the animal expressing

the behaviour and the identity of social partners. Another way of thinking of this repeatable 'opponent effect' is that 'eliciting a specific behaviour in others' (Aggression in Chapters 1 and 2, SSB in Chapter 4) is itself a 'personality' trait to which social partners are responsive (social responsiveness, Webster and Ward 2011; Taborsky and Oliveira 2012; Wolf and McNamara 2013; Wolf and Krause 2014). This demonstrates the importance of recognizing that observed social behaviours are expressions of multiple interacting phenotypes (Moore et al. 1997) and by extension genotypes (Moore et al. 1997, Wilson et al. 2009, 2013).

An important missing component of the above-mentioned analysis is that the traits of conspecifics underpinning social partner effects remain unidentified. We know that opponent identity explains a certain amount of variation in the focal individual's behaviour, but we do not know what characteristics of the opponent are responsible for the observed effects (Bijma 2014). The contribution of specific traits causing the indirect effect can, fortunately, be quantified when adopting the 'trait-based' approach (Moore et al. 1997; McGlothlin and Brodie 2009). Indeed, this approach has been developed specifically by quantitative geneticists interested in measuring the 'environmental' (or social) gradient responsible for indirect effects. For example, does the focal individual respond to opponents varying in size, behaviour, or perhaps pheromone profiles? By measuring repeatable phenotypic traits of the opponent (including behaviours), we were able to tease apart which traits were driving the opponent effect. We did so using explicitly both approaches in Chapter 4. We found that in water striders (Chapter 4), the social partner's same sex sexual behaviour (SSB) reduced the tendency of males to show SSB themselves. However, in this case, the partner effect was not due to the repeatable part ('personality') of the partner SSB. We concluded that other repeatable

phenotypic traits of males must have affected SSB in conspecific males, for example activity levels or chemical signals. The second approach, used in Chapters 1 and 2, was an extension of the variance partitioning approach to estimate correlations between opponent effects on social behaviours and focal effects on other traits (i.e. whether individuals that elicit more aggressiveness in others are themselves more or less active, explorative, etc. than individuals eliciting less aggressiveness in others). This, like the trait-based approach, allows us to identify the traits of conspecifics causing the indirect effect. In crickets, repeatable behaviours expressed by opponents (activity, exploration, and aggressiveness) indeed elicited aggressiveness in conspecifics. Interestingly, behavioural traits were more important than other traits normally hypothesized to represent an important mediator of agonistic behaviour in the animal contest literature, such as body size (Briffa et al. 2015). In both crickets and water striders, social partners responded to behaviours rather than other characteristics of conspecifics, such as their weight or the relative size difference between the two contestants. This highlights the importance of level-specific analyses considering the separate effects of personality and plasticity of partners in research investigating social environment effects. Identifying the specific component of a phenotype that affects conspecifics will allow scientists to address explicitly the behavioural traits causing social selection and social evolution.

From phenotypes to genotypes: consequences of IGEs for the maintenance of genetic variation in behaviour and their role in shaping evolutionary trajectories

Identifying which behavioural traits cause indirect effects is of paramount importance because it is the repeatable part of the phenotype that has the potential to be underpinned by

genetic variation, and thus relevant for evolution. Published estimates show that labile traits such as behaviours typically harbour variation due to additive genetic effects (ranging from 0.14 to approximately 0.5; Stirling et al. 2002, van Oers et al. 2005, Postma 2014, Dochtermann et al. 2015). Because behaviours are known to be heritable, the presence of substantial indirect effects of phenotypes documented in Chapters 1 and 4 suggested a strong potential for additive genetic variation in partner effects (IGEs). In Chapter 2, we thus set out to partition repeatable among individual behavioural variance in indirect effects into its additive genetic and environmental components. We found that indirect effects on aggression were indeed partly underpinned by genetic variation: IGEs explained almost 30% of the indirect effects. Therefore, the social environment was heritable. Another way to think about IGEs is that an individual 'inherits its social environment' in the sense that an individual carries genes that elicit behaviours in others (e.g., genes for inducing aggressiveness). Importantly, when accounting for IGEs, the 'traditional' estimate of total heritability (h^2) of a social trait (aggression) is no longer appropriate as the genetic variance of the social environment must be included in the calculation. The total heritable variation of a trait in a population includes not only the additive genetic variance (due to 'direct effects', DGEs), but also IGEs, as well as their covariance (see Chapter 2 for details). In our study, (Chapter 2) we found a 3.2-fold decrease in total heritability when accounting for IGEs. Our example specifically highlights that the important element affecting the total heritability estimates is the correlation between DGEs and IGEs on the same trait. In crickets, the genetic correlation between DGEs and IGEs for aggression was strong and negative, such that individuals genetically predisposed for higher levels of aggression also were genetically predisposed to suppress aggressiveness in opponents. Negative genetic correlations

can impose major evolutionary constraints, as they effectively reduce the amount of heritable variation that is available to selection (McGlothlin et al. 2010, Bijma 2011, 2014). The response to selection (R), given the heritability of a trait (h^2) and the selection differential (S) is calculated in the breeder's equation by $R = h^2*S$ (Falconer and Mackay 1996). In our case therefore there will be virtually no microevolution of aggression possible, as very little genetic variation in aggression is available due to the effects of IGEs (see above). Despite aggression being heritable, IGEs may thereby clarify why (genetic) variation can be maintained even in cases where there is strong directional selection acting on the focal trait, explaining why evolutionary stasis is so common in natural populations under selection.

Researchers interested in understanding 'animal personality' variation from an adaptive perspective largely overlook the importance of indirect (genetic) effects caused by social interactions in their studies of the maintenance and evolutionary consequences of variation in social behaviours. IGEs arising from social interactions can both provide a major source of heritable variation on which selection can act, as well as a represent an evolutionary constraint (Wilson et al. 2011b), and may thus explain why 'personality' variation might be maintained in natural animal populations. While these concepts are widely used by quantitative geneticists interested in predicting the evolution of traits, it is clear from our results that they are in dire need of being incorporated in behavioural ecology studies.

Going multivariate: complex relationships between traits

Another central theme that emerged in my dissertation is that studies of the evolution of behaviour should explicitly recognize that behavioural phenotypes are multivariate and include

responses of the social environment. Individuals express multiple traits that are correlated genetically (and phenotypically) with each other, which may impose constrains to their evolution (Arnold 1994, Blows 2007). These concepts are not new to evolutionary biologists, but only recently have they been applied to the different levels of covariation in behaviours (Dochtermann and Dingemanse 2013, Araya-Ajoy and Dingemanse 2014). Among-individual correlations in behaviours, or 'behavioural syndromes', are widely documented across taxa (Sih et al. 2004a, 2004b, Garamszegi et al. 2012). For instance, social behaviours such as aggression often correlate with behaviours expressed in other contexts (e.g. bold and active individuals are generally more aggressive, Garamszegi et al. 2012). We therefore implemented multivariate analyses of behavioural phenotypes (or genotypes) in Chapters 1, 2, and 3. For example, if we had considered traits in isolation (e.g. exploration, Chapter 2), we would have predicted high evolutionary potential for these traits given their high heritability estimates. However, once we included the genetic correlation with other traits showing IGEs (e.g. aggression), we discovered that even when traits are themselves not directly involved in social interactions, their evolution can become 'anchored' to a trait though IGEs and either become constrained (i.e., in the presence of negative correlations between DGEs and IGEs) or facilitated (i.e., in the presence of positive correlations between DGEs and IGEs). Thus, if among-individual correlations exist between a social behaviour with indirect effects and another non-social behaviour, the social environment has the potential to indirectly affect the evolution of other traits. Importantly, this also provides a previously overlooked mechanism for the maintenance of personality traits expressed in a non-social context (e.g. risk-taking behaviour, explorative tendency), which becomes only evident when taking a multivariate approach.

We also extended this multivariate approach to the integration between behaviours and other traits, such as life-history. In Chapter 3, we asked whether genetic relationships between life-history traits were mediated by behaviours as recently suggested in the pace-of-life (POL) framework (Réale et al. 2010b). To address this, we built upon results from mixed models with other tools such as path analysis (Chapter 3). This enabled us to test explicitly which of the trade-offs between life history traits were mediated by behaviour al traits. Furthermore, with this approach we could detect 'hidden relationships' that were not apparent in the simple bivariate correlation matrices alone. For example, in Chapter 3, we discovered a tight relationship between aggression and exploration, even though they were seemingly uncorrelated at the genetic level. As supported by the path analysis on the genetic matrix, aggressiveness and exploratory tendency strongly depended on size, and both behaviours mediated the effects of size on lifespan. Consequently, the independent evolution of behaviours may still be constrained despite them not showing any direct genetic relationship. This example highlights the importance of (multilevel) path analysis to better understand the biological causal pathways underlying (genetic) correlations between traits, including behaviours.

The phenotypic gambit

Evolutionary processes can only be fully understood when both phenotypic and genetic data are available (Roff 2002). However, most studies of animal personality rely on the assumption that the among-individual behavioural variation in a population implicitly reflects the underlying genetic variation, and thus use estimates of repeatability as proxies for

heritability to make evolutionary predictions. The assumption that the phenotypic architecture reflects the genetic one is known as the 'phenotypic gambit' (Grafen 1984) (or Cheverud's conjecture, Cheverud 1988). The gambit stems from the often logistically difficult task of obtaining reliable quantitative genetic parameters in behavioural ecology studies, and the belief that the genetic architecture does not matter for drawing conclusions concerning adaption in the field of behavioural ecology (Grafen 1984). Heritability estimates, and estimates of genetic correlations, require large sample sizes and knowledge of relatedness among individuals, which are not easy to obtain especially in field studies. For labile traits such as behaviours, the expected environmental (co)variance is large (Falconer and Mackay 1996), and thus researchers have called for caution about the generality of the phenotypic gambit (Kruuk et al. 2003). Although a recent meta-analyses shows that 50% of the among-individual variation in behaviour is heritable (Dochtermann et al. 2015), several factors may contribute to non-genetic sources of variation. Permanent environmental effects are an important source of non-heritable variation. These include maternal and paternal effects, epigenetics and environmental effects that have long-term effects on phenotypes (Roff 1997). Permanent environmental effects caused by developmental environments vary among individuals and thus, when only phenotypic estimates are available, contribute to 'personality' variation though not contributing to evolutionary change. Importantly, environmental sources are also relevant for predicting evolutionary change, because if not accounted for correctly they can become conflated with genetic variance, and heritability estimates (Kruuk and Hadfield 2007). Laboratory studies such as ours thus provide a useful setup when large numbers of individuals

are required to estimate the genetic (co)variation available in a population, to be able to formulate correct evolutionary predictions.

The additive genetic variation available in a trait will also affect the magnitude of its genetic covariation with other traits. However, when the environmental component is large, phenotypic correlations may poorly reflect genetic correlations (Krebs and Davies 1997, Brommer 2013). The genetic correlation is "hidden" beneath several additional layers of other factors (i.e., permanent environment, error) affecting the phenotype. Each of these factors may have a large effect, and thus determine the resulting phenotypic correlation. A meta-analysis showed that phenotypic correlations between behaviours reliably reflect the direction of underlying genetic relationships, but not necessarily their magnitude (Dochtermann 2011). Evolutionary theory predicts that genetic correlations can strongly determine the direction and outcome of evolutionary responses to natural selection (Lande 1979, Lande and Arnold 1983). From an evolutionary viewpoint, an important distinction is therefore whether the integration between different traits, such as behaviours and life-history (Chapter 3) occurs at the genetic level. Genetic life history trade-offs are thought to be an important mechanism maintaining behavioural variation as postulated by the pace-of-life hypothesis (detailed above). However, negative genetic correlations reflecting trade-offs between traits are often masked by positive environmental correlations, as suggested by life-history theory (van Noordwijk and de Jong 1986, Reznick et al. 2000). This mismatch between levels was one of the main findings in Chapter 3, where we compared the genetic correlations with the among-individual correlations. We found important differences between patterns of covariance between life-history traits and behaviours at the genetic and individual level (the latter presenting the typical target of

behavioural ecology studies of POL), which were mediated by permanent environmental effects. For example, the positive effect of aggression on lifespan detected at the genetic level was not present at the individual level because the larger permanent environmental contribution was driving the relationship. The relationship between behaviours (aggression and exploration, discussed above) represents another example of caution needed when taking the (multivariate) phenotypic gambit on traits with a large environmental component, such as behaviours and life-history. This suggests that extreme caution is required in predicting evolutionary trajectories of multivariate behavioural phenotypes without information on their additive genetic architecture.

Conclusions

My dissertation lies at the interface of two fields of evolutionary biology: behavioural ecology and quantitative genetics; it answers outstanding questions regarding the maintenance of (genetic) variability and evolution of individual behaviour raised by behavioural ecologists, with approaches and theory developed by quantitative geneticists. Importantly, my dissertation appreciates the key importance of social interactions between individuals, a characteristic of all living organisms. I addressed variation in the social environment as both a cause and a consequence of behavioural variation. I did so by exploring the (genetic) architecture underlying interacting phenotypes, by taking into account the potential impact of indirect (genetic) effects. Acknowledging the social environment as a genetically influenced environment that can itself evolve will improve the understanding of the evolutionary consequences of indirect (genetic) effects on behaviours, i.e. social evolution. By integrating the

social environment in a 'multivariate' view of the phenotype (genotype), the framework outlined in this dissertation can be broadly applied to all traits associated with traits involved in social interactions (such as life-history traits), and may greatly help explaining the maintenance of genetic variation in behaviour.

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AUTHOR CONTRIBUTIONS

Chapter 1: *Interacting with the enemy: indirect effects of personality on conspecific aggression in crickets.*

FS and NJD conceived the study. FS collected the data. FS analysed the data with input from NJD, AJW, and YGAA. FS wrote the first draft of the manuscript. All authors contributed to revisions and approved the final manuscript.

Chapter 2: *Indirect genetic effects: a new explanation for the maintenance of personality variation*

FS and NJD conceived the study. FS collected the data. FS analysed the data with input from NJD, AJW, and PTN. FS wrote the first draft of the manuscript. All authors contributed to revisions and approved the most recent version of manuscript.

Chapter 3: *Behavioural mediators of genetic life-history trade-offs in field crickets*

FS and NJD conceived the study. FS collected the data. FS analysed the data with input from NJD, AJW, and PTN. FS wrote the first draft of the manuscript. All authors contributed to revisions and approved the most recent version of manuscript.

Chapter 4: *Do social partners affect same-sex sexual behaviour in male water striders?*

CSH and NJD conceived the study. CSH collected the data. CSH and FS analysed the data with input from NJD. CSH wrote the first draft of the manuscript. All authors contributed to revisions and approved the final manuscript.

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2009-2011: Masters of Science (Evolutionary Biology), Universita' degli Studi di Padova, Padova, Italy

2006-2009: Bachelor of Science (Biology), Universita' degli Studi di Padova, Padova, Italy

Peer reviewed journal publications

Santostefano F, Wilson AJ, Araya-Ajoy YG, Dingemanse NJ. "Interacting with the enemy: indirect effects of personality on conspecific aggression in crickets." *Behavioural Ecology (2016): arw037.*

Han CS, Santostefano F, Dingemanse NJ. "Do social partners affect same-sex sexual behaviour in male water striders?." *Animal Behaviour 116 (2016): 53-59.*

Biro PA, Fanson KV, Santostefano F. "Stress-induced peak (but not resting) metabolism correlates with mating display intensity in male guppies" *Ecology and Evolution (2016), Online.*

Niemelae PT, Santostefano F. "Social carry-over effects on non-social behavioural variation: mechanisms and consequences" *Frontiers in Ecology and Evolution 3 (2015): 49.*

Under Review

Santostefano F, Wilson AJ, Niemelae PT, Dingemanse NJ. "Behavioural mediators of genetic life-history trade-offs in Field crickets" submitted to *Evolution*

In preparation

Santostefano F, Wilson AJ, Niemelae PT, Dingemanse NJ. "Indirect genetic effects: a new explanation for the maintenance of personality variation"

Presentations

Santostefano F, Wilson AJ, Niemelae PT, Dingemanse NJ. "Behavioural mediators of genetic life-history trade-offs in Field crickets". 2016 May. *Oral presentation at the IGB Berlin, Leibniz institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany*

Santostefano F, Wilson AJ, Niemelae PT, Dingemanse NJ. "Behavioural mediators of genetic life-history trade-offs in Field crickets". 2016 February. *Oral presentation at the Behavioural Ecology Meeting, Chioggia, Italy*

Santostefano F, Wilson AJ, Araya-Ajoy YG, Dingemanse NJ. "Interacting with the enemy: indirect effects of personality on conspecific aggression in crickets". August 2015, *Oral presentation at the Behaviour Conference, Cairns, Australia*

Santostefano F. "Social environment effects on behavioural variation in Field crickets". July 2015, *Oral presentation at the Cogcom Workshop, Vienna, Austria*

Santostefano F, Dingemanse NJ. "Individual variation in behaviour and the role of social environment". July 2014. *Oral presentation at the European Conference on Behavioural Biology, Prague, Czech Republic.*

Santostefano F, Dingemanse NJ. "Individual variation, indirect genetic effects & social environments in crickets". December 2013. *Oral presentation at Exeter University, Cornwall Campus, Penryn, United Kingdom.*

Santostefano F, Dingemanse NJ . "Interacting phenotypes and the maintenance of between-individual behavioural variation in field crickets". November 2013. *Poster at the International Conference on Individual Differences, Groeningen, The Netherlands.*

Santostefano F, Dingemanse NJ . "Interacting phenotypes and the maintenance of between-individual behavioural variation in field crickets". August 2013. *Poster at the European Society of Evolutionary Biology Conference, Lisbon, Portugal.*

Teaching and Mentoring

Master and MEME students' project supervision

Tuzun, N: Coping with social environment: aggression in response to relative opponent size in field crickets

Meli, S: Do behavioural syndromes predict dominance hierarchies in field crickets?

Shridar, VH: consequences of the associations between state and exploratory behaviour in field crickets *Gryllus bimaculatus* on the maintenance of personality

Velado Lobato, P: Effect of social environment on individual variation in aggressive behaviour in the field cricket *Gryllus campestris.*

Ariens, S: Effect of the social environment on agonistic interacting phenotypes in *Gryllus campestris.*

Honors and Awards

International Max Planck Research School Student Travel Grant 2016

ISBE Student Travel Grant 2016

CogCom Student Travel Grant 2015

International Max Planck Research School Student Project Grant 2015

International Max Planck Research School Student Lab Visit Grant 2014

International Max Planck Research School Merit Scholarship 2012

STATUTORY DECLARATION AND STATEMENT

Ehrenwörtliche Versicherung

Ich versichere hiermit an Eides statt, dass die von mir vorgelegte Dissertation von mir selbstständig und ohne unerlaubte Hilfe angefertigt ist.

München, den ....
Francesca Santostefano

Erklärung

Hiermit erkläre ich, dass die Dissertation nicht ganz oder in wesentlichen Teilen einer anderen Prüfungskommission vorgelegt worden ist. Im Weiteren erkläre ich, dass ich mich nicht anderweitig einer Doktorprüfung ohne Erfolg unterzogen habe oder ohne Erfolg versucht habe, eine Dissertation einzureichen oder mich einer Doktorprüfung zu unterziehen.

München, den ....
Francesca Santostefano