The ecology and evolution of individual behavioural variation

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Thesis submitted to the University of Nottingham for the degree of

Doctor of Philosophy

Abstract

Central to this thesis is the investigation of how several factors (e.g. morphology, ecology, and social conditions) co-vary with consistent individual behavioural variation. Additionally, conceptual work explores the reach of consistent inter-individual behavioural variation in several novel contexts. In doing so, the aim is to contribute novel findings to the quickly growing compendium of knowledge concerning 'animal personality' in various settings.

In summary, during this PhD the following research has been performed:

- Through the use of lab-reared juvenile three-spined stickleback (*Gasterosteus aculeatus*) whose parents came from different habitats, I investigated the relative contribution of plasticity and (epi)genetic mechanisms on three behaviours (boldness, activity, and sociality). While these behaviours did not differ between juveniles from both populations, correlations of body length with specific behaviours did. (Chapter 2)
- During fieldwork on the Scottish island of North Uist, seven populations of wild three-spined stickleback were surveyed for boldness, exploratory behaviour, and activity. Furthermore, the defensive morphology of these fish was quantified following an armour-staining protocol. Bold fish were found to be less armoured, directly contradicting the phenotypic compensation hypothesis, while more active fish were more armoured, which contradicts the idea that armour is energetically costly. (Chapter 3)
- Spurred on by the findings on morphology-behaviour correlations, the ecology of the studied populations was assessed with a focus on parasitism

by three common macro-parasites of three-spined stickleback, predation by trout, competition with the nine-spined stickleback, and the availability of resources. I found that all these factors, to some extent, influence individual behavioural variation. Importantly, this study provides the first observational evidence that less-explored factors, such as resource availability and interspecific competition, can be highly relevant influences on individual behaviour. (Chapter 4)

- Next, a study on the effect of group behavioural composition on group foraging behaviour was done. With the help of a research visit to Aarhus University, similar experiments were performed on stickleback and social spiders (*Stegodyphus dumicola*). Group behavioural composition had a strong effect in both study systems, but experience was a more relevant influence on group foraging behaviour in stickleback. By using two different study organisms from quite disparate animal taxa, this allows me to develop hypotheses concerning the evolutionary origin and maintenance of individual behavioural variation and its tentative link with sociality. (Chapter 5)
- Lastly, the phenomenon of consistent individual variation in behaviour is explored in novel contexts through conceptual essays (Chapter 6).
    - Do bacteria offer a suitable system to study 'personality'?
    - How can animal personality be leveraged to improve animal welfare?
    - Are non-antagonistic interspecific interactions an overlooked yet important factor in the study of animal personality?

Acknowledgments

None of this would have been possible without:

The UK unit: First and foremost a big thank you to my supervisors, Ben Chapman, John Brookfield, and Andrew MacColl, for guiding me, answering questions, and giving me the freedom to shape parts of this project according to my slightly unconventional ways. Gratitude, kind sirs. Of course, other members of the department have been instrumental in this quest as well. Special mention goes to Sara Goodacre and Tom Reader, for being kind assessors and helping me get to Denmark, or resolve statistical issues. My fellow students have been a kind and generous source of support and banter. Kudos to B109: Jim, Stu, Dan, Jon, Laura, Marie, Sarah. Thanks lads and ladies. The visiting Brazilian buddies Rafael and Henrique went above and beyond in assisting me, for which I will always be grateful. Obrigado. The life and blood of any department or lab, the technical staff, is of utmost importance for the success of any student or staff member. Alan, Ann, Dan (yep, again), Elaine, and others: thank you so much. All your hard work does not go unnoticed and is greatly appreciated.

The Danish department: My research stay at Aarhus University was a great experience, not in the least due to the incredibly nice, welcoming, and supportive people that helped me there. Trine Bilde was absolutely essential in and proved to be a marvellous hostess and valuable research partner, indeed. The same goes for Virginia, who has been friendly and helpful to a degree I never could have hoped for.

Marie's aid in caring for my eight-legged study partners was priceless and highly appreciated. All other members of the superb spider-lab in Aarhus were wonderful and of great help in shaping the project I undertook there.

The Belgian base: It goes without saying, yet it will be said, that the absolute bedrock, the grand foundation of everything I have ever done, everything I will ever achieve, is my family. Their support and unconditional love is the fuel that keeps me going. My parents and brother drag me into the light when the shadows threaten to swallow me. Their existence means more to me than they will ever know. Beyond direct lines, the extended clan of aunts, uncles, nephews, nieces, and all novel attachments to the family tree, are ever supportive and genuinely caring.

To all those above, and to those I forgot to mention (a thousand apologies!):

Thank you!

Publications

A list of peer-reviewed publications based on the work presented in this thesis:

De Winter, G.; Stratford, J.P. & Chapman, B.B. (2015). Using bacteria to study consistent variation in individual behavior. *Behavioral Ecology*. **26**(6): 1465 – 1469.

De Winter, G.; Martins, H.R.; Trovo, R.A. & Chapman, B.B. (2016). Different behaviour-body length correlations in two populations of juvenile three-spined stickleback (*Gasterosteus aculeatus*). *Behavioural Processes*. **122**: 75 – 79.

De Winter, G.; Martins, H.R.; Trovo, R.A. & Chapman, B.B. (2016). Knights in shining armour are not necessarily bold: defensive morphology correlates negatively with boldness, but positively with activity, in wild populations of threespine stickleback, *Gasterosteus aculeatus*. *Evolutionary Ecology Research.* **17**(2): 279 – 290.

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0. Prologue

… Hear me, dear muses…

Science is beautiful. And yet, many of those who hope to pursue this beauty are taught to bury the kernel of awe and wonder under heavy, unimaginative boulders of convention. Inchoate scientists are encouraged to keep their heads down, follow trodden paths, and respect tradition. While there is value in established routes, it can be found in the unexplored lands beyond them as well. This, at least, is my conviction. Therefore, kind muses, give me the strength and ability to produce a work that encompasses solid scientific efforts but also includes less conventional sidesteps.

Allow me to forego burrowing like a hedgehog lest I forget how to jump like a fox.

The realm of ideas and concepts is often neglected or undervalued in modern scientific training. Essays, perspectives, and their ilk, are all too often side-projects, certainly deemed unsuitable for aspiring scientists. This plane of inquiry, however, is where I feel most at home. Gems are waiting to be unearthed there (let me succumb to convention in this instance: Blagosklonny & Pardee, 2002). Let me hunt for them. In return, I shall do my utmost to sculpt whatever may follow into a form that appeases traditionalists and iconoclasts alike.

This sculpture shall be made out of numbers and language. Unfortunately, in scientific writing, so we are told, there is no room for metaphors, similes and the like. Our great forebears, the ancient natural philosophers, would cringe. They did not

separate science from philosophy or literature. Instead, they embraced the common driver for all these: the desire to know, to understand, to learn.

Yet now we face a linguistic straight-jacket. Emotionless accuracy rules the letters and paragraphs of science, and has goaded our understanding of the natural world to ever increasing heights. But do prose and science need to be mutually exclusive? I, for one, do not believe so.

However, generous muses, give me the strength and inspiration to write in a way that is scientifically accurate but allows the beauty of science to shine through. Let me produce the following pages in an impersonal and objective manner, yet let the readers experience some of the privilege I felt in pursuit of this opportunity.

If it pleases you, gentle muses, grant me these requests, and I shall, as always, remain your humble servant…

I. General Introduction:

Causes, consequences, and context of consistent individual variation in behaviour

I.I. Individual behavioural variation – A meeting ground for behaviour, ecology and evolution

Many species harbour a surprising and previously unexpected variety in consistent individual behaviour, and its origin and maintenance is a puzzle. Before proceeding, however, a few words need to be addressed on terminology. Concepts and terms in research concerning individual behaviour, such as personality, behavioural type, temperament and behavioural syndrome, are used alongside, or in place of, each other (Réale et al. 2007; Biro & Stamps 2008; Stamps & Groothuis 2010a; Budaev & Brown 2011; David & Dall 2016; Roche et al. 2016). Fortunately, the recent surge in research concerning behavioural variation among individuals and populations has led to refinement in these terms' definitions and agreement concerning their use. Table 1.1 provides a succinct glossary of related terms as they are used in the rest of the thesis (based primarily on Koolhaas et al. 1999; Bergmüller & Taborsky 2010; Stamps & Groothuis 2010b; Wolf & Weissing 2012).

Table 1.1: Glossary of terms.

Term	**Definition**
Animal personality	Denotes the fact that individual animals often show behavioural variation within and between populations, consistent over time and/or across situations (synonym: temperament).
Behavioural reaction norm	The set of behavioural phenotypes that an individual can produce across a range of environmental conditions and internal stimuli.

Behavioural syndrome	A group of behaviours that is correlated at population level in different contexts and situations (e.g. the aggressiveness-boldness syndrome).
Behavioural type	An individual's position in multivariate behavioural syndrome space, i.e. the phenotypic expression of a range of behavioural traits.
Coping style	A coherent set of behavioural and physiological stress responses, consistent over time and across contexts (sometimes used as synonym for both behavioural type and behavioural syndrome).
Phenotypic compensation	Hypothesis that stipulates that behaviour and morphology are attuned to each other, e.g. risk-taking individuals are expected to possess a more developed defensive morphology.
Social niche specialisation	When an individual chooses, or is forced into, a specific social role, it behaves accordingly. In group context this can lead to consistent differences in individual behaviour depending on social role.
State	The collection of features that should be taken into consideration by an animal when making behavioural decisions that can affect fitness. Examples are morphology, physiological condition, social information, learning experiences, and environmental variables.

Consistent variation in individual behaviour presents a puzzle. To maximize fitness in the wide variety of environmental conditions that many organisms experience during their lifetime, limitless behavioural flexibility would provide a very useful mechanism. Instead, individual animals tend to display a rather limited plasticity in behaviour and the behavioural differences among individuals are often conserved in different contexts (Dingemanse & Réale 2005). It has become increasingly clear that to resolve this mystery of consistent individual behavioural variation, ecological and evolutionary factors cannot be ignored (Dingemanse & Réale 2005; Réale et al. 2007; Réale et al. 2010; Wolf & Weissing 2012; Dall et al. 2012).

The first syntheses and integrative studies have recently appeared. For example, Bergmüller & Taborsky (2010) develop the social niche hypothesis which stipulates that the social conditions an animal is exposed to can contribute to consistent individual behavioural variation in populations. Finally, Chapman et al. (2011b) and Jandt et al. (2014) investigated behavioural variation in both individuals and the colonies they form, linking behavioural variation at multiple scales in eusocial colonies. These initial integrative steps have already shown that there are many interacting factors, feedback loops and contextual considerations to be taken into account when studying the evolution and ecology of individual behavioural variation. Assimilating these into an inclusive framework will highlight not only the importance of consistent individual behavioural variation in ecology and evolutionary biology, but also allows the identification of new research avenues (Réale et al. 2010; Dall et al. 2012; David & Dall 2016).

In this general introduction, the factors that affect the evolution of individual variation in behaviour will be discussed. Any behavioural trait is the outcome of internal and external factors (Stamps & Groothuis 2010b), which are often difficult to separate entirely and fall on a broad spectrum between fully intrinsic or extrinsic. The potential impact of individual behavioural variation on large-scale evolutionary and ecological processes forms the next part of this review. Again, expecting a clear division ignores their potential overlap in space and time, which has only recently been recognized. Finally, the relevance of context, interaction and feedback will be emphasized.

Ultimately, the goal of this broad review is to accentuate the relevance of an integrated ecological and evolutionary view on variation in individual behaviour extending across space, time and levels of biological complexity.

Causes of individual behavioural variation

Consistent variation in individual behaviour has been noted in an extensive variety of taxa, from cockroaches (Planas-sitja et al. 2015), to sharks (Jacoby et al. 2014), and meerkats (English et al. 2010). This observation has spurred two main lines of enquiry: why does this consistent variation exist when greater plasticity would generally be advantageous? And how does it persist (Stamps 2007; Réale et al. 2010; Schuett et al. 2010; Bergmüller & Taborsky 2010; Wolf & Weissing 2010)?

One way to address this is to study potential causes of individual variation in behaviour, and several different candidates have been proposed. Since numerous contributing factors are still relatively unexplored, the following list is by no means exhaustive.

Genes

Recent advances in genetic and genomic technologies initiated an increasing effort to pinpoint and describe the genetic basis for individual behavioural variation (van Oers et al. 2005; Bell & Aubin-Horth 2010; Dochtermann & Roff 2010; van Oers & Mueller 2010; van Oers & Sinn 2011). Of course, aspects of the other potential causes discussed later likely also have a genetic foundation, but here the focus lies specifically on the identification of genes, genetic interactions and genomic regions that directly affect behavioural variation.

Using pedigree analyses and breeding designs usually provides preliminary indications for a genetic basis of individual behavioural variation (Dingemanse et al. 2010; Dochtermann & Roff 2010; Dingemanse et al. 2012). For example, in lab-reared three-spined sticklebacks (*Gasterosteus aculeatus*) (Bell 2005), wild great tits (*Parus major*) (Dingemanse et al. 2002), and bighorn ewes (*Ovis canadensis*) (Réale et al. 2000), assaying the behaviour of parents and their offspring has resulted in heritability estimates for the behaviours that were tested (for an overview of the heritability of personality, see Dochtermann et al. 2015). But these studies are exceptions as, so far, few studies have explicitly addressed the heritability of individual variation in consistent behaviour (for an overview, see van Oers & Sinn 2011). Much of this work is based solely on phenotypic characteristics and assumes that these accurately reflect the underlying genetic architecture. However, our increasing ability to investigate genes and genomes creates the opportunity to take behavioural ecology and evolution beyond this assumption of straightforward genotype-phenotype mapping, which is also known as the 'phenotypic gambit' (Springer et al. 2011; van Oers & Sinn 2011; Rittschof & Robinson 2014).

An additional complicating factor is that the genetic groundwork underlying individual behavioural variation may be rife with pleiotropic interactions which result in genetic correlations between traits (Estes 2005; Dochtermann & Dingemanse 2013). Therefore, identifying genes for behavioural variation without taking into account potential genetic correlations prohibits the assignment of causality in genotype-phenotype mapping (Estes 2005).

Life-history

The behaviour of an organism is influenced by events early in life, even prior to birth. Indeed, *in utero* conditions affect behaviours such as aggression, exploration and activity. In fact, individual behavioural variation could be partially shaped even prior to conception through maternal and epigenetic effects that reach across generations (Biro & Stamps 2008; Stamps & Groothuis 2010a). In side-blotched lizards (*Uta stansburiana*), for example, yellow-throated barred mothers lay larger eggs than orange-throated striped ones. Large eggs, in turn, produce bulkier offspring that display escape behaviours more suited to their morphology (reversals in yellow-throated barred lizards versus jumping in striped orange-throated lizards) (Lancaster et al. 2010).

After birth, individual variation in behaviour may reflect life-history trade-offs involving physiological traits. Growth rate and metabolism have been suggested as relevant influences on consistent individual behavioural variation (Stamps 2007; Biro & Stamps 2008; Careau et al. 2008). It is, for example, argued that individual differences in growth rate can persist due to growth-mortality trade-offs that result in equal fitness outcomes for individuals with different growth rates (Stamps 2007). This variety in growth rates could proximately explain correlations between behaviours. Growing faster means having higher food requirements, which in turn might explain more active and/or bold behaviour. An example of this is the correlation between boldness and growth rate in three-spined stickleback (Ward et al. 2004). This notion is extended in the hypothesis that behavioural traits are linked to life-history productivity, which includes fecundity alongside growth (Wolf et al.

2007; Biro & Stamps 2008), as exemplified by the complex interaction between exploratory behaviour and reproductive success in wild great tits where the correlation between exploratory behaviour and reproductive success depends on weather conditions (Dingemanse et al. 2004). The complex physiological underpinnings of life-history productivity have two consequences: first, it is hard to change, and second, it works best if consistent (Biro & Stamps 2008). Therefore, we can expect individual differences in the physiology underlying life-history productivity. Then, if some personality traits are connected to certain life-history characteristics, so this hypothesis proposes, individual differences in life-history traits offer a (partial) explanation for the existence of individual behavioural variation. These links between behaviour, physiology and life-history are encapsulated in the pace-of-life syndrome concept which posits that different ecological conditions induce different metabolic and other physiological responses in related species and populations, affecting behavioural variation (Réale et al. 2010b). Support for this hypothesis in natural conditions, however, is equivocal (see e.g. Závorka et al. 2015 where the lab-based measures of activity did not correlate with survival in the wild in brown trout (*Salmo trutta*)).

It would be a mistake, however, to think that only those life-history traits that exert their influence from an early age onwards matter. Even after sexual maturity, certain experiences and associated learning can influence correlations between behaviours or between behavioural and physiological characteristics (Stamps & Groothuis 2010a), such as how social conditions can affect both risk-taking and leadership in three-spined stickleback (Jolles et al. 2014a), as well as the repeatability of bold behaviour (Jolles et al. 2016).

Parasites

Another proposed causal factor in the evolutionary generation and maintenance of individual behavioural variation is parasitism. Long ignored as potential cause for individual behavioural variation (Barber & Dingemanse 2010; Kortet et al. 2010), it is well-known that parasites can affect host behaviour to increase their chance of transmission (Poulin 2013; Araujo et al. 2016). In fact, it has even been proposed that latent infection with the common brain parasite *Toxoplasma gondii* affects human culture through altering personality traits such as neuroticism (Lafferty 2006). On the other hand, host behaviour can influence the number and species of parasites it is exposed to. For example, birds showing more active escape behaviour display a higher prevalence of certain parasites (Garcia-Longoria et al. 2014) and primate species with a higher tendency to explore or learn via social mechanisms carry a higher parasite burden, suggesting that traits conducive to cultural development are associated with parasite exposure (Mccabe et al. 2015).

Parasites can impact the cost-benefit ratio of a particular (combination of) behaviour(s), both directly and indirectly. For example, exploratory individuals can expose themselves to new parasites, and social individuals could increase their exposure to parasites that are transmitted between individuals through contact. Yet being exploratory and/or social can also have benefits such as a stronger immune system, or a better food supply (Barber & Dingemanse 2010). It can pay to avoid infection directly, but this comes with potential costs and additionally depends on the host's immune phenotype. Another consideration is that parasites can affect behavioural syndromes. If several behavioural traits are linked to infection risk,

particular trait combinations could be cemented by a strong selection pressure imposed by local parasite communities. Parasites can also decouple existing syndromes if they affect some, but not all, associated behaviours (Barber & Dingemanse 2010; Poulin 2013). This option is rarely investigated and the mechanisms behind this still requires elucidation (one can imagine different physiological bases for different behaviour so that a parasite affecting a certain physiological trait affects one behaviour but not others). More indirectly, parasitic infections impact the host's state, and through affecting growth rate, fecundity or immune function, parasites can have a profound impact on an organism's life-history, which, as mentioned earlier, can be tied to behavioural traits (Barber & Dingemanse 2010; Kortet et al. 2010).

Specific local environmental conditions regulate the parasite community composition, and previous infections and learned behaviour could lead to variation in avoidance behaviours, such as when previous exposure leads to behavioural mechanisms to avoid infection, e.g. reduced exploration or sociability (Barber et al. 2016).

Predator-prey interactions

Predation risk is thought to be a major influence in shaping behavioural syndromes and types (Dall et al. 2004; Dingemanse & Réale 2005; Wolf et al. 2007; Pettorelli et al. 2011). Predator presence or absence can shift selection pressures imposed on individuals in a population, as can the specific type of predator.

Recent experimental work shows that predator exposure can sculpt the behavioural syndromes and types in a prey population when predator presence exerts a selection pressure on specific (combinations of) behaviours (Relyea 2002; Bell & Sih 2007; Brydges et al. 2008; Dingemanse et al. 2009; Harris et al. 2010). Furthermore, there is evidence for context-specificity, wherein a predator can influence the coupling of behavioural traits. In three-spined stickleback, for example, predator presence induces a consistent correlation between boldness and aggression (Bell & Sih 2007), and boldness and activity (Brydges et al. 2008), possibly generated through predator-induced selection for individuals that behave consistently towards both predators (i.e. degree of boldness) and conspecifics (i.e. degree of aggression) (Bell & Sih 2007). Another example of how the presence of a predator can provide a context that affects trait combinations is the negation of personality-based dispersal in mosquitofish (*Gambusia affinis*) under predation risk (Cote et al. 2013). These experimental investigations tend to focus on the prey's perspective, but predator-prey dynamics are not a one-way street (Sih et al. 2012). Variation in prey phenotype could affect individual behavioural variation in predators. Predator-prey dynamics exhibit frequency-dependent selection, which has been suggested as potential contributor to the maintenance of 'personalities' over evolutionary time (Wolf & McNamara 2012). It is not inconceivable, then, that prey can mould predator behaviour as well. Very few studies have addressed this option. A recent study on three-spined stickleback and northern pike has, however, found evidence for behavioural reciprocity between prey and predator, as well as consistent differences in both prey and predator behaviour (Mcghee et al. 2014). Distributing the focus to both sides of the predator-prey equation could prove illuminating (Lima 2002; Pruitt et al. 2012b).

Finally, for both prey and predator, each other's presence or absence feeds back onto other potential generators of behavioural variation. For instance, exposure to predation can lead to (learned) avoidance behaviour that may reach across generations via, for example epigenetic inheritance or parental teaching, and affect behavioural traits and associated life-history parameters (Stamps & Groothuis 2010a). Predators, on the other hand, can learn to recognize their prey and adopt different hunting strategies with diverse metabolic requirements (e.g. Pruitt et al. 2012b).

Social interactions

Individuals do not only engage in interspecific antagonistic interactions such as parasitism and predation, but are also subjected to numerous intraspecific encounters. Social context can be an important factor in moderating the variation present in individually consistent behaviour (Webster & Ward 2011). The social niche hypothesis stipulates that social interactions can be important contributing factors to the rise and maintenance of consistent individual variation (Bergmüller & Taborsky 2010). By aligning itself with, or being forced into, a certain social role, an individual's behaviour may be shaped accordingly. Competitive and cooperative interactions, and the mixture of both, might lead to persistent individual behavioural variation and trait correlations. For example, in social spiders (*Stegodyphus mimosarum*) repeated social interactions generate among-individual differences in behaviour and reinforce within-individual consistency of behaviour (Laskowski & Pruitt 2014).

However, separating cause and effect is difficult. Does behavioural variation arise through social interaction, or are social dynamics outlined by the participants' behavioural types? Experimental studies point both ways (for the former, see e.g. Laskowski & Pruitt 2014; Modlmeier et al. 2014, for the latter e.g. Laskowski & Bell 2014). This influence of social interaction appears to be taxa-, context-, sex-, and individual-specific (Harris et al. 2010; Schuett et al. 2010; King et al. 2013). Additionally, the interplay between behaviour and social dynamics need not be constrained to direct partakers. The mere presence of certain behavioural types can alter social behaviours of others (Hamilton & Ligocki 2012), and simply being part of a group can alter the repeatability of personality traits (Jolles et al. 2016). Recent advances in network analysis allow a look beyond dyadic relationships which were, and still are, the main focus in studies of social interaction. By widening the scope to (part of) the social network of an individual, questions about the relationship between an organism's network position and its behaviour come into view (Krause et al. 2010; Jacoby et al. 2014).

One would think that eusocial colonies provide a highly suitable study system to investigate the effects of social interactions on individual behavioural variation, but the majority of studies in this field focus on genetic relatedness and its effects on various aspects of colony life. Nevertheless, the behaviour of individuals and its influence on fellow colony-dwellers, even within the same caste, has recently caught the attention of researchers (Pruitt et al. 2011; Jeanson & Weidenmüller 2014; Keiser et al. 2014; Keiser et al. 2016; Wright et al. 2016), which has revealed that even within castes distinct behavioural types can occur. A final area that has been scarcely explored is the effect of non-antagonistic interspecific social interactions such as mutualism on shaping individual behavioural variation (but see Wilson et al. 2014 for

behavioural variation in cleaner fish and their clients, where increased feeding effort of the cleaner fish correlates with a lower propensity to cheat in cleaner-client interactions).

Abiotic environment

Many aspects of the abiotic environment constitute selective pressures that can shape an organism's behavioural traits. Food availability and habitat stability, for example, have been implicated in the induction and maintenance of behavioural types and syndromes. These aspects, however, depend on other features such as temperature, humidity, etc. Therefore, to list, or test, every abiotic factor might prove a near-endless pursuit.

One facet of the abiotic environment that seems quite important in generating and maintaining individual behavioural variation is variability (Brydges et al. 2008; Dalesman et al. 2015). Environments vary in space and time, and different types of variation can have diverging effects on the development and stability of behavioural types and syndromes (Stamps & Groothuis 2010b; Wolf & Weissing 2010). Fluctuating environments can even result in opposite fitness consequences for behavioural traits over time. In great tits (*Parus major*), for example, the relationship between parental personality and offspring survival depends on changes in environment that affect food availability (Dingemanse et al. 2004). The effect of spatiotemporal environmental variation is highly context-specific. Furthermore, individuals can differ in their sensitivity and responsiveness towards changing environmental conditions, which means that the behavioural effect of such

fluctuations could in itself depend on other behavioural traits (Sih & Bell 2008). Additionally, organisms are not entirely defenceless against environmental changes. They are able to, albeit often to a limited extent, choose or modify their physical environment (Stamps & Groothuis 2010b). In eusocial colonies, for example, the conditions larvae are exposed to could influence the interindividual behavioural variation present in adult workers (Jeanson & Weidenmüller 2014). On top of this, the general environment of the colony itself can influence the behavioural type composition of its occupants (Pruitt & Goodnight 2014).

This abiotic influence on variation in individual behaviour is not just a direct one. The environment affects other potential causes of individual variation in behaviour such as parasite and predator occurrence, or life-history. The relative harshness of the surroundings can mediate social interactions, and, through epigenetic interactions, the environment may even fashion the transcription of genes across generations, such as when scarcity of food affects the behaviour of (grand)children (Stamps & Groothuis 2010a). Clearly, the influence of abiotic environmental factors is another well-connected root in the tangled system of potential factors underlying variation in individual behaviour.

Ecological and evolutionary consequences

Only recently, the potentially far-reaching ecological and evolutionary consequences of individual behavioural variation have started to receive attention. In the following

section I outline recent findings concerning the implications of animal personality for major ecological and evolutionary processes.

Dispersal and migration

Dispersal and migration are processes of great importance in ecology and evolution, both in the long and short term. From the rapid incursion of invasive species, through climate-change-induced home-range changes, to patterns of biodiversity, dispersal and migration are relevant for all of these.

The tendency to disperse or migrate often correlates with behavioural traits such as boldness, exploration and aggressiveness (Cote et al. 2010; Sih et al. 2012). In most cases, this link is highly consistent within populations, leading to the concept of a 'dispersal syndrome' (Sih et al. 2004a; Sih & Bell 2008; Wolf & Weissing 2012). But not only permanent dispersal could depend on behavioural type, (partial) migration might as well (Chapman et al. 2011a). Different stages of both processes could rely on different behavioural traits. For example, the decision to leave might depend on sensitivity to environmental or social cues, the journey on exploratory behaviour, and colonization success on boldness or aggression. This connection between the journey's stage and behaviour can be reflected by a consistent dispersal syndrome or a specific mix of behavioural types in newly established populations that reflects the specific set of behaviours required to successfully initiate and complete the journey (Cote et al. 2010; Sih et al. 2012; Wolf & Weissing 2012).

Through influencing dispersal and migratory tendencies, individual variation in behaviour can be a key aspect of ecological and evolutionary processes. If only a specific behavioural subset of individuals disperses or migrates, this leads to differences in behavioural composition between the source population and its offshoots, or at the core and the edges of a single population, for example bolder round gobies (*Neogobius melanostomus*) that are more likely to be found at the invasion front (Myles-Gonzalez et al. 2015). This differential dispersion or migration can impact large-scale metapopulation dynamics (Cote et al. 2010; Sih et al. 2012; Wolf & Weissing 2012). This, in turn, can affect biological invasions (Sih & Bell 2008), re-introduction programmes (Cote et al. 2010; Wolf & Weissing 2012) and responses to climate change (Sih et al. 2012; Wong & Candolin 2015). Even extinction and survival rates can be affected, as can between-population differences at the geno- and phenotypic level (Sih et al. 2012).

Social evolution

The social environment of an individual is, as mentioned earlier, a potential cause for individual behavioural variation. However, this is only one side of the social coin (Wolf & Krause 2014). Social interactions are also ubiquitous in, and relevant for, various evolutionary and ecological processes.

Differences in social behaviours can spur the evolution of social sensitivity and responsiveness. After all, it pays to recognize the behaviour of your neighbours and to know how to respond appropriately. This can trigger a co-evolutionary process between a social cue and the response, favouring behavioural consistency and

coordination, as well as an increase in social competition, potentially promoting high levels of cooperation in sub-groups (Sih & Bell 2008; Wolf & Weissing 2012; Wolf & Krause 2014). Could individual behavioural variation be a hidden foundation of eusociality? To my knowledge, no one has yet explicitly investigated this possibility. An intriguing hint is that social spiders display increased within-species variation in aggression and boldness compared to closely related sub-social species (Pruitt et al. 2012a). Also, within eusocial colonies, inter-individual behavioural differences tend to be beneficial as they increase the range of worker response behaviours and introduce rare behaviours that might prove useful in unusual conditions (Jeanson & Weidenmüller 2014). Furthermore, extending the personality concept to the level of the colony itself adds another layer of analysis and potential evolutionary influence (e.g. Chapman et al. 2011b; Modlmeier et al. 2014a; Wright et al. 2016). Strong cooperation additionally increases a group's problem-solving ability. Diverse groups are more likely to harbour an organism well-equipped to deal with a specific problem (the 'pool of competence hypothesis'), and diverse mechanisms of information acquisition and use translate into a heightened potential for swarm intelligence (Wolf & Krause 2014). General growth in social complexity might induce a selection pressure favouring greater cognitive abilities (Sih & Del Giudice 2012; see also the 'social brain hypothesis', Dunbar 1998). However, increasing social complexity can also lead to more intricate communication mechanisms and the concomitant rising chance of error could result in decreased group performance (Wolf & Krause 2014).

All this suggests that individual behavioural variation can have a substantial impact on the social evolutionary trajectory within and between species. Also noteworthy is that the eco-evolutionary consequences of individual variation in behaviour are often connected (for example social system and dispersal tendency where local

population density can drive certain ‘personalities’ to disperse or migrate, see Cote et al. 2010; Sih et al. 2012; Found & St. Clair 2016).

Adaptation

If we assume that some behaviours have a genetic basis and that consistent behavioural differences between individuals affect fitness and possess some degree of heritability, they might provide a substrate for selection. In other words, they can be a starting point for adaptation and divergence within and between populations (Dingemanse & Réale 2005; van Oers et al. 2005; Wolf & Weissing 2012; Trillmich et al. 2015).

The connection between individual behavioural differences and fitness has recently been attracting consideration, but much work remains to be done, certainly in natural conditions (Dingemanse & Réale 2005; Niemela & Dingemanse 2014). An additional difficulty is that this link strongly depends on environmental circumstances. What works in one environment, might not do so in another. Besides, traits are rarely selected for or against in isolation. Often, traits (behavioural and otherwise) are correlated so that selection on one affects the evolutionary path of other(s) (Dingemanse & Réale 2005; van Oers et al. 2005; van Oers & Mueller 2010). Another possibility is that selection acts upon multiple targets and engenders trait correlations via selecting for or against certain trait combinations. Recent advances in genomics could potentially provide tools to investigate these options and to elucidate when, if, and how (behavioural) traits are, or have been, influenced by selective pressures and, additionally, to see when the phenotypic gambit is a safe bet

(van Oers et al. 2005; Bell & Aubin-Horth 2010; van Oers & Mueller 2010; van Oers & Sinn 2011; Rittschof & Robinson 2014). Another intriguing line of enquiry ties in with the controversial concept of multi-level selection. Does the behavioural composition of colonies in eusocial species, for example, affect their fitness? Initial hints for a positive answer are offered by studies showing that behavioural diversity within a eusocial colony contributes to its success in ants and social spiders (Modlmeier et al. 2012; Pruitt & Goodnight 2014). Vice versa, can the relative success of a group or colony shape the behaviour of its constituents?

All in all, behavioural traits, much like others, are not exempt from the inquisitive eye of natural selection. Furthermore, combinations of such traits, and correlations with other traits, can pose targets for selection as well. Unravelling how individual behavioural variation can be a substrate for selection also requires attention for the (unavoidable) context-dependency.

Speciation, the speed of evolution, and evolvability

Not only does behavioural variation provide a potential source for selection, it might also influence the evolutionary trajectory that follows. In other words, it can influence the process of speciation as well as the tempo and mode of evolution (Sih et al. 2004b; Wolf & Weissing 2012; Ingley & Johnson 2014).

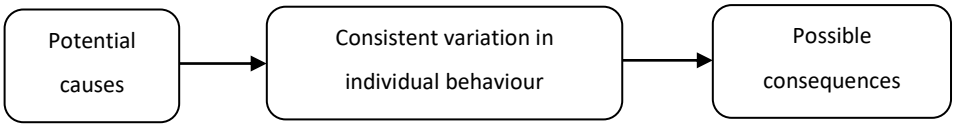
Regarding the tempo and mode of evolution, and, related, evolvability, it serves to recall that traits are rarely unitary targets for selection but are instead entrenched in an intricate network of correlations. Behavioural traits can be correlated with each

other and with other trait types (Sih et al. 2004b). If these links have a genetic basis, this implies that evolutionary change in one trait can constrain (or facilitate?) heritable changes in another one which need not be under direct selection (Sih et al. 2004b; Wolf & Weissing 2012; Dochtermann & Dingemanse 2013). The occurrence of correlations between behavioural traits and others (behavioural or not) appears to be context-dependent (Stamps & Groothuis 2010a), which means that the evolutionary path of a trait is influenced not only by internal (e.g. trait correlations) and external factors, but also by the specific interaction(s) between them, such as when an external factor affects how internal factors influence the selection on a trait under consideration. Additionally, individual variation in behaviour can directly influence speciation, whether it is by acting as a 'magic trait' in mate choice or by shaping differential dispersal or migration tendencies (Ingley & Johnson 2014). Furthermore, we need not constrain ourselves to a single species' evolutionary journey, as behavioural variation affects interactions between different species, antagonistic or otherwise. An arms race between host and parasite, for example, is not only influenced by behavioural variation, but can in turn lead to greater evolvability and host complexity than might be present otherwise (Zaman et al. 2014).

Context, interaction, and feedback

In considering potential causes and consequences of individual variation in behaviour it becomes clear that we are dealing with a highly complex, integrated phenomenon (Fig. 1.1). In fact, one of the recurring themes is the importance of context, interaction and feedback. Many of the causes and consequences are linked, often to a degree that makes it hard to untangle them.

a)



b)

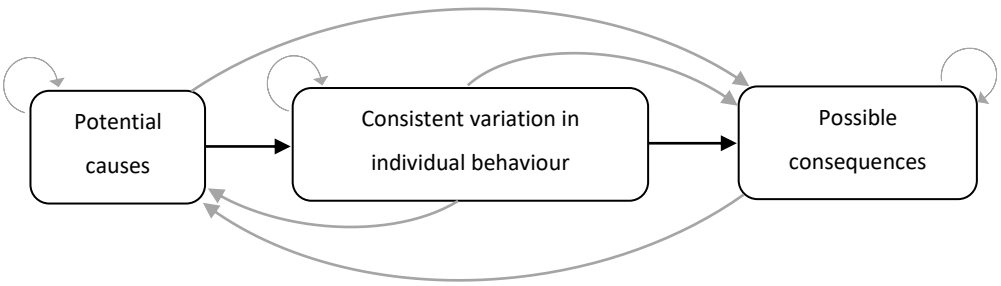


Figure 1.1: a) conception of a stereotypical causal chain concerning consistent variation in individual behaviour ('animal personality'); b) including potential feedbacks and interactions (grey arrows) results in a more realistic web of interactions rather than a straight sequence.

Context sensitivity occurs across the whole causal chain surrounding individual behavioural variation. Both biotic and abiotic factors influence a wide range of

processes such as gene expression, development, the occurrence of parasites and conspecifics, interactions between species, and so on. These, in turn, affect the expression of behaviour and even the (non-)existence of behavioural types and syndromes. In fact, the potential feedbacks between state and behaviour in producing consistent individual variation is a focal point in recent theoretical developments (Sih et al. 2014). Interestingly, while the development and implications of individual behavioural variation are highly context-dependent, behavioural types and syndromes themselves are fairly robust when facing change. In fact, their definition includes persistence across contexts (Sih et al. 2004b; Réale et al. 2007; Dingemanse & Wolf 2010). This might sound contradictory, but it is not. The establishment and consequences of a context-robust characteristic can be highly context-dependent. Behavioural types and syndromes are a chief example of this. Consistent across contexts when present, their presence and precise nature are strongly affected by context.

Causes and consequences of individual behavioural variation can also provide context for each other. An intriguing consideration is that these interactions are not limited to the causes and consequences of behavioural differentiation, but include the behavioural traits themselves. Feedback mechanisms allow them to influence their own development directly and indirectly via modulation of the factors that shape them (Sih et al. 2014). Behavioural traits are not powerless victims, but active participants.

To complicate matters more, the occurrence and specifics of context-dependency, interactions and feedback mechanisms are intertwined as well, adding another level of intricacy. Furthermore, the recent concept of behavioural types and syndromes at other levels of biological organization spurs questions concerning the equivalence of

this web of context, interaction and feedback at multiple levels (e.g. individual and colony behaviour, see Chapman et al. 2011b; Pruitt et al. 2013; Jandt et al. 2014).

Conclusion: Disentangling the web

Based on recent studies and conceptual developments, an integrated picture of behavioural variation embedded within ecology and evolution is emerging. This picture is complicated and convoluted, especially when realizing that it represents only one organizational level. If we seek to understand behavioural variation in, say, both individuals and groups, it merely represents a two-dimensional slice of a three-dimensional sculpture.

However, in no way does this mean that understanding consistent variation in individual behaviour is beyond our current capacities. Individual behavioural variation, at whichever level, is increasingly recognized as an important mediator in various ecological and evolutionary processes. (Sih & Bell 2008; Wolf & Weissing 2012). A surge of recent research has helped greatly in understanding the roots of this variation. Studying its importance in a broader ecological and evolutionary context has lagged behind slightly, but is catching up. Finally, the latest developments include the possibility of behavioural types and syndromes at different levels of biological organization.

The web of links and interactions surrounding individual behavioural variation supports the pleas for a broad, integrated approach (Réale et al. 2007; Dall et al. 2012; Dall & Griffith 2014). While these calls often focus on the 'animal personality'

perspective, including additional organizational levels, such as groups and colonies, might prove useful (Dall et al. 2012).

This integrated approach begets a new generation of research avenues. The investigation of a single cause or consequence remains definitely worthwhile as many questions remain wanting of an answer, but a second type of question that explicitly incorporates the high linkage in the web of factors spun around behavioural variation needs to be addressed too. Examples of such questions are:

- How do genes, life-history, predation, parasitism, social interactions and the abiotic environment interact in determining the occurrence, consistency and variability of behavioural types and syndromes?

- Under what conditions do behaviours, directly or indirectly, reinforce or dampen their own expression, or that of correlated behaviours, through feedback mechanisms?

- How does individual behavioural variation influence large-scale ecological and evolutionary processes, and how does this alter its own evolutionary path?

- What determines the behavioural structure of a group and how is this linked to the behaviour of its members?

- How is behavioural variation at different organizational levels connected, and what is the relative importance of individual/group behaviour in ecological and evolutionary processes?

Of course, these questions should be tailored to specific system(s) and behaviour(s) and particular predictions should be developed based on models and studies concerning specific behaviours and influencing factors.

Individual behavioural variation is nested in a causal web of intertwined ecological and evolutionary strands. As such, it provides an exceptional opportunity for the integration of these disciplines.

I.II. PhD aims

While the general topic of this PhD is quite broad and already covered in the above review, specific questions that I hope to have contributed to are:

- How well-established are individual personalities in early stages of ontogeny? How important are parental vs. current environmental conditions? (Chapter 2)

- Does consistent individual behavioural variation co-vary with morphology in wild populations? Does our study system provide support for the phenotypic compensation hypothesis in a natural environment? (Chapter 3)

- How do different ecological factors (with a focus on parasitism, predation, resource availability, and interspecific competition) contribute to consistent behavioural variation within and between populations of wild animals? (Chapter 4)

- How does behavioural variation between individuals shape group behaviour? How consistent is the effect of constituent members on the group's dynamics? How (dis)similar are the answers to these two questions in different study systems? In other words, are there general, cross-taxonomic rules governing the evolution and maintenance of individual behavioural variation and its effect on group behaviour? (Chapter 5)

- Can the conceptual background developed in animal personality research be extended into other fields of biological enquiry, such as microbiology, animal welfare, or the dynamics of mutualisms? (Chapter 6)

To investigate these queries, the main focal animals were three-spined stickleback (*Gasterosteus aculeatus*), both in laboratory (Chapters 2 and 5) and field conditions (Chapters 3 and 4). Additionally, a research visit to Aarhus University allowed me to work with the social spider *Stegodyphus dumicola* in order to add a cross-taxonomic perspective to the work concerning social effects of personality (Chapter 5). Finally, acquainting myself with literature dealing with behaviour in microbiological context, animal welfare, and mutualistic interactions, resulted in a foundation for conceptual queries (Chapter 6).

II. Budding personalities?*

Different behaviour-body length correlations in two populations of juvenile three-spined stickleback

* A version of this chapter has been published as:

De Winter, G.; Martins, H.R.; Trovo, R.A. & Chapman, B.B. (2016) Different behaviour-body length correlations in two populations of juvenile three-spined stickleback (*Gasterosteus aculeatus*). *Behavioural Processes.* 122: 75 – 79.

Abstract

Behavioural variation among individuals has received a lot of attention by behavioural ecologists in the past few years. Its causes and consequences are becoming vast areas of research. The origin and maintenance of individual variation in behaviour within and among populations is affected by many facets of the biotic and abiotic environment. The question we investigate here is whether the parental population affects variation and repeatability of behaviour in juveniles raised in identical conditions.

Here, two populations of lab-reared juvenile three-spined sticklebacks (*Gasterosteus aculeatus*) are tested for variation in, and repeatability of, three behavioural axes (boldness, exploration, and sociability). Given the identical rearing conditions, the only difference between these populations is the parental habitat. In both populations, correlations between behaviour and body length are found. Interestingly, these differ between the populations. In one population body length was negatively correlated with exploratory behaviour, while in the other one body length correlated negatively with sociability.

Considering the identical environment these juvenile fish were exposed to, these findings suggest a potential (epi)genetic foundation for these correlations and shows that, in three-spined sticklebacks, the proximate basis for correlations between body length and behaviour appears quite malleable.

II.I. Introduction

Despite the importance of variation in evolution, individual variation in behaviour has traditionally been considered as non-adaptive noise around an adaptive mean by behavioural ecologists (Dall et al. 2004). Recently, this assumption has been questioned, driving research on the adaptive nature of individual behavioural variation.

One approach to investigate the evolutionary importance of ecological factors on individual behavioural variation is to perform common garden experiments. By eliminating environmental fluctuations, differences between the populations in the presence and type of behavioural syndrome provide a strong clue for the importance of (epi)genetic effects. Additionally, this allows an assessment of the constraint and adaptive hypotheses. The first assumes a shared proximal link between behaviours. This implies that trait correlations are hard to uncouple. In the context of behavioural variation, this hypothesis stipulates that behavioural syndromes should be similar across populations. The adaptive hypothesis in contrast argues that selection favours context-specific correlations. Following this view, behavioural correlations are thought to be more easily shaped by selection, leading to greater behavioural differences between populations (Bell 2005).

Here, the aim is to test whether juvenile sticklebacks (*Gasterosteus aculeatus*) reared in a shared laboratory environment display consistent behavioural correlations and whether these correlations, if present, differ among different parental source populations.

Additionally, the presence or absence of correlations between behaviour and body length can also depend on ecological factors, as the correlation between body size and certain behaviours is not universal (Walling et al. 2004; Bell & Sih 2007; Harris et al. 2010; Ingley et al. 2014). In line with the adaptive hypothesis, we propose that juveniles from different source populations might exhibit different body length-behaviour correlations. For example, smaller fish might be driven by their metabolism to increase their feeding efficiency (i.e. the metabolic hypothesis, see Brown & Braithwaite 2004). However, prevailing ecological conditions, e.g. the presence of predators, can determine what the most successful way to achieve this is (e.g. shoaling vs. solitary exploration). As shoals can attract undesirable attention from predators (Ford & Swearer 2013), we hypothesize that juveniles from a parental predator-free population are more inclined to shoal with decreasing body length to maximize their feeding efficiency, whereas relatively small juveniles from a parental population with significant predation benefit more foraging-wise from being exploratory and not necessarily from being sociable.

II.II. Methods

Study animals

In this study we used the lab-reared progeny (aged 4 months) of two wild Icelandic freshwater *Gasterosteus aculeatus* populations (Holsvatn: 64°31'N, 22°8'W;

Vífilsstaðavatn: 64°04’N, 21°52’W; four breeding pairs per population). The juvenile fish (n = 29 per population) were kept in 1m*0.45m*0.3m tanks in temperature-controlled rooms (14.5 ± 1 °C), exposed to a 16L:8D photoperiod. The first five days after hatching, the fry were fed with *Paramecium* spp. Afterwards they received a daily dose of brine shrimp (*Artemia salina*) until day 64, when their diet was changed to a daily meal of chopped frozen bloodworm (Chironomidae larvae).

Behavioural experiments

Each individual fish was assayed for three behaviours (boldness, exploration, and sociability) on two occasions. During the experimental procedures, the focal fish were housed in individual tanks (0.12m*0.2m*0.12m) after being measured with standard callipers (± 0.1mm). We assayed one behaviour per day in a set order (boldness, exploration, sociability), and after three days we repeated the assays. The total time of the behavioural trials per population was six days (i.e. two trials for three behaviours). Half of the water in the individual tanks was refreshed twice a week. The fish continued to be fed each morning throughout the experiments and were returned to their original tanks after completion of the behavioural trials. All sides of the individual and experimental tanks were rendered opaque with dark plastic bags to minimise the observer effect and potential social cues from conspecifics. Following each trial, the water in the experimental tanks was entirely replaced to avoid accumulation of food waste. All tests were filmed with Panasonic HX-A100 cameras.

Boldness

To assess boldness, the experimental individual was placed near the refuge in a 0.2m*0.35m*0.2m tank. At the opposite side of the tank, food was provided at the beginning of the trial after an acclimatization period of five minutes. When the fish took a bite, a predator strike was simulated by sliding a bird-shaped paper plane over the experimental tank on a thin rope (see also Bell 2005; Bell & Sih 2007) (Fig. 2.1). In absence of feeding, the 'bird' was released after 5 minutes. Following the simulated attack, behaviour was recorded for a further 5 minutes.

Through video analysis, we quantified the time it took to emerge from the refuge and the total time the focal fish spent in the food zone (defined as the refuge-less tank half where the food was offered). Each of these measures was obtained after the simulated attack.

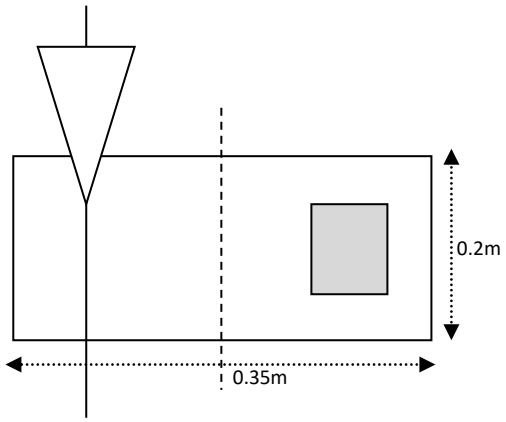


Figure 2.1: Schematic view of the experimental set-up for the boldness trials. The refuge (grey) is located in the right side of the tank. The left side is the food zone, over which a simulated predator was released.

Exploration

The exploration assay took place in a 0.3m*0.45m*0.3m tank. After the focal fish was transferred from its individual tank, it was observed for 5 minutes. The tank contained a refuge and a novel object (an upright piece of dark plastic rain gutter). During analysis of the trial videos, the tank was divided into 100 zones (Bell & Stamps 2004; Dingemanse et al. 2009) (Fig. 2.2). Exploratory behaviour was quantified as the number of different zones visited.

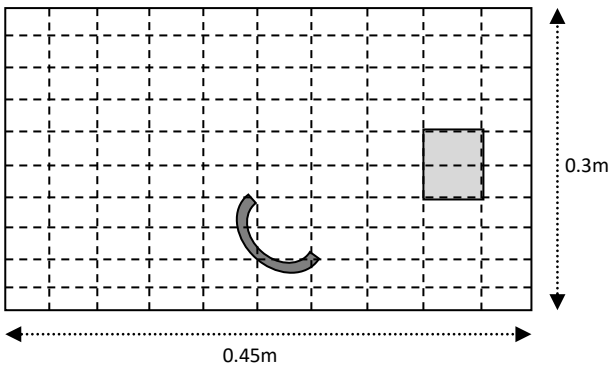


Figure 2.2: Schematic view of the experimental set-up for the exploration trials. The tank contained a refuge (light grey) and novel object (dark grey). The dotted grid illustrates the zones as used during video analysis.

Social behaviour

Social behaviour was assessed by introducing the focal fish into an experimental tank (0.2m*0.35m*0.2m) that incorporated a smaller transparent tank (0.12m*0.2m*0.12m) holding three haphazardly selected conspecifics from the same

population (Ward et al. 2004; Laskowski & Bell 2014) (Fig. 2.3). Over the course of 5 minutes, the behaviour of the focal fish was recorded. In the subsequent analysis, the tank area of the focal fish was divided into two zones (one third of the total tank closest to the stimulus shoal starting one body length away from the partition and the remaining area farther away). The time spent in the zone close to the stimulus conspecifics was measured, as well as the time spent within one body length of the stimulus tank. Further, the number of times the focal fish touched the transparent tank housing its conspecifics was counted.

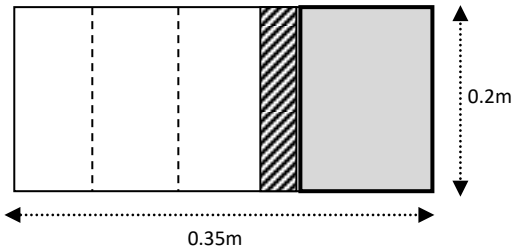


Figure 2.3: Schematic view of the experimental set-up for the sociability assay. Grey denotes the tank holding the stimulus individuals and the dotted lines delineate the zones (L-R: far, medium, and close) used in the analysis. The diagonal lines represent the zone within 1 body length of the stimulus tank.

Data analysis

Video processing

The experimental videos were pre-processed using the freeware program VirtualDub (http://www.virtualdub.org/) to standardize and prepare them for analysis. All videos were rendered in grayscale to enhance contrast and frame rate was standardized at 2.5 fps. Next, fish were tracked using the free image processing program ImageJ (http://imagej.nih.gov/ij/). Rendering the pre-processed videos into a series of images (1 image per frame) allowed tracking of the focal fish with an accuracy of 1 set of x-y coordinates per 40ms. Based on these coordinates, the behavioural measures were calculated.

Statistical analysis

Using principal component analysis, the different parameters describing boldness were summarized into a single component score. The same procedure was applied for sociability. This way, multiple comparisons can be avoided and the behavioural scores are standardized (Bell & Stamps 2004; Bell 2005).

First, the individual repeatability of the three behaviours was assessed using the intra-class correlation coefficient, following Lessells & Boag (1987), with standard errors calculated following Nakagawa & Schielzeth (2010).

Next, we investigated the presence of behavioural syndromes via Spearman rank correlation tests for each possible combination of behaviours, both across and within populations. P--values have been adjusted for multiple testing with the false discovery rate (Benjamini & Hochberg 1995).

After testing the normality of the residuals with a Shapiro test, sources of variation for each of the three behaviours were assessed via a generalized linear mixed model (GLMM) with appropriate default error distribution (Gaussian for exploration (normally distributed data) and gamma for boldness and sociability (right-skewed data)). Population, body length and their interaction were included as fixed effects, and individual as random effect for which we provide an estimate of the variance. The models were run with backward elimination of non-significant fixed effects.

Finally, correlations between body length and the three behavioural measures were assessed with Spearman's Rank correlations (data were not normally distributed) in each population.

All analyses were run in R i386 3.1.2 and statistical tests were two-tailed with a critical value of $p = 0.05$ throughout.

II.III. Results

Only one behaviour was significantly repeatable in one population (exploration in Vífilsstaðavatn: $r = 0.454 \pm 0.091$, $p = 0.005$). We found no evidence for a behavioural syndrome, either across or within populations (all $p > 0.15$).

The first principal component for boldness (PCB) explained 68.9% of the variation and was characterized by a negative loading for emergence time (-0.707) and a positive one for total time in the food zone (0.707). The first principal component for sociability (PCS) explained 90.5% of the variation and was characterized by a positive loading for time spent close (0.543) to the stimulus shoal and negative loadings for time within one body length of the stimulus conspecifics (-0.594) and times touching the separation (-0.594). Hence, a high score can be interpreted as being social, while a negative score points towards aggressiveness (presence within one body length and biting attempts are often used as signs of aggression in three-spined stickleback, e.g. Bell & Stamps 2004; Bell 2005; Bell & Sih 2008).

For both exploration (number of zones visited) and sociability (PCS) a significant interaction between population and body length was found (Table 2.1). For the same two behavioural measures, body length in itself proved to be a significant factor. The individual effect was also significant for exploration and sociability. For boldness, none of the measured variables was significant.

Table 2.1: Sources of variation in the three behaviours (boldness, exploration and sociability) as analysed via GLMM (significant p-values in bold).

	Boldness		Exploration		Sociability	
Fixed effects	X^2_{df}	p	X^2_{df}	p	X^2_{df}	p
Population	0.764_1	0.382	0.778_1	0.378	2.749_1	0.0974
Body length	3.597_1	0.058	14.010_1	**0.0002**	4.619_1	**0.0494**
Population * body length	0.0502_1	0.819	7.702_1	**0.005**	4.576_1	**0.0324**
Random effect	Var	SEM	var	SEM	Var	SEM
Individual	0.126	0.047	48.65	0.916	0.004	0.002

In Population 2 (Vífilsstaðavatn) body length correlated negatively with the number of zones explored ($\rho = -0.533$, $p < 0.0001$), while in Population 1 (Holsvatn) no significant correlation between body length and exploration was found ($\rho = -0.052$, $p = 0.701$) (Fig. 2.4). Contrastingly, in Holsvatn body length correlated negatively with PCS ($\rho = -0.349$, $p = 0.007$), but in Vífilsstaðavatn no correlation between body length and social behaviour was found ($\rho = -0.13$, $p = 0.332$). Neither population showed a significant correlation between body length and boldness ($\rho = -0.072$, $p = 0.590$ for Holsvatn and $\rho = -0.249$, $p = 0.059$ for Vífilsstaðavatn).

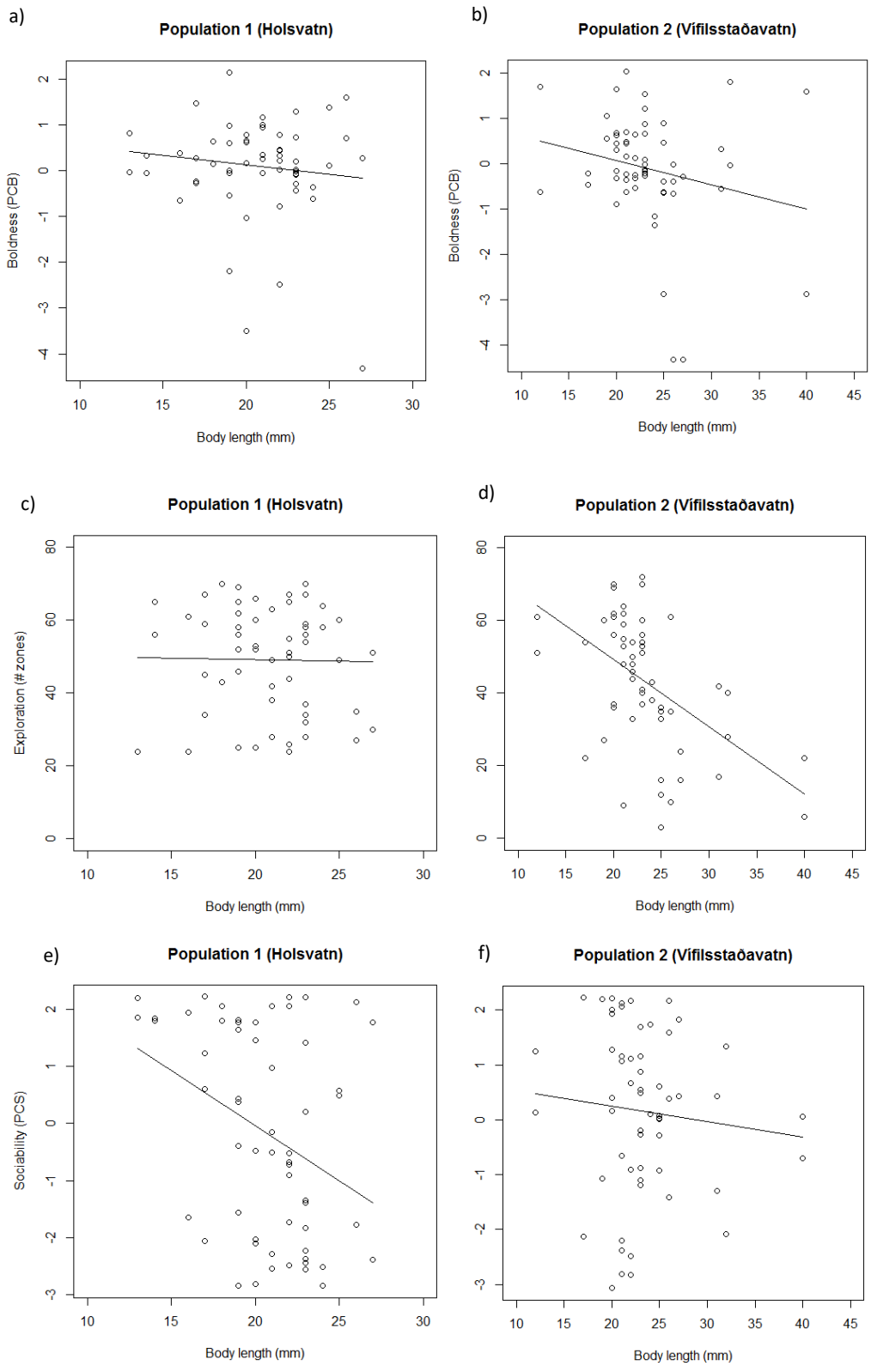


Figure 2.4: Correlation between body length and boldness (a –b) ,; between body length and exploration (c – d) and between body length and sociability (e – f) for Holsvatn (left: a, c, and e), and Vífilsstaðavatn (right: b, d, and f).

II.IV. Discussion

While no behavioural differences between, or syndromes within, the populations were found, the results of this study indicate that in two lab-reared populations of juvenile sticklebacks, correlations of behaviour with body length exhibit different patterns.

The connection between social behaviour and body length in Holsvatn suggests that smaller fish are more likely to stay close to three stimulus conspecifics, but keep a distance of at least one body length. In contrast, time within one body length of the stimulus fish, and number of times touching the separation increase with body length. This can be interpreted as a display of two aspects of social behaviour. One is shoaling tendency, or the inclination to stay relatively close to potential shoal mates. The other is aggressive behaviour, either through approaching conspecifics closer than one body length or actively trying to attack them (Bell & Stamps 2004; Bell 2005; Bell & Sih 2007). Interpreted this way, smaller individuals in Holsvatn exhibit a higher tendency to shoal, whereas large ones are more aggressive. In Vífilsstaðavatn, smaller fish are more explorative than their larger-bodied conspecifics.

As indicated here and in other studies, the link between body size and certain behaviours is not universal (Walling et al. 2004; Bell & Sih 2007; Harris et al. 2010; Ingley et al. 2014). One possible explanation for the difference between both populations here is predation. The presence of predators has been linked to the generation of animal personality and behavioural syndromes (Bell & Sih 2007; Brydges et al. 2008). Historical data from the Ecological Survey of Icelandic Lakes

indicates that Holsvatn does not house any salmonid predators, while in Vífilsstaðavatn the presence of both Arctic char (*Salvelinus alpinus*) and Brown trout (*Salmo trutta*) has been recorded. One possible interpretation for the results obtained here rests on the metabolic hypothesis (Brown & Braithwaite 2004). Smaller fish have a higher metabolic rate than larger conspecifics, resulting in the need to increase their feeding efficiency compared to larger-bodied fish, which can be achieved through shoaling (Ranta & Kaitala 1991; Foster et al. 2001). In populations under predation pressure, however, shoals might attract unwanted attention (Ford & Swearer 2013). In this case, small fish might benefit from being exploratory assuming that predators are drawn primarily to larger-sized conspecifics.

Since the experimental sticklebacks were all of the same age, body length did not reflect an age difference as it might have in other studies (e.g. Brown & Braithwaite 2004). Additionally, it should be noted that individual behaviour need not be consistent across the entire lifespan (Bell & Stamps 2004). A future research avenue is to collect longitudinal data to see whether exploratory and/or social stickleback grow faster or slower and whether this is accompanied by behavioural changes. This was not pursued here since the experimental fish were processed as part of another study. We also acknowledge the relative small sample size and limited genetic variation in our study.

Overall, this study tentatively supports an adaptive hypothesis of personality through establishing the malleability of body length-behaviour correlations and suggests possible (epi)genetic roots for behavioural differences between populations. To solidify this support for an adaptive hypothesis requires further work that incorporates more populations, more breeding pairs, and an extension of the experiments over multiple generations raised in common garden conditions.

III. Knights in shining armour are not necessarily bold*

Defensive morphology correlates negatively with boldness, but positively with activity, in wild three-spined sticklebacks

* A version of this chapter has been published as:

De Winter, G.; Martis, H.R.; Trovo, R.A. & Chapman, B.B. (2016). Knights in shining armour are not necessarily bold: Defensive morphology correlates negatively with boldness, but positively with activity, in wild three-spined sticklebacks (*Gasterosteus aculeatus*). *Evolutionary Ecology Research.* **17:** 279 – 290.

Abstract

The three-spined stickleback, *Gasterosteus aculeatus*, varies in armour phenotype from fully armoured to non-armoured morphs. Here, we aim to investigate the connection between individual variation in behaviour and armour morphology within and across populations.

During spring 2015, we assayed the behaviour of 250 sticklebacks from seven populations on the Scottish island of North Uist, located in the Outer Hebrides.

The 'phenotypic compensation' hypothesis holds that individuals with poorer defences should behave more timidly. Yet the energetic costs associated with bearing armour should reduce both the activity and exploration behaviour of more heavily armoured individuals.

We assayed individual stickleback for boldness, exploration, and activity. We then stained the fish with Alizarin red, which allowed the characterisation of the armour phenotype of each fish.

Across populations, bold fish were less armoured than their shy counterparts and active fish were more armoured. Exploratory behaviour was not correlated with armour phenotype.

III.I. Introduction

Recent research has highlighted the existence of individual behavioural consistency across a wide range of traits such as boldness (Brown & Braithwaite 2004; Ward et al. 2004; Chapman et al. 2011a), exploration (Fraser et al. 2001; Dingemanse et al. 2002; Careau et al. 2011) and mating behaviour (Chapman et al. 2009). This has been observed in a wide variety of taxa, including mammals (Réale et al. 2000; English et al. 2010; Careau et al. 2011), birds (Dingemanse et al. 2002; Foltz et al. 2015; Mccowan et al. 2015), fish (Brown & Braithwaite 2004; Ward et al. 2004; Chapman et al. 2011a), and invertebrates (Chapman et al. 2011b; Grinsted et al. 2013; Planas-sitja et al. 2015). This phenomenon, known as 'animal personality' (Dall et al. 2004; Dingemanse & Réale 2005), is perhaps surprising given that behavioural plasticity is beneficial to maximize individual fitness in the heterogeneous environments that most organisms face during their lifetimes (Dingemanse & Réale 2005). Hence a key question in contemporary behavioural and evolutionary biology is to address the existence and prevalence of animal personality within wild populations of varying ecology (Dingemanse & Réale 2005).

Many models of animal personality are derived from the link between behaviour and state, where the concept of state refers to the various features of an animal that are relevant factors in behavioural decisions that affect fitness. One of these features is morphology (Wolf & Weissing 2010). If morphology contributes to an animal's state, which, in turn, affects behaviour, one could predict that certain behaviours and morphological features may covary. One empirical example of this is the link between boldness and defence morphology in aquatic snails (*Radix balthica*),

whereby bold snails have more defensible shells (i.e. rounder shells with wider aperture) (Ahlgren et al. 2015). Similarly, bold brown anole lizards (*Anolis sagrei*) autotomize their tails more readily than shy ones when subjected to an unrestricted diet (Kuo et al. 2015), and bold crucian carp (*Carassius carassius*) express more pronounced inducible defences in response to sustained exposure to a piscivorous predators (Hulthén et al. 2013). These findings are suggestive of 'phenotypic compensation', whereby bold individuals compensate for their risky lifestyle by exhibiting more pronounced anti-predation morphological features (Ahlgren et al. 2015). In broad terms, this hypothesis suggests that the morphology of organisms is attuned to optimize the cost/benefit ratio of displaying certain behaviours. Hence, if an organism consistently displays a certain level of behaviour (e.g. being bold, exploratory, active,…) we expect that its morphology is such that it maximizes the benefits and minimizes the cost of performing that behaviour. So, bold, risk-taking individuals are expected to possess morphological features that allow them to deal with these risks, as suggested by the above examples (Hulthén et al. 2013; Ahlgren et al. 2015; Kuo et al. 2015). Since both morphology and behaviour can possess a certain plasticity, the direction of causality can run both ways in this hypothesis. Behaviour can be adapted to morphology, but the reverse is also possible. Given this hypothesis, we expect that three-spined sticklebacks (*Gasterosteus aculeatus*) that are more prone to predation (i.e. bolder fish) are more heavily armoured. The large variation in armour phenotypes among three-spined sticklebacks, from fully armoured marine fish to freshwater populations with greatly reduced armour, provides a great resource to address this hypothesis. Since armour is likely less plastic than behaviour we focus on the direction of causality that implies that the armour of an individual influences its behaviour, rather than the reverse.

Furthermore, being heavily armoured may impact the energy requirements and hydrodynamics of swimming. Movement-related behaviours such as exploration and activity might therefore also be affected by armour phenotype. For example, a trade-off between armour and swimming hydrodynamics means that heavily armoured fish cannot reach the fast-start performance and fleeing velocity less armoured ones can (Andraso 1997; Bergstrom 2002). Findings in lizard species of the Cordylidae family support this. The degree of armour among and within these species is highly variable and correlates negatively with distance run from a predator (Losos et al. 2002), leading to the assertion that active flight and armoured defence are incompatible anti-predator strategies. While we did not assess escape behaviour, we propose that similar considerations could apply to exploratory behaviour and activity. In a more general context, metabolism and growth rate, both of which are intimately tied to energetic requirements, underlie a set of explanations concerning the occurrence of behavioural types and syndromes (e.g. Biro & Stamps 2008; Careau et al. 2008). Therefore, the energetic costs of armour can affect the behaviour of an individual via a direct effect on movement efficiency, as well as indirectly via its effect on metabolism and/or growth rate. With regards to activity and exploration, we expect that fish with more developed armour display lower levels of movement-related behaviours due to energetic costs.

The link between morphology and behaviour might also shed light on the existence and maintenance of trait correlations. Regardless of the specific proximate mechanism(s), the existence of behaviour-morphology covariation can (but need not) indicate selection pressures that favour and reinforce certain trait correlations.

Here, we report a test of the 'phenotypic compensation' and 'energetic cost' hypotheses through an investigation of the highly variable armour morphology of

wild stickleback populations on the Scottish island of North Uist. We predict that bolder individuals should be more armoured than shy ones, both across and within populations. Furthermore, we extend our investigation to include exploratory behaviour and activity, and postulate that both are negatively influenced by the degree of armour.

III.II. Methods

Fish capture and maintenance

Over a four week period in April and May 2015, 250 three-spined sticklebacks were collected from seven freshwater lakes on the Scottish island of North Uist (Table 3.1, Fig. 3.1). Traps were set late afternoon and the fish collected the next morning. Such catch methods may result in non-random capture of behavioural types (Biro & Dingemanse 2009), but this is difficult to avoid in natural conditions. While we did not find a method that could overcome this issue, our focus on population comparisons means that even if our caught individuals are a non-random sample (i.e; only the boldest individuals), the relative differences between our samples of the populations are likely to be maintained in our experimental sets of individuals.

Table 3.1: Sample sites

Loch	Code	Coordinates	Sampled individuals (total, female (F), and male (M))
na Buaile	BUAI	57°38'N 7°11'W	40 (33F / 7M)
Chadha Ruaidh	CHRU	57°36'N 7°12W	40 (24F / 16M)
an Daimh	DAIM	57°35'N 7°12'W	20 (11F / 9M)
na Reival	REIV	57°37'N 7°31'W	30 (17F / 13M)
Scadavay	SCAD	57°35'N 7°14'W	40 (23F / 17M)
nan Struban	STRU	57°34'N 7°21'W	40 (20F / 20M)
Tormasad	TORM	57°33'N 7°19'W	40 (40F / 0M)

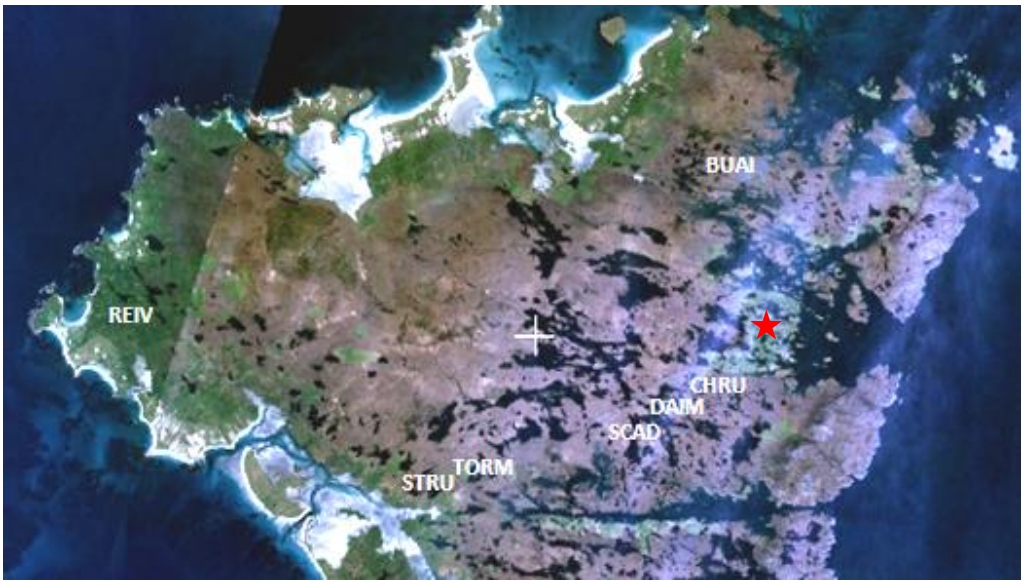


Figure 3.1: Geographical location of the sample sites on the island of North Uist. The red star denotes the site of the field station.

During the behavioural experiments (which were performed in a fixed order potentially leading to test order effects (Bell 2013), but again our across-population focus mitigates this), the fish were kept in individual tanks (0.12m*0.20m*0.12m) in

the field station (Drumcharry house, Lochmaddy, 57°61'N 7°17'W). The behavioural assays started after one day of acclimatization and lasted for four days with one trial performed each day. The water in the experimental and individual tanks was obtained from a nearby fishless loch (57°61'W 7°17'W, pH: 6.68, EC: 243 Ms/cm), and its temperature was monitored daily (average: 6.85°C, min-max: 6 - 8°C). The fish were exposed to a natural photoperiod (approx. 10L:14D) and not fed during the experimental period. Following each assay, half of the water in the individual tanks was refreshed to prevent the build-up of waste and remove possible chemical cues from previous fish.

All of the performed research adhered to the Association for the Study of Animal Behaviour Guidelines for the Use of Animals in Research, the legal requirements of the country in which the work was carried out, and all institutional guidelines.

Behavioural trials

Boldness

Each fish was tested twice on consecutive days to assess responsiveness to risk (i.e. individual boldness). Behavioural observations took place remotely using a Panasonic HX-A100 camera and the Panasonic Image app to ensure a minimal influence of the observer on the behaviour of the fish. At the beginning of the trial each focal individual was placed in an experimental tank (0.20m*0.40m*0.30m) and given an acclimatization period of three minutes. After this time had elapsed, the tank was

rapidly covered with a cardboard plaque to elicit a fright response. The cardboard was removed in a single quick motion upwards after three seconds. Fish characteristically froze, and individuals varied in the time taken to return to normal movement. We chose this latency to return to movement after a fright stimulus as measure of boldness (Chapman et al. 2010; Piyapong et al. 2010) and calculated the mean time taken to return to movement (defined as continuous motion for a distance equal or greater than one body length) over both trials. Each trial had a ceiling value of 300 seconds. To generate a 'boldness score' in which bold individuals had a high score and shy individuals a low score, we subtracted the mean latency to move from the ceiling value.

Exploration and activity

To assess exploratory behaviour and activity, we carried out a further series of trials. The boldness and exploration/activity trials were separated by approximately 24 hours. One focal individual was placed in an experimental tank (0.55m*0.45m*0.20m). After an acclimatization period of five minutes, recording and live tracking of the movement of the four experimental individuals simultaneously, each in a separate experimental tank, was initiated using four mounted cameras (Panasonic WV-CP314E CCTV cameras with Computar T4Z2813CS-IR 2.8 – 12mm lenses) and specialized software (Ethovision XT recording, Noldus Information Technology, Wageningen, the Netherlands). One refuge and three other habitat structures, all made out of white plastic cups to prevent unwanted interactions with the tracking software, were present in each tank. Testing took place

under standardized light conditions comparable to natural twilight which allowed optimal tracking and prevented reflections.

The coordinates of each fish in its randomly assigned experimental tank were recorded by the Ethovision software. Based on these data, activity was quantified as the total time moving and exploratory behaviour as the area of the tank that the focal fish visited during the five minute trial. The exploration and activity scores used in the analysis are the means of both trials. The size of the explored area in square meters was obtained through processing the heatmaps generated by the Ethovision software in ImageJ (http://imagej.nih.gov/ij) (Fig. 3.2).

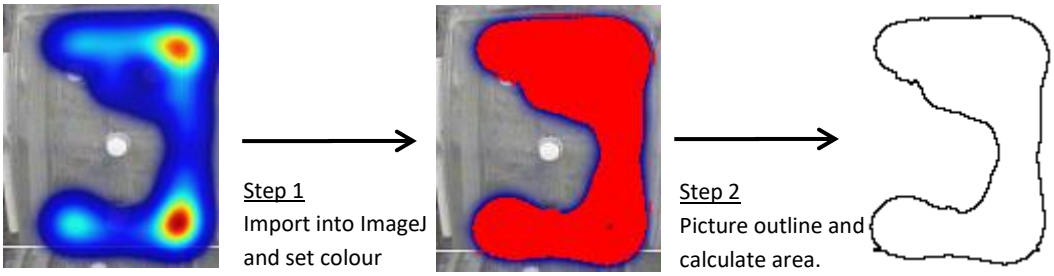


Figure 3.2: The heatmaps generated by Ethovision (left) were imported into ImageJ, where they were processed (middle and right) in order to calculate the size of the area visited by the focal fish.

Quantifying defensive morphology

Following the completion of the behavioural assays, fish were euthanized with MS222 (tricaine methanesulfonate). Next, they were rehydrated and fixated in a solution of 10% buffered formalin. After two weeks of fixation, all fish were washed

with distilled water and incubated in a 1% KOH solution for one hour. Subsequently, they were bleached (5 ml of H2O2 in 1l of KOH solution) overnight. Their bones and armour were then stained with 0.5% Alizarin red solution. After washing the stained fish with distilled water and 1% KOH solution, they were laterally photographed (Pentax K-50, smc DA 18-55mm F3.5-5.6 lens) and stored in 40% isopropyl alcohol.

The photographs were analysed with ImageJ and as measures of armour we quantified the number of lateral plates, the height of the largest of these plates, the total number of spines (dorsal and pelvic), and the length of the first dorsal spine (if present) (Fig. 3.3). These parameters were corrected for body length by using the residuals from the regression of body length with each of the parameters.

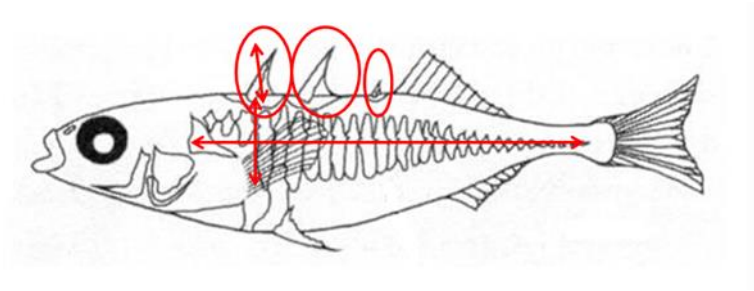


Figure 3.3: Illustration of the measured defensive morphological parameters.

Data analysis

The morphological parameters were then summarized into a single armour score (PC Armour) using principal component analysis. Next, we assessed the presence of a correlation between armour score and boldness, exploration, and activity (all three behaviour scores are the means of the two trials), both among and within

populations. Since exploration was normally distributed, we used a Pearson product-moment correlation; for boldness and activity (not normally distributed) we performed a Spearman's rank-order test.

Behavioural differences between the sexes (as determined via dissection) were tested using a two-sample t-test in case of normal data (exploration), and a Wilcoxon rank sum test for non-normal data (i.e. boldness and activity).

Finally, to assess sources of variation for each of the three behaviours, we formulated general linear models with PC Armour, body length, sex, population, and all possible two-way interactions as fixed effects. When the residuals were not normally distributed (right-skewed for boldness) we used a gamma error distribution. The models for exploration and activity had normal residuals and were run with a gaussian error distribution.

All analyses were performed using R i386 3.2.1.

III.III. Results

The principal component analysis performed on the morphological traits resulted in a first principal component that explained 73.84% of the variance (Table 3.2). All morphological variables loaded negatively on this component; therefore a high score reflects a low armour phenotype. In further analysis, this PC1 score is inverted (multiplied by -1) so that a high score (i.e. high 'PC Armour') reflects a more armoured phenotype.

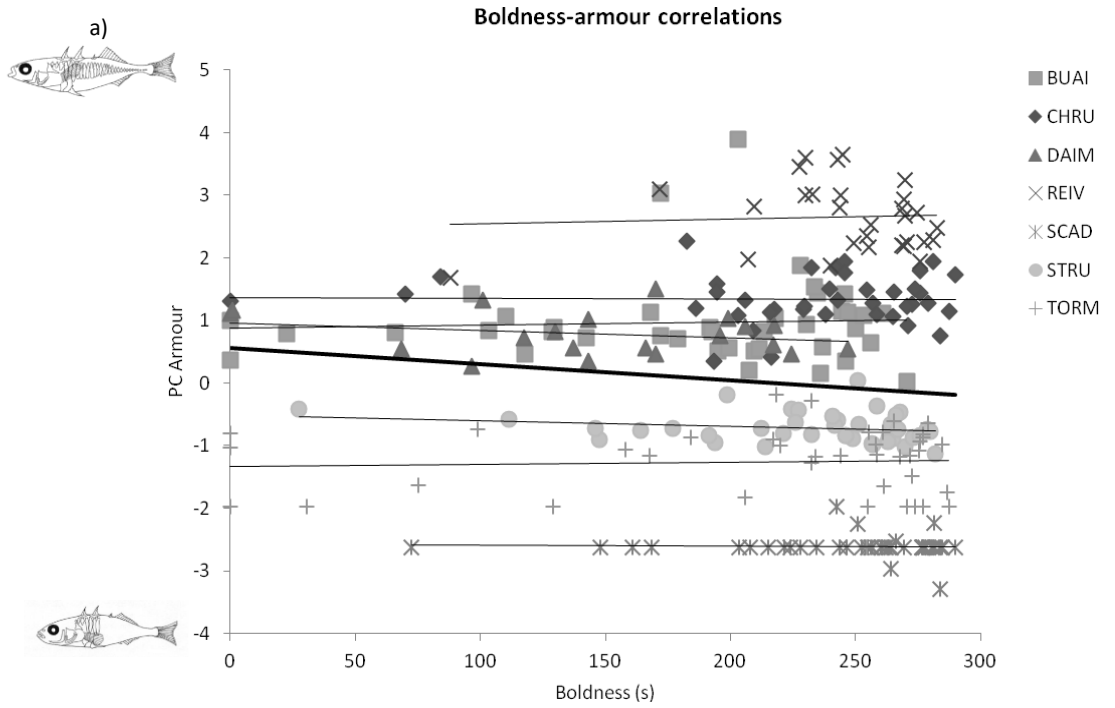
Table 3.2: Results of the principal component analysis on the morphological parameters.

Residuals	PC1 loadings
Number of lateral plates	-0.530
Height largest plate	-0.515
Number of spines	-0.546
Length first dorsal spine	-0.395
Proportion of variance explained	73.84%

Across populations, boldness was significantly negatively correlated with PC Armour, which means that individuals in bold populations were less armoured than those in shy ones (Fig. 3.3a, Table 3.3). No significant within population correlations were found. Exploration was not correlated with armour phenotype across populations (Table 3.3). In one population (CHRU), however, exploratory behaviour did show a negative correlation with armour (Table 3.3). Activity was significantly positively correlated with armour so that armoured fish were also more active (Fig. 3.3b, Table 3.3). The same positive relationship between activity and armour was also found in CHRU and REIV (Fig. 3.3b, Table 3.3).

Table 3.3: Correlations between armour phenotype and boldness, exploration, and activity (significant p-values in bold, * denotes p-values significant following false discovery rate for multiple testing).

	Boldness		Exploration		Activity	
	ρ	p	r	p	ρ	p
Across populations	**- 0.155**	**0.014**	-0.076	0.235	**0.254**	**< 0.001***
Within populations						
BUAI	0.054	0.740	-0.142	0.382	0.057	0.725
CHRU	0.053	0.745	**-0.36**	**0.024**	**0.707**	**<0.001***
DAIM	- 0.157	0.508	0.145	0.543	-0.343	0.139
REIV	- 0.247	0.197	0.061	0.753	**0.453**	**0.014***
SCAD	- 0.082	0.615	-0.032	0.844	-0.090	0.579
STRU	- 0.231	0.151	0.176	0.276	0.193	0.233
TORM	- 0.016	0.923	-0.164	0.313	0.217	0.179



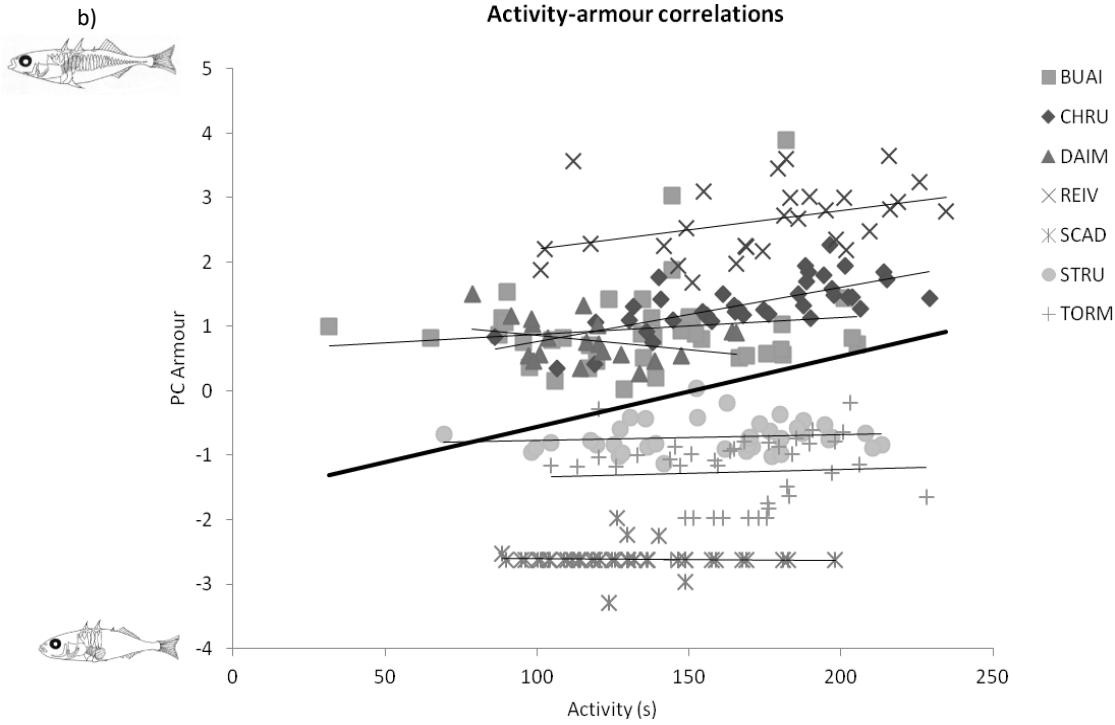


Figure 3.3: Correlation between behaviour and armour phenotype across populations for (a) boldness, and (b) activity. Each population has its own symbol and the overall correlation is illustrated by the bold black line.

Across populations, none of the three behaviours differed significantly between sexes (boldness: W = 6071, p = 0.219, exploration: t = 0.488, p = 0.626, activity: W = 6478, p = 0.647). Within populations, only DAIM (t = -3.963, p = 0.001), REIV (t = 3.211, p = 0.004) and SCAD (t = -2.114, p = 0.042) displayed significant behavioural differences between males and females, all in exploratory behaviour (Fig. 3.4). In REIV, males were less exploratory than females; in DAIM and SCAD, this pattern was reversed.

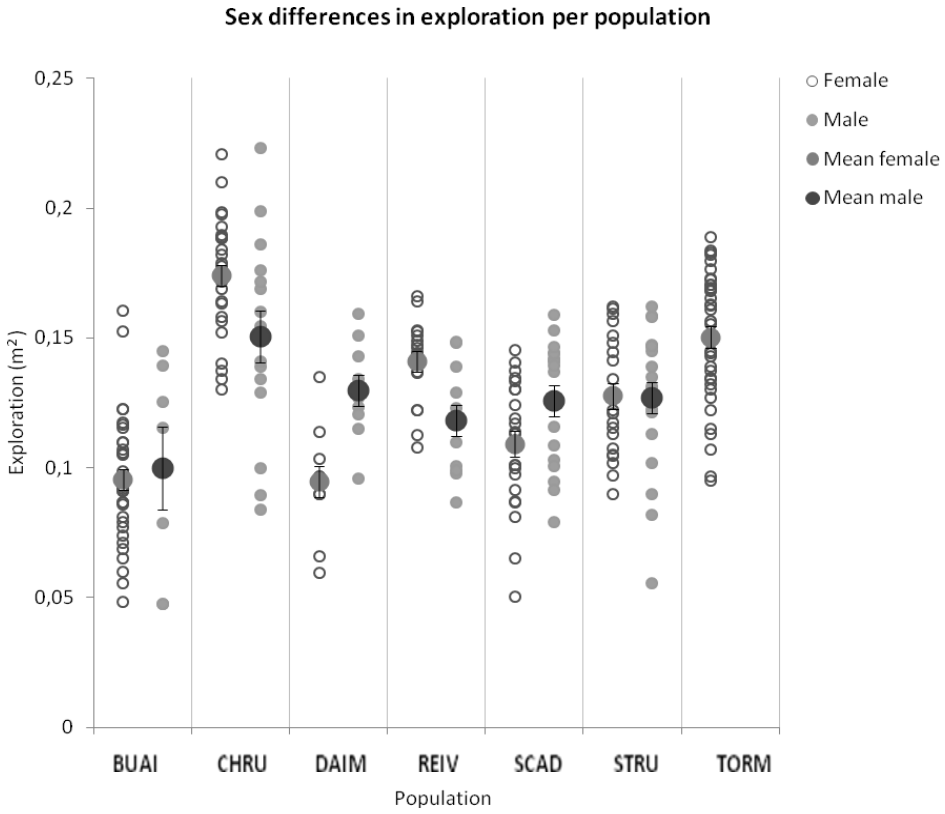


Figure 3.4: Difference in exploratory behaviour between males and females for each population (only females were caught in TORM).

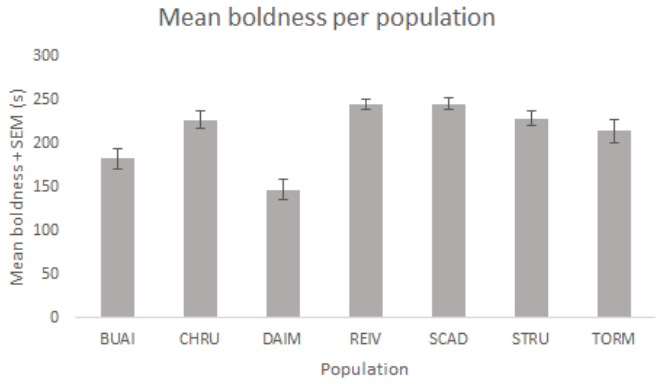
All behaviours were significantly repeatable (boldness: 0.531 ± 0.022, p <0.0001; exploration: 0.366 ± 0.021, p<0.0001; activity: 0.383 ± 0.0211, p<0.0001) (following Lessells and Boag 1987; Nakagawa and Schielzeth 2010).

The models revealed that population is a main source of variation for all behaviours (Table 3.4, Fig. 3.5). Additionally, body length significantly affected exploration and boldness, with smaller individuals being both less exploratory and less active than their larger conspecifics. According to our models, activity is also influenced by sex, but this is not borne out by our earlier analyses (see above). Significant interactions were between body length and population for boldness, between sex and population for exploration, and between PC Armour and population, as well as between PC Armour and body length for activity (Table 3.4).

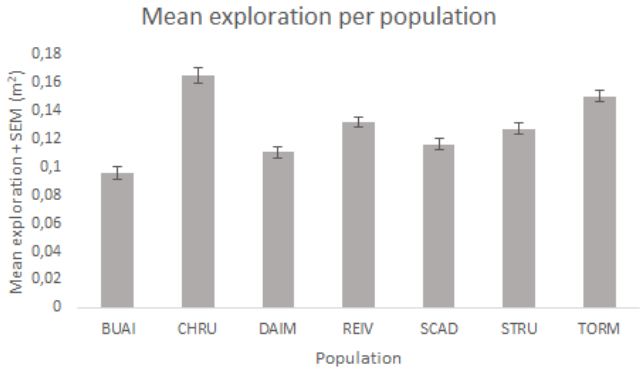
Table 3.4: Results from the general linear model for each of the tested behaviours. Significant p-values in bold.

	Boldness		Exploration		Activity	
	χ^2_{df}	p	χ^2_{df}	p	χ^2_{df}	p
PC Armour	0.228_1	0.633	0.001_1	0.983	1.793_1	0.181
Body length	0.726_1	0.394	8.883_1	**0.003**	15.482_1	**<0.0001**
Sex	0.136_1	0.712	0.606_1	0.436	4.787_1	**0.029**
Population	46.726_6	**<0.0001**	137.110_6	**<0.0001**	65.436_6	**<0.0001**
PC Armour * body length	0.708_1	0.400	3.166_1	0.075	4.748_1	**0.029**
PC Armour * sex	0.209_1	0.647	0.015_1	0.904	0.891_1	0.345
PC Armour * population	2.073_6	0.913	3.916_6	0.688	14.252_6	**0.027**
Body length * sex	2.361_1	0.124	0.019_1	0.890	0.574_1	0.448
Body length * population	15.394_6	**0.017**	4.974_6	0.547	12.583_6	0.050
Sex * population	9.624_5	0.087	17.985_5	**0.003**	3.848_5	0.571

a)



b)



c)

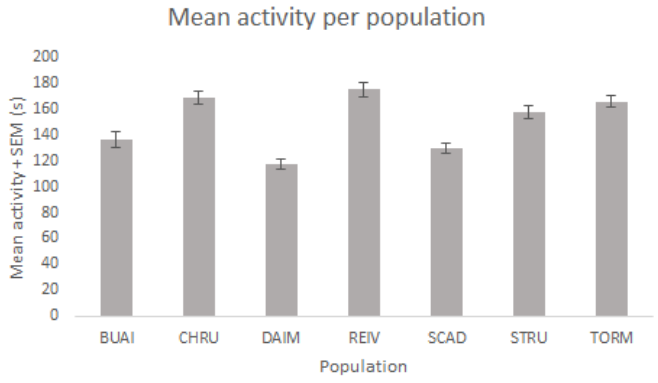


Figure 3.5: Mean boldness (a), exploration (b), and activity (c) for each of the sampled populations (+ SEM).

III. IV. Discussion

Our results directly contradict our stated hypotheses. Bold fish are less armoured, and more active fish are more armoured. Armour phenotype as characterized here did not correlate with exploratory behaviour.

The phenotypic compensation hypothesis led us to suggest that risk-taking individuals possess a more pronounced anti-predator morphology; hence we proposed that bolder stickleback would be more armoured. Instead, we found the opposite. However, our finding is not without precedent. Comparing three-spined stickleback that differed in pelvic phenotype from two Canadian lakes did not yield an across-population correlation between risk-taking behaviour and anti-predator morphology but did find such a relation within one of the populations (Grand 2000). Additionally, in juvenile, lab-reared stickleback there is a strong (but non-significant) suggestion that boldness correlates negatively with armour (Lacasse & Aubin-Horth 2012). Our results suggest that this might hold in natural conditions as well, prompting a reconsideration of the extent to which the phenotypic compensation hypothesis can explain behaviour-morphology correlations in natural conditions. Within populations, however, we found no clear correlation between boldness and armour phenotype, which suggests that the boldness-armour correlation we observed is likely the result of ecological factors that differ substantially between populations, but not within them (e.g. predation, food availability,…), that affect both boldness and armour development. An alternative, or complementary, consideration is that the sample size for the across population test is substantially

larger than those of the within-population tests. As such, the power of the former is higher than that of the latter.

We found a positive correlation between activity and armour, contradicting our energetic cost hypothesis. This suggests that the energetic costs of armour are either negligible or mitigated by potential benefits of being active. In two populations we also observed a positive relation between armour and activity. In the other populations we did not find a significant correlation. The same consideration as with boldness might apply here: the ecological factors a population is subjected to are more significant influences on behaviour than armour phenotype. Hence, if mitigation of the energetic costs of armour takes place, the extent of it is likely to be influenced by environmental factors. The fitness consequences of exploratory behaviour in great tits (*Parus major*), for example, fluctuate in a natural changing environment (Dingemanse et al. 2004).

Perhaps the fish in certain populations are subjected to one or several specific environmental influences, or combinations thereof, that allow sufficient mitigation (and, in some cases even a reversal compared to what would be expected under the energetic cost hypothesis). One likely option is food availability. Previous work, both theoretical and experimental, has already shown that both food availability and predictability can influence boldness (e.g. Chapman et al. 2010; Luttbeg & Sih 2010) and exploratory behaviour (Chapman et al. 2010). Our results suggest that this might the case for activity as well. When ample food is available, fish might be able to acquire sufficient energetic resources to maintain high activity levels even with a well-developed armour.

Additionally, possessing armour could lead to a higher metabolism which, in turn, affects hunger levels, possibly leading to more active behaviour in order to discover food patches. This idea is in line with proposals coupling metabolism to behavioural variation (Careau et al. 2008; Careau et al. 2011; Careau & Garland 2014). We did not, however, find a correlation between exploration and armour phenotype. One reason for this might be that interpreting behaviour is notoriously difficult. Our measure for exploration, the proportion of a novel environment explored, involves an aspect of risk and might thus also involve a certain facet of boldness. In fact, our quantification for exploratory behaviour combines elements of risk (i.e. boldness) with elements of movement (i.e. activity) (The behaviours are all positively correlated with ρ = roughly 0.25 for the two boldness correlations and $r = \pm$ 0.5 for the activity-exploration correlation). Maybe the negative correlation between armour and boldness and the positive one between armour and activity negate each other in our exploration assay, but this is, of course, highly speculative.

The overruling effect of population ecology is not limited to behaviour. For example, when investigating the stress reactivity of marine and freshwater stickleback, the ecological factors are highly significant for both cortisol levels and stress reactivity (Di Poi et al. 2016). The authors suggest that the divergence in stress reactivity could have its roots in different natural selection pressures (e.g. predation) between the sampled populations, mirroring our suggestions concerning population differences in behaviour.

We did not find behavioural differences between males and females across populations, and significant differences between the sexes were only found in three populations for exploratory behaviour. This contrasts with other studies which found that males in guppies (*Poecilia reticulata*), three-spined stickleback, and the live-

bearing fish genus *Brachyraphis* tend to be bolder and more exploratory than females (Harris et al. 2010; King et al. 2013; Ingley et al. 2014). The differences we found did not show a consistent pattern, so we surmise that behavioural differences between the sexes depend on the prevailing (or past) ecological conditions of the specific population or reproductive status at the time of testing.

Our work involved wild-caught fish. This means that the variation we observe in both behaviour and morphology arises via a complex web of interactions including genetic and plastic effects. Even maternal effects cannot be ignored (see e.g. Lancaster et al. 2010). As such, our results should be interpreted carefully and extrapolation to underlying causal mechanisms should be done with care. These effects could be studied in more detail through common garden experiments of subsequent generations. Nevertheless, the relationships we observe point the way towards future work that may help unravel the complex causal web in which individual variation in behaviour is embedded. A fruitful research avenue, for example, is to investigate the ecology of these populations in more detail. This may provide more information and could help to gain understanding of the behaviour-morphology-ecology triad.

Overall, we find that, across wild populations of three-spined stickleback, bolder fish are less armoured and higher activity is linked with a more pronounced armour phenotype. There is no clear correlation between armour phenotype and exploratory behaviour. Within population correlations between any of the three tested behaviours and armour are rare and are likely the result of specific (combinations of) environmental factors. Such potential dependency of behaviour-morphology covariations on ecological facets cautions against extrapolating findings from one or a few populations to establish a general hypothesis.

IV. The ecology of behavioural variation:

Parasites, resources, interspecific competition, and predation

Abstract

Consistent individual differences in behaviour ('animal personality') are by now well established. The contemporary challenge is studying the proximate and ultimate drivers of such variation under natural conditions. Yet linking ecological variables to behavioural variation in the wild is logistically demanding and requires cross-population comparisons of behaviour and ecological aspects.

The radiation of three-spined stickleback (*Gasterosteus aculeatus*) on North Uist allows us to investigate the influence of several ecological factors that have been postulated to affect behavioural variation: predation, parasitism, resource availability, and interspecific competition. We assay fish from seven populations for boldness, exploration, and activity, and relate this to individual parasite load and population-level parasite prevalence, predator presence, resource availability, and interspecific competition.

Our analyses reveal that key behaviours covary with ecological conditions under wild conditions. Fish from high parasite lochs were less exploratory and medium parasitism at loch level correlated with high boldness. Predation correlated with decreased activity, and high resource lochs contained bolder, more active individuals. Finally, in lochs with interspecific competitors, fish were less exploratory and less active. We additionally provide the first observational evidence that the effects of parasitism, resource availability, and to a lesser extent interspecific competition, outweigh those of predation upon behavioural variation in wild populations.

IV.I. Introduction

In recent years, the study of consistent individual behavioural variation (known as 'animal personality') has become a major topic of interest (Dingemanse & Réale 2005; Réale et al. 2007). The origin and evolutionary maintenance of this consistent variation in individual behaviour, both within and between populations, has proven to be a complex conundrum. However, researchers are beginning to make headway in uncovering the causes and consequences of the phenomenon. Many aspects of the biotic and abiotic environment influence behavioural variation and consistency within- and between-populations. Previous studies have illuminated the roles of parasites (Garcia-Longoria et al. 2014; Kortet et al. 2015), predation (Relyea 2002; Bell & Sih 2007; Brydges et al. 2008; Dingemanse et al. 2009; Harris et al. 2010), the abiotic environment (Brydges et al. 2008), and other factors such as the social setting (Schürch et al. 2010; Colléter & Brown 2011; Hamilton & Ligocki 2012; Carter et al. 2014; Jolles et al. 2014; Laskowski & Bell 2014). However, most studies that investigate how ecology shapes behaviour focus on a single environmental factor. Here, we utilize the well-developed study system of three-spined stickleback (*Gasterosteus aculeatus*) to provide a thorough assessment of how multiple ecological factors influence behavioural/personality traits in a single species. We combine data on four important ecological parameters (parasitism, predation, resource availability, and competition) and deliver an unprecedented look at how behaviour and animal personality covary with ecology in natural conditions.

Parasites can have powerful selective effects and numerous empirical studies show that parasitic infection affects numerous behaviours in fish (Barber & Dingemanse

2010). Parasites can affect the cost-benefit ratio of expressing a particular behaviour both directly and indirectly (Barber & Dingemanse 2010; Kortet et al. 2010). It can pay to directly avoid infection, but this comes with potential costs. For example, being neophobic or shunning social interactions can limit exposure to parasites, but this can negatively affect fitness-influencing aspects such as food acquisition or reproduction. Additionally, parasite-mediated selection on behavioural traits could act differentially upon individuals due to varying immune phenotypes, which has been speculated to drive the evolution of consistent individual behavioural variation (Kortet et al. 2010). For example, it has been argued that well-developed immune systems allow individuals to pay a smaller cost for parasite infection and that, due to positive feedback mechanisms, these individuals are expected to be more bold and active than conspecifics with less efficient immune systems (Kortet et al. 2010). Another consideration is that parasites can affect behavioural trait combinations (i.e. behavioural syndromes). If several behavioural traits are linked to infection risk, particular trait combinations could become strongly linked via the selection pressure imposed by local parasite communities. Vice versa, parasites can decouple existing syndromes if they affect some, but not all, associated behaviours (Barber & Dingemanse 2010). Despite much discussion in the literature of the possible effects of parasites on consistent individual behavioural variation, empirical studies linking parasite intensity to personality variation across populations in the wild are rare (but see Kortet et al. 2015).

Of the diverse ecological factors that may shape patterns of personality/behavioural syndrome variation in natural populations, predation is possibly the best studied (e.g. Bell 2005; Bell & Sih 2007; Brydges et al. 2008; Dingemanse et al. 2009). The presence or absence of a predator, as well as the specific type of predator, can shift

selection pressures imposed on individuals in a population, which may have consequences for the adaptive value of behavioural consistency and also individual and correlated personality traits. Recent experimental work shows that predator exposure can sculpt the behavioural syndromes and types in a prey population (Relyea 2002; Bell & Sih 2007; Brydges et al. 2008; Dingemanse et al. 2009; Harris et al. 2010), and that the effect of predation on behaviour is strongly context-dependent. Studies are equivocal, and both higher (Harris et al. 2010; Ingley et al. 2014; Kortet et al. 2015) and lower (Brydges et al. 2008; Herczeg et al. 2009) mean boldness in populations under predation pressure have been reported. Activity seems to be negatively correlated with predator presence (Kortet et al. 2015). Furthermore, evidence suggests that predation can influence the coupling of behavioural traits, i.e. the strength and structure of behavioural syndromes. In three-spined sticklebacks, for example, predator presence induces a consistent correlation between boldness and aggression (Bell & Sih 2007), and boldness and activity (Brydges et al. 2008).

A conceptual model stresses the importance of resource availability as both a direct and indirect influence on behaviour (Luttbeg & Sih 2010). Directly, the model suggests that conditions of intermediate favourability (i.e. medium predation risk and medium resource availability) provide fertile grounds for behavioural syndromes. Conditions at the extreme ends of the ecological favourability spectrum would lead to behavioural convergence on a single strategy, e.g. in high resource areas the model predicts a behavioural convergence on high boldness (Luttbeg & Sih 2010). A study in song sparrows (*Melospiza melodia*) supports this as food supplementation increased territorial aggression, particularly in rural birds (Foltz et al. 2015). Beyond availability, the predictability and distribution of food sources also

affects behaviour. For example, unpredictability in food supply early in life increased boldness and exploration, but decreased sociability, in guppies (*Poecilia reticulata*) (Chapman et al. 2010). More indirectly, resource availability affects life-history parameters such as growth and metabolism, both of which have been implicated as proximate factors influencing the development and maintenance of animal personality (Stamps 2007; Careau et al. 2008). Additionally, available resources contribute, for example, to the compensatory abilities of infected individuals, thereby modulating the influence of parasites (Kortet et al. 2010). Similarly, if resource availability affects risk-taking behaviour such as boldness, this may influence an individual's predation vulnerability.

Another factor that has emerged as a potentially important influence on behavioural variation is competition (Dall et al. 2004; Dingemanse et al. 2004; Dingemanse & Réale 2005; Cote et al. 2008; Bergmüller & Taborsky 2010; Schuett et al. 2010). Most of these models and studies focus on intraspecific competition for mates (Bergmüller & Taborsky 2010; Schuett et al. 2010), food (Dall et al. 2004; Dingemanse et al. 2004; Dingemanse & Réale 2005), or space (Cote et al. 2008). Interspecific competition, on the other hand, is rarely studied in this context. However, previous work on three- and nine-spined sticklebacks (*Gasterosteus aculeatus* and *Pungitius pungitius* respectively) has shown that interspecific interactions between these two stickleback species are affected by individual boldness in both species (Webster et al. 2009). Regardless of species, bold individuals consumed proportionally more prey, and active individuals spent more time in open water than in vegetated habitat (Webster et al. 2009). These findings suggest substantial overlap in resource and habitat use between these species, corroborated by earlier work (Hart 2003; MacColl et al. 2013), that marks them as strong potential competitors. Interestingly, this work

shows that individual-level behavioural variation affects the strength and outcome of interspecific competition between these two stickleback species (Webster et al. 2009).

Our study system lends itself well for studies concerning the ecological influences on behavioural variation since the stickleback radiation on North Uist resulted in many isolated populations, spread over a relatively small area (Giles 1983b; MacColl et al. 2013). These populations show a high degree of variation in several ecological variables. Fish from seven lochs were assessed for three behavioural properties (boldness, exploration, and activity). Information on infection with three parasite species (*Gyrodactyllus arcuatus, Diplostomum sp*. and *Schistocephalus solidus*) was collected. Parasites of the genus *Gyrodactylus* are widespread on the scales of marine and freshwater fish, and are directly contagious between hosts (Barber et al. 2000). Meta-cercariae of the trematode *Diplostomum sp.* invade the eye-lens of hosts and infect fish via environmental uptake (Barber et al. 2000). The internal parasite *Schistocephalus solidus* is acquired via feeding on snails and occupies the body cavity of infected fish where it makes substantial demands on host energy reserves before being ingested by its final avian host (Barber et al. 2000) Additional data on predator presence (brown trout, *Salmo trutta fario*), resource availability, and competitor (*Pungitius pungitius*) presence were acquired. This allows us to assess the impact of four proposed influences (parasitism, predation, resource availability, and interspecific competition) on the ecology and evolution of individual behavioural variation in natural conditions.

IV.II. Methods

Three-spined sticklebacks were collected using freshwater minnow traps during April – May 2015 from seven freshwater lakes on the Scottish island of North Uist (Table 4.1). Traps were set late afternoon and the fish collected the next morning.

Table 4.1: Sample sites with coordinates, assigned code, number of sampled individuals, and our categorization of parasitism, predation, resource availability, and competitor presence.

Loch	Code	Coordinates	Sampled individuals	Parasitism	Predation	Resource availability	Competitor presence	Mean body length (mm ± SEM)
na Buaile	BUAI	57°38'N 7°11'W	40	High	None	Medium	None	33.830 ± 0.457
Chadha Ruaidh	CHRU	57°36'N 7°12W	40	Medium	None	Low	None	34.428 ± 0.616
an Daimh	DAIM	57°35'N 7°12'W	20	Low	Present	Low	Present	34.160 ± 0.576
na Reival	REIV	57°37'N 7°31'W	30	High	None	High	Present	43.553 ± 0.658
Scadavay	SCAD	57°35'N 7°14'W	40	Medium	Present	Low	Present	32.927 ± 0.278
nan Struban	STRU	57°34'N 7°21'W	40	High	Present	Medium	None	31.885 ± 0.434
Tormasad	TORM	57°33'N 7°19'W	40	Low	Present	Medium	None	35.722 ± 0.411

Following collection, the fish were transported to the experimental rooms in the field station (Drumcharry house, Lochmaddy, 57°61'N 7°17'W) where they were kept in individual tanks (0.12m*0.20m*0.12m) and exposed to a natural photoperiod of approximately 10L:14D. After one day of acclimatization, behavioural assays were performed for four days, with one trial per day (i.e. two days of boldness test

followed by two days of exploration/activity tests, or vice versa). The testing order of the individuals was randomized. The water used in the experimental and individual tanks was obtained from a nearby fishless loch (57°61'W 7°17'W, pH: 6.68, EC: 243 Ms/cm). The temperature of the water in the tanks was monitored and recorded each day during the trials (average: 6.85°C, min-max: 6 - 8°C). Every two days, half of the water in the individual tanks was refreshed.

All of the performed research adhered to the Association for the Study of Animal Behaviour Guidelines for the Use of Animals in Research, the legal requirements of the country in which the work was carried out, and all institutional guidelines.

Behavioural trials

For each population, every focal individual was tested twice for each behaviour (boldness, exploration, and activity) on consecutive days. All sides of the experimental tanks were rendered opaque with white plastic sheets to minimise the observer effect and remove potential visual social cues from conspecifics. During the experiments we maintained a photoperiod of 10L:14D of natural lighting provided through slightly dimmed windows.

Boldness

To assess boldness, the focal individual was placed in an experimental tank (0.20m*0.40m*0.30m) and allowed three minutes to acclimatize. After this acclimatization period, a fright stimulus was provided by rapidly covering the top of the tank with cardboard. Pilot trials showed that fish uniformly responded by freezing and that the time to return to movement (defined as continuous motion with a displacement greater than one body length) exhibited substantial individual variation. So as a measure of 'boldness' we recorded the time it took for fish to return to movement after being frozen in response to a fright stimulus (Chapman et al. 2010; Piyapong et al. 2010; De Winter et al. 2016). Since the majority of pilot fish returned to movement under five minutes, we chose a ceiling value of 300 seconds.

Using two separate experimental tanks, two fish were tested simultaneously, observed by two observers. To minimize the observer effect, the behavioural observations were carried out through live remote viewing using a Panasonic HX-A1000 camera and the Panasonic Image app. Time to return to movement was recorded manually. Following each ten tests, half of the water in both experimental tanks was replaced.

Exploration and activity

Exploratory behaviour and activity were assayed in four experimental tanks (0.55m*0.45m*0.20m). After introducing a focal individual into each tank, an

acclimatization period of five minutes was granted before recording and live tracking the movement of the experimental individuals (Ethovision XT recording, Noldus Information Technology, Wageningen, the Netherlands) using four mounted cameras (Panasonic WV-CP314E CCTV cameras with Computar T4Z2813CS-IR 2.8 – 12mm lenses). The tanks contained a refuge and three habitat structures, all made out of white plastic cups to prevent unwanted interactions with the tracking software. To allow optimal tracking and prevent reflections, testing was performed under standardized light conditions mimicking twilight.

During the live tracking, the coordinates of each fish in its randomly assigned experimental tank were recorded once per second by the software. Based on the gathered data, 'activity' was quantified as the total time moving. 'Exploration' was characterized by calculating the area of the tank that the focal fish visited during the five minute trial. The heatmaps generated by the Ethovision software were processed in ImageJ (http://imagej.nih.gov/ij/) to determine the size of the visited area in square meters.

Parasites, predation, resources, and competition

Following the behavioural trials, basic morphological parameters were quantified and parasite presence was evaluated. After euthanasia with tricaine methanesulfonate (MS222), standard body length (± 0.1mm) and weight (± 0.0001gr) were measured using standard callipers and a balance. Next, the fish were assessed for the presence and number of the monogenean ectoparasite *Gyrodactylus arcuatus* and the eye fluke *Diplostomum sp*. Finally, the fish were labelled and stored

in 70% ethanol. Upon return to the university, the fish were sexed and dissected. The presence, number and total weight of the tapeworm *Schistocephalus solidus* was recorded.

To enable population-level comparisons, we next categorized parasite, predation, and resource data into discrete categories, following earlier studies that often divide their focal populations into high vs. low predation or high vs. low parasitism (Bell & Sih 2007; Brydges et al. 2008; Herczeg et al. 2009; Kortet et al. 2015). The detail of our data allowed us to refine our categories slightly. Based on the parasite counts, the sampled populations were classified as follows: low parasitism (L) (< 25% of sampled fish infected with at least one parasite), medium parasitism (M) (25 – 50% of sampled fish infected with at least one parasite), and high parasitism (H) (> 50% of sampled fish infected with at least one parasite). To prevent *a priori* bias concerning the severity of effects of specific parasite species, no special consideration was given to parasite species in establishing these categories. This represents a snap-shot of parasitism rather than a long-term categorization, but parasites tend to have a relatively rapid effect on behaviour (Barber et al. 2000).

To quantify predation, data on presence or absence of brown trout was obtained from the local North Uist Angling Club (MacColl et al. 2013; MacColl & Aucott 2014), and by gill-netting (Magalhaes et al. 2016). Based on these data, the studied populations were categorized as either no predation (N), or predators present (P).

Resource availability was quantified by using the PC score for environmental factors known to correlate with aquatic productivity, such as invertebrate abundance which is an important food source for our study species (De Roij & MacColl 2012; MacColl et al. 2013). The values are averages of annual readings that started in April 2006 and

have shown to be quite consistent over time (MacColl et al. 2013). Concentrations of Ca, Na, Mg, and K, were obtained via inductively coupled plasma mass spectrometry (ICP-MS). Dissolved organic carbon (DOC) and chlorophyll a concentrations were obtained via total organic carbon analyser and spectrophotometry respectively. PC1 explained 76.75% of variation. We categorized lochs with a score above 1 as high resource availability (H), lochs with a score between 1 and -1 as medium (M), and lochs with a score below -1 as having a low resource availability (L).

Finally, as a measure for interspecific competition, we use data on *Pungitius* presence in the sampled lochs (MacColl et al. 2013), and divided the lochs into *Pungitius* absent (N) or present (P) categories.

Data analysis

Boldness scores were deducted from the ceiling value of 300s in order to ensure that bold fish have higher scores than shy fish. The consistency (i.e. repeatability) of the behavioural measures was calculated according to Lessells & Boag 1987, with standard errors obtained following Nakagawa & Schielzeth 2010. Spearman Rank's correlations were performed to assess the correlations between body length and the mean of each of the tested behavioural parameters.

All analyses were performed in R 3.2.1. Tests were two-tailed and significance was inferred when $p = 0.05$ throughout. To assess the effects of parasitism, predation, and resource availability on each of the tested behavioural parameters at population level, we used Kruskal-Wallis tests (data were not normally distributed) for each

behaviour-environmental parameter combination. We initially fitted general linear models for each behaviour with the ecological variables as fixed effects. Unfortunately, these models were overfitted which is why we opted for Kruskal-Wallis tests. Following this, we applied the false discovery rate method to adjust the p-values to correct for multiple testing (Benjamini & Hochberg 1995). To assess the significance of the differences between categories for the significant environmental factors, we ran a post-hoc Tukey HSD.

To investigate the individual level effects of the different parasites, sex and body length, generalized linear mixed models were fitted for each behaviour with each parasite, sex, and body length as fixed effects, and population and individual as random effects. We included the interaction with sex for each parasite in the initial model. We provide a variance estimate for the random effects.

To estimate effect sizes, we calculated Cohen's *d* for multiple groups based on standardized mean differences (Cohen 1988). As a rough guide when using this method, values <0.2 are considered small effects, 0.5 is considered medium, and values >0.8 are large effects (Cohen 1988).

IV.III. Results

All populations but one (DAIM) showed consistent bold behaviour. However, concerning exploration DAIM was the only population displaying behavioural consistency. Consistent activity was found in three populations (BUAI, REIV, and STRU) (Table 4.2).

Table 4.2: Repeatabilities for the tested behavioural measures with standard error and p-value (significant values in bold).

	Boldness			Exploration			Activity		
Loch	r	se	p	r	se	p	r	se	p
BUAI	**0.336**	**0.071**	**0.015**	-0.142	0.052	0.813	**0.307**	**0.068**	**0.024**
CHRU	**0.672**	**0.071**	**<0.0001**	0.138	0.038	0.193	0.217	0.054	0.084
DAIM	-0.017	0.008	0.528	**0.612**	**0.108**	**0.001**	0.301	0.095	0.088
REIV	**0.4**	**0.088**	**0.012**	0.29	0.076	0.055	**0.643**	**0.085**	**<0.0001**
SCAD	**0.493**	**0.08**	**0.0005**	0.191	0.049	0.113	-0.079	0.027	0.688
STRU	**0.452**	**0.079**	**0.001**	0.183	0.048	0.124	**0.42**	**0.078**	**0.003**
TORM	**0.768**	**0.057**	**<0.0001**	0.105	0.03	0.254	0.066	0.02	0.338

Spearman's rank correlation tests indicated that both exploratory behaviour (ρ = 0.183, p <0.0001) and activity (ρ = 0.331, p<0.0001) were positively correlated with body length (Fig. 4.1). No correlation was found between boldness and body length (ρ = -0.006, p = 0.893).

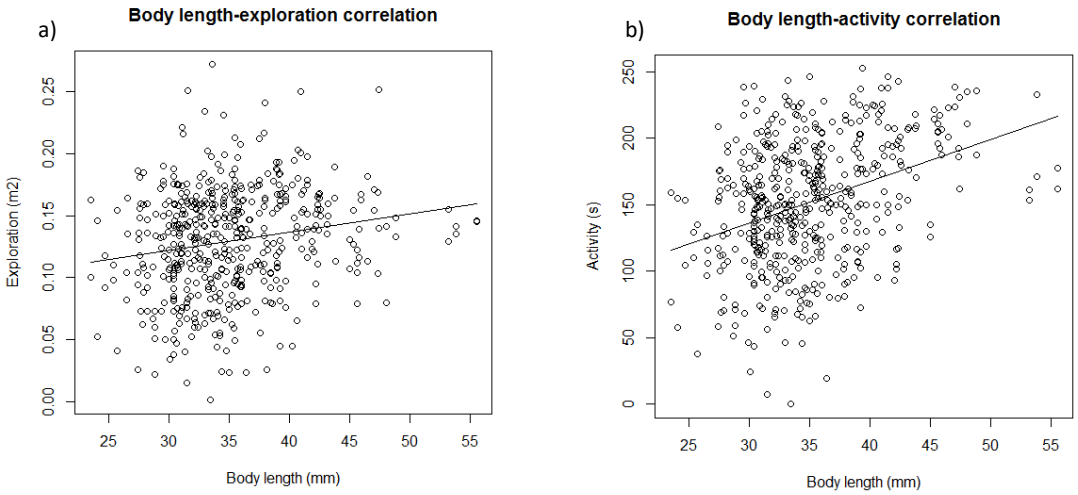


Figure 4.1: Correlation between body length and exploration (a), and body length and activity (b).

At the population level, parasitism had a significant effect on boldness ($\chi^2_{df} = 17.122_2$, $p = 0.0006$) and exploration ($\chi^2_{df} = 28.368_2$, $p < 0.0001$). The presence of predators affected only activity ($\chi^2_{df} = 9.453_1$, $p = 0.004$). Both boldness ($\chi^2_{df} = 9.683_2$, $p = 0.013$) and activity ($\chi^2_{df} = 23.335_2$, $p < 0.0001$) were influenced by resource availability, and the presence of interspecific competitors had an impact on exploration ($\chi^2_{df} = 18.33_1$, $p < 0.0001$) and activity ($\chi^2_{df} = 12.825_1$, $p = 0.0008$).

Post-hoc testing revealed that fish were significantly bolder in populations with medium parasitism than in high or low parasite lochs (mean difference medium-high: 19.985 ± 7.986s, $p = 0.034$; mean difference medium-low: 44.519 ± 9.274s, $p < 0.0001$) (Fig. 4.2). In low parasite lochs, fish were significantly less bold than fish in high parasite lochs (mean difference: 24.534 ± 8.691s, $p = 0.014$) (Fig. 4.2). Fish from high parasite lochs were also significantly less exploratory than their counterparts in low (mean difference: 0.020 ± 0.005m^2, $p < 0.0001$) and medium (mean difference: 0.023 ± 0.004m^2, $p < 0.0001$) parasite lochs (Fig. 4.2).

Fish from lochs with predators were less active than fish in populations with no predation (mean difference: 11.924 ± 4.24s, $p = 0.005$) (Fig 4.2).

Individuals from high resource populations were significantly bolder than fish from medium (mean difference: 36.717 ± 11.186s, $p = 0.003$) and low (mean difference: 26.872 ± 11.421s, $p = 0.05$) resource populations (Fig. 4.2). Fish activity was significantly higher in high resource lochs compared to medium (mean difference: 21.682 ± 6.678s, $p = 0.004$) and low resource (mean difference: 32.685 ± 6.819s, $p < 0.0001$) (Fig. 4.2). Fish in medium resource lochs were more active than those in low resource lochs (mean difference: 11.003 ± 4.442s, $p = 0.036$) (Fig. 4.2).

Finally, the presence of interspecific competitors correlated with less exploratory (mean difference: 0.015 ± 0.004 m^2, p = 0.0002) and less active (mean difference: 15.257 ± 4.36s, p = 0.0005) fish (Fig. 4.2).

At the level of individual fish, none of the parasites explained significant behavioural variation (Table 4.3), only body length did for exploration ($\chi^2_{df} = 8.897_1$, p = 0.003) and activity ($\chi^2_{df} = 20.715_1$, p < 0.0001) (see also Fig. 4.1). Random effect variances (+ SEM) were as follows: boldness: population: 1093 ± 12.495, individual: 2603 ± 19.284; exploration: population: 0.0005 ± 0.009, individual: 0.0001 ± 0.004; activity: population: 270.9 ± 6.221, individual: 266.4 ± 6.168.

Table 4.3: Summary statistics for each parasite species as obtained via glmm (see Methods for full model details).

	Boldness		Exploration		Activity	
	χ^2_{df}	p	χ^2_{df}	P	χ^2_{df}	P
Gyrodactylus arcuatus	0.167_1	0.828	0.500_1	0.479	2.018_1	0.155
Diplostomum sp.	0.095_1	0.758	1.123_1	0.289	1.733_1	0.188
Schistocephalus solidus	0.019_1	0.889	0.304_1	0.581	1.567_1	0.211

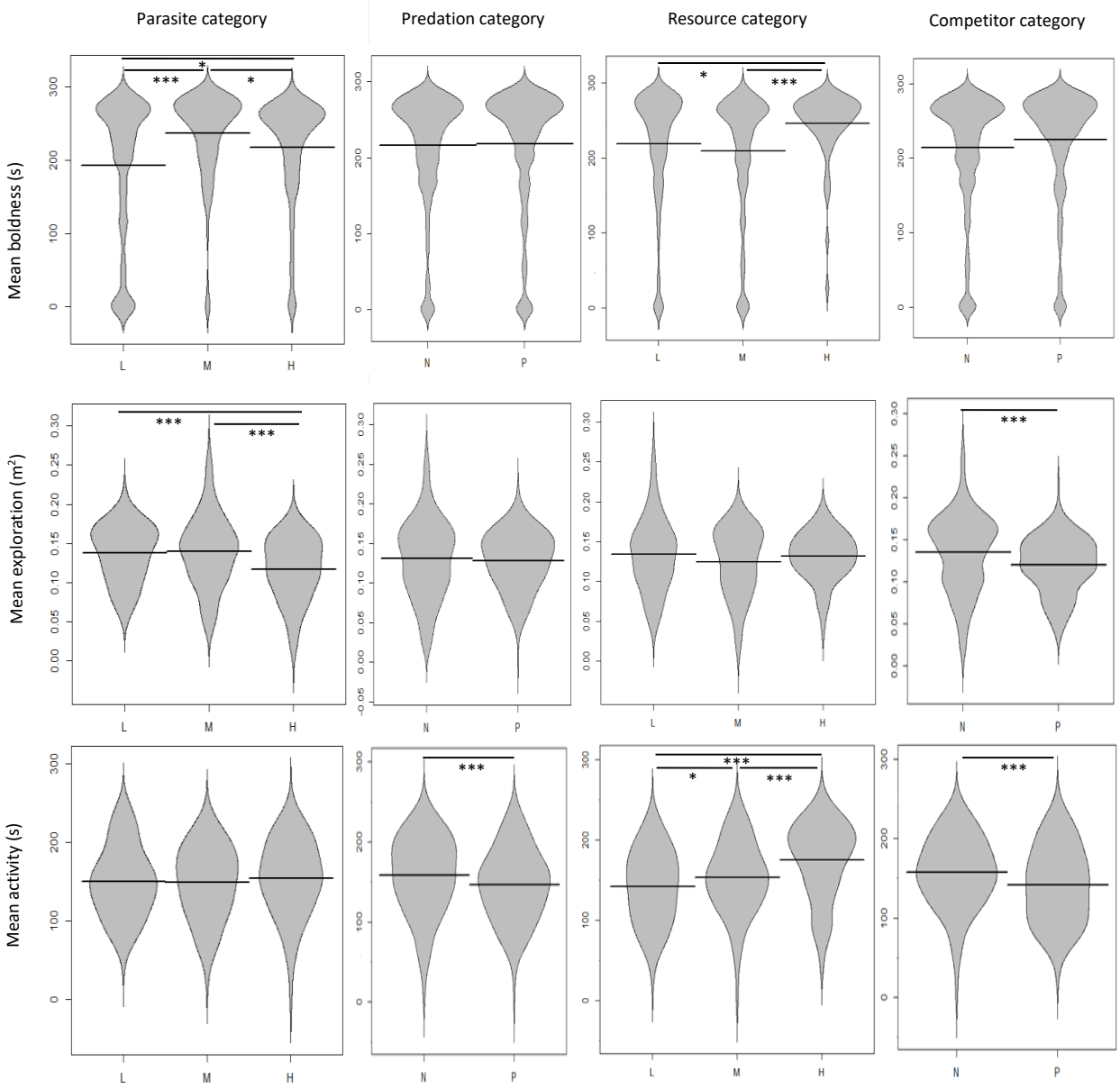


Figure 4.2: Differences in mean behaviour (top: boldness, middle: exploration, bottom: activity) per category (left: parasites, low (L), medium (M), and high (H); second column: predation, none (N), predator present (P); third column: resources, low (L), medium (M), and high (H); right: interspecific competition, none (N), competitor present (P)). The black line is the mean value; overlaid with kernel density estimations (grey). (* = $p<0.05$, ** = $p<0.01$, *** = $p<0.0001$)

The effect sizes of the different factors for each behaviour are given in table 4.4. Parasitism and resource availability exert substantial effects, followed by interspecific competition. Predation has only a minor effect on a single behaviour.

Table 4.4: Effect sizes (Cohen's *d*) for the ecological factors given for the three behaviours. Bold values denote significant factors from Kruskal-Wallis tests.

	Cohen's *d*		
Ecological factor	Boldness	Exploration	Activity
Parasitism	**0.564**	**0.556**	0.124
Predation	0.025	0.047	**0.253**
Resource availability	**0.517**	0.240	**0.700**
Pungitius presence	0.135	**0.359**	**0.323**

IV.IV. Discussion

Our data suggest a powerful role for ecology in shaping behavioural variation in natural populations of three-spined stickleback with different ecological factors having different effects. None of the ecological factors affected all three measured behaviours.

Parasitism affected boldness and exploration. Fish in lochs with medium parasitism were bolder than those in lochs with either high or low parasite prevalence. Models suggest that if animals are able to invest in their immune system as compensation for parasitism, parasite infection favours high levels of boldness due to a positive feedback between immunocompetence and resource intake mediated by bold

behaviour (Kortet et al. 2010). Our results suggest that this may hold up to a point. In other words, our finding of the highest boldness in medium parasitism lochs suggests that there might be a 'compensation threshold'. Interestingly, on an individual fish level, none of the parasites we quantified (*Gyrodactylus arcuatus, Diplostomum spp.,* and *Schistocepahlus solidus*) significantly affected boldness, exploratory behaviour or activity. Other studies, in contrast, have shown that three-spined sticklebacks infected with *Schistocephalus solidus* are bolder when facing a potential predator (Giles 1983a; Milinski 1985; Giles 1987). However, these studies were performed under laboratory conditions, thereby potentially neglecting the influence of continuous predation pressure, resource availability, and competition that wild populations are subjected to. Alternatively, the specific observational procedures used can affect the measurement and quantification of behavioural traits.

There are two additional points to take into account. First, the effects of different parasite species might counteract each other. However, we found no significant effect for any parasite species; therefore we cannot provide empirical support for this hypothesis. Second, parasites (either specific species or overall parasite burden) may affect behaviours that were not assayed here. For example, infection with *Schistocephalus solidus* has been shown to reduce shoaling tendency (Barber & Huntingford 1995) and alter foraging behaviour (Milinski 1984; Tierney 1994) in three-spined sticklebacks.

Also at an individual level, body length, but not parasite load, was a significant predictor of exploration and activity, with larger fish being more exploratory and more active. This contradicts the metabolic hypothesis which suggests that the higher metabolism of smaller-bodied fish spurs behaviour that increases the chance of finding novel food sources (Brown & Braithwaite 2004). But, body size can also be

a proxy for age in fish, so it could be that our results reflect a behavioural difference among age classes, rather than among fish within a specific age class. Boldness was not affected by body size, mirroring results obtained in earlier studies on three-spined stickleback (Walling et al. 2004) and the live-bearing fish genus *Brachyraphis* (Ingley et al. 2014). However, unlike Ingley (2014), we found no effect of sex on bold behaviour. A final consideration at individual level is that our tests for exploration/activity involved repeated exposure to the same experimental tanks. This could have led to a habituation effect which might account for the lack of repeatability in these traits.

A recent study in Eurasian minnows (*Phoxinus phoxinus*) found that fish from a high parasite, high predation population were bolder and less active than those from a low parasite, low predation population, but the effect of the parasite (*Diplostomum phoxini*) on its own on host behaviour was not significant (Kortet et al. 2015), which mirrors results in rainbow trout (*Oncorhynchus mykiss*) (Klemme et al. 2016), and our own findings from the individual level analysis. Another study on minnows showed that purposeful infection with *D. phoxini* did not alter boldness, activity, or exploration, but did affect the repeatability of these behaviours (Kekäläinen et al. 2014). Based on this study and our results, we suggest that the behavioural differences in activity between the two populations in Kortet et al.'s 2015 study are largely due to the difference in predation, rather than parasite exposure while both parasitism and predation might play a role in boldness and exploratory behaviour.

We found that fish from populations with predators were less active than those from other populations. Previous work on North Uist stickleback (Spence et al. 2013) did not find an effect of predation on refuge emergence time, only an effect of morph, which is supported by our results from the boldness trials. Other studies have found

both a positive (Harris et al. 2010; Ingley et al. 2014; Kortet et al. 2015) and negative (Brydges et al. 2008; Herczeg et al. 2009) effect of predator presence on boldness. Predator presence has also been linked to a decrease in activity of the predated Eurasian minnow (*Phoxinus phoxinus*) (Kortet et al. 2015), which is corroborated by our results. When facing a predator that hunts visually and is attracted to movement, it might indeed be behaviourally beneficial for the prey to spend less time moving around (Ioannou & Krause 2009). It should be noted that our results are for predation by trout. Other potential predators, such as birds or invertebrates, could also play a role, which presents an opportunity for further research, namely to investigate how different predators affect individual behavioural variation.

Fish from populations with a high resource availability tended to be bolder and more active than those in medium or low resource lochs, contrasting with results for boldness in rainbow trout (Biro et al. 2003), but aligning with findings for active prey pursuit in Atlantic cod (*Gadus morhua*) (Moberg et al. 2011). Food acquisition can be framed as a trade-off with predation risk. Therefore, some argue that increased food abundance should correlate with a decrease in risk-taking behaviour (i.e. boldness) (Biro et al. 2003). However, boldness is often linked with aggression in three-spined stickleback (Huntingford 1976; Bell 2005; Dingemanse et al. 2007) and aggressive behaviour might be too costly to maintain in low resource conditions. In song sparrows (*Melospiza melodia*), for example, an experimental supplementation in available food increased territorial aggression (Foltz et al. 2015). One additional aspect of resource availability for future studies to consider is resource distribution. Being bold, active or exploratory is more useful in a patchy resource distribution rather than a homogenous one. Additionally, the predictability of food availability has also been shown to affect boldness in the Trinidadian guppy *Poecilia reticulata*

(Chapman et al. 2010). Our results thus represent a snap-shot at a single moment in a fluctuating environment. Previous fluctuations in resource availability and distribution could have influenced the behavioural types present in the sampled populations.

The presence of interspecific competitors did not affect boldness, but negatively influenced both activity and exploration. In lochs where three-spined sticklebacks shared their environment with nine-spined sticklebacks, the former were less exploratory and less active. High intraspecific competition is thought to increase aggression (Dall et al. 2004; Dingemanse et al. 2004; Sih et al. 2004a), but the behavioural effects of interspecific competition have received little attention so far. However, if interspecific competitive interactions between three- and nine-spined stickleback are accompanied by aggression or induce other costs, it might be beneficial for individuals to limit interspecific competitive interactions. One way to achieve this is to reduce exploratory behaviour and/or activity. The increased rate of environmental change and the associated changes in species ranges mean that introduced or invasive species are a growing concern. Integrating behavioural studies on how novel potential competitors influence the behavioural variation in both resident and invading species is therefore a timely issue that deserves consideration (Sih et al. 2012).

Interestingly, the effect sizes in our study (see table 4.4) suggest that parasitism and resource availability are the most powerful ecological factors shaping behavioural variation in natural populations of three-spined stickleback, followed by the presence of interspecific competitors. Counterintuitively, the effect of predation is minimal. It affects only a single behaviour (activity) in our study and even then its effect size is modest, yet predation is perhaps the most often studied ecological

influence on animal personality (Bell & Sih 2007; Brydges et al. 2008; Dingemanse et al. 2009; Herczeg et al. 2009; Harris et al. 2010; Kortet et al. 2015). The link between parasitism and animal personality has been getting more attention recently (Barber & Dingemanse 2010; Kortet et al. 2010; Kortet et al. 2015), but studies in natural conditions are still quite rare (Kortet et al. 2015). Furthermore, our findings suggest that resource availability and certainly interspecific competition have been severely neglected in the study of ecological correlates of behavioural variation in both wild and laboratory populations (but see Webster et al. 2009; Chapman et al. 2010; Foltz et al. 2015).

Future work could investigate other behaviours (e.g. shoaling tendency, aggression, and food preference) and/or focus on neglected factors that deserve more attention such as resource availability and interspecific competition. Lab-based infection experiments could elucidate interactions between parasite infection and resource availability on behavioural expression and consistency. Additionally, including habitat characteristics such as resource distribution or shelter availability could prove interesting. However, future endeavours will face an unavoidable trade-off between the control and manipulability of artificial conditions and the vicissitudes of fieldwork (Niemela & Dingemanse 2014). Furthermore, the effects we have found can have their roots in genetic changes, plasticity, or, more likely, both. Early life experiences, for example, can influence how exposure to predators or parasites affects behavioural responses later in life (Stamps & Groothuis 2010b; Wolf & Weissing 2010) Similarly, some models stress state-dependency, which means that the sensitivity to, and subsequent behavioural effects of, environmental changes can hinge upon the individual state (i.e. features that affect the costs and benefits of

behavioural actions, see Sih et al. 2015) of an organism (Luttbeg & Sih 2010). This sensitivity and plasticity, in turn, might have a genetic basis.

In conclusion, this study incorporates three purported major influences on behavioural variation in natural populations. We have assayed the behaviour of wild fish from seven lochs that differ in several environmental factors. Our results indicate that different ecological factors affect different (sets of) behaviours and provide a detailed look at the complexity of behavioural variation and its ecological correlates in natural conditions.

V. The power of others:

The effects of behavioural composition and experience on group performance in a vertebrate and invertebrate species with different social systems

Abstract

Consistent individual behavioural variation is a topic of great interest in contemporary biology. A pertinent question concerns how individual variation affects group dynamics. Given that group living is widespread in nature, this has broad relevance across a wide variety of taxa. Both the composition of behavioural types of groups, and group experience have been shown to affect aspects of group behaviour. Here we experimentally examine the potential interaction between these two factors in two taxonomically different study systems, the three-spined stickleback (*Gasterosteus aculeatus*) and the social spider *Stegodyphus dumicola.* We assess the effects of behavioural composition and group experience, as well as their potential interaction, on group behaviour by composing groups of different behavioural composition and retesting their foraging behaviour over a period of time for both species

Our analyses reveal that, in sticklebacks, both composition and experience affected latency to find food, group cohesion, and the number of fish that fed in a social foraging trial. In social spiders, the effect of experience was less evident. In both species, groups with a mixed behavioural type composition outperform those with a homogenous make up via more rapid feeding and an increased number of individuals that participated in feeding events. But, importantly, our design allows us to provide experimental support for the hypothesis that these factors (group composition and experience) operate via different mechanisms and do not interact.

V.I. Introduction

In recent years, the existence of consistent variation in individual behaviour within populations ('animal personality') has become an intense research focus in behavioural ecology. What was once widely seen as mere noise around an adaptive mean has now become a topic of scrutiny (Dall et al. 2004; Dingemanse & Réale 2005; Réale et al. 2007). An individual that is highly plastic in its behaviour can maximize its fitness in the many heterogeneous environments it is likely to encounter during its lifetime (Dingemanse & Réale 2005). Yet, such unbridled plasticity is rare as it is expected to be costly to have very high plasticity given, for example, the need to use and maintain complex regulatory and sensory mechanisms (DeWitt et al. 1998; Dall et al. 2004). In fact, the rule seems to be the occurrence of discernible behavioural types that consistently differ between individuals in the behaviour they display within and across contexts (Dall et al. 2004; Dingemanse & Réale 2005; Réale et al. 2007; Duckworth 2010). So why is consistent individual variation in behaviour so widespread in nature?

This question has been approached from various angles and many aspects of an organism's phenotype and environment have been related to consistent behavioural variation, from physiology (Biro & Stamps 2008; Careau et al. 2008; Stamps & Groothuis 2010), to predation and parasitism (Dall et al. 2004; Dingemanse & Réale 2005; Wolf et al. 2007; Barber & Dingemanse, 2010; Kortet et al. 2010; Pettorelli et al. 2011), to the abiotic environment (Brydges et al. 2008). The social environment of an organism is also considered to be a relevant factor for the development and maintenance of individual variation in behaviour (Bergmüller & Taborsky 2010). The

influence of this wide array of ecological and other factors on the occurrence and pattern of animal personality within and between populations strongly hints at an adaptive function for the existence of behavioural types and syndromes (Wilson 1998; Dingemanse & Wolf 2010; Wolf & Weissing 2010).

One of the most recent fields of enquiry that has attracted attention concerns the effects of animal personality in a social context (Bergmüller & Taborsky 2010; Krause et al. 2010; Webster & Ward 2011; Montiglio et al. 2013; Wolf & Krause 2014; Farine & Montiglio 2015). Organisms do not live in isolation and often spend part of their lives, or their entire lives, in a group of conspecifics. As an individual's behaviour affects the behaviour of other individuals, it also influences group-level behaviours that rely on the interaction between multiple members of the group, for example, cooperative foraging, defence, or breeding. Social effects of behavioural types, wherein the behaviour of an individual affects that of other individuals, have been found in different species with varying social systems, from shoaling three-spined sticklebacks (*Gasterosteus aculeatus*) (Pike et al. 2008), to cooperatively breeding cichlids (*Neolamprologus pulcher*) (Hamilton & Ligocki 2012), flocking great tits (*Parus major*) (Aplin et al. 2013; Aplin et al. 2014), and the permanently social spider *Stegodyphus dumicola* (Modlmeier et al. 2014).

One way to investigate the relationship between individual behavioural types and group performance is to compare the collective feeding success of individuals among groups that differ in behavioural composition. In guppies (*Poecilia reticulata*), for example, it has been shown that groups of bold or mixed behavioural types (i.e. 'bold' and 'shy') are faster to approach a novel feeder device than shy groups, and that in mixed groups more individuals acquire food than in single-type groups (Dyer et al. 2009). Even in invertebrates, such as the social spider *Stegodyphus dumicola*

and the ant *Temnothorax longispinus,* this effect has been noted (Modlmeier et al. 2012; Keiser & Pruitt 2014; Wright et al. 2015). The proposed explanation for this observation involves producer-scrounger dynamics (Kurvers et al. 2010). Following the rationale of this hypothesis, shy (scrounger) individuals should tend to follow bold (producer) ones and, in doing so, they are more likely to discover a new food source than if they would find themselves in a completely shy group. Bold individuals, on the other hand, are less likely to seek company (Ward et al. 2004) and, when given a choice, shy individuals prefer to follow other shy individuals (Nakayama et al. 2016). As such, in an entirely bold group only one or a few individuals will discover the novel food source (Dyer et al. 2009). Mixed groups are therefore predicted to perform best.

Besides group composition, experience also influences group performance. In three-spined stickleback (*Gasterosteus aculeatus*), familiarity (a form of group experience) affects fine-scale social organization and the discovery of resources (Utne-Palm & Hart, 2000; Atton et al. 2014). The idea is that increased experience results in a more efficient use of social cues and environmental information, which, in turn, improves group performance Additionally, a study in juvenile stickleback suggests that decreased aggression between familiar fish contributes to a more equal division of resources (Utne-Palm & Hart 2000). In guppies, individuals prefer to follow familiar individuals and that, in turn, can lead to social learning and the acquisition of information (Laland & Williams 1997). Furthermore, guppies appear to learn the route to a novel food source more swiftly when it is shown by familiar trained demonstrator fish rather than unfamiliar demonstrators (Swaney et al. 2001). These findings suggest that an improvement in the use and/or transmission of (learned)

information might lie at the root of experience's effect on group behaviour and performance.

Thus far, the effects of group composition and experience on group performance have been studied mostly in isolation (but initial explorations hint at possible interactions, see Laskowski & Bell 2014; Laskowski & Pruitt 2014; Laskowski et al. 2016). Here we focus on the joint effect of these two factors to investigate whether they interact. To address this, we designed an experiment to assess both factors simultaneously. By establishing groups of known behavioural composition and repeatedly testing them for group performance, we were able to investigate the effect of group composition, experience, and their interaction, on the foraging behaviour of the experimental groups. Since the effect of experience is mostly attributed to (social) learning (Utne-Palm & Hart 2000; Atton et al. 2014), we hypothesize that the effect of experience on group behaviour (in our case, more rapid and equally distributed feeding) is enhanced in groups of mixed behavioural phenotypes due to individuals assessing (or 'learning') the behavioural types of others and aligning themselves accordingly into a social role (cf. the social niche hypothesis, see Bergmüller & Taborsky 2010), and/or learning about environmental cues. In other words, bold individual take on the role of 'producers', while shy ones settle into a role of 'scroungers'. In homogenous groups, individuals might still learn about the behaviour of others as experience increases, but this effect is less in this case since individuals are less likely to perform a social role that is at odds with their behavioural type (i.e. bold individuals are less prone to adopt 'shy' roles than individuals that were shy to begin with, so either bold individuals explore by themselves, or shy individuals do not explore at all in behaviourally homogenous groups). We acknowledge that this limits the potential role of plasticity, but a recent

study in *S. dumicola* does suggest that social niche disruption negatively affects individual and group performance, which, in turn, is proposed to be due to the costs of changing social niche (Laskowski et al. 2016).

We implemented this experimental design in two taxonomically different study systems: three-spined sticklebacks (*Gasterosteus aculeatus*) and social spiders (*Stegodyphus dumicola*). These species differ markedly in ecology and social system, but are both frequently used in studies investigating aspects of animal personality. Implementing a similar experimental design across two different study systems provides an opportunity us to gain broader insights into the links between individual and group behaviour.

If 'producer-scrounger' dynamics result in better performance of mixed groups (Dyer et al. 2009), we expect groups of mixed composition (i.e. bold and shy fish/spiders) to perform better on a foraging task than groups composed solely out of bold or shy individuals. Experience (including group familiarity and relatedness) may reduce conflict and promote synergy and cooperation, we therefore expect experienced groups to outperform unexperienced groups. It is not known whether experience interacts with group composition differentially. If experience reinforces the effects of behavioural composition of the group, we would expect experienced mixed phenotype groups to perform better than unexperienced mixed phenotype groups, and mixed groups should show relatively higher improvement in group behaviour (according to our metrics) over time compared to single-phenotype groups, mediated by an increase in experienced. This effect could also be found in spiders, however, because these are social spiders, familiarity (as type of experience) is, in most contexts, an a priori condition (most often in the form of relatedness) so that (social) learning processes are either optimized or less relevant (i.e. only the

environmental aspects may matter) in a foraging context, both of which could lead to a relatively smaller effect of experience on group composition over time, as well as a reduced effect of experience independently of its influence on group composition.

V.II. Methods

All of the performed research adhered to the Association for the Study of Animal Behaviour Guidelines for the Use of Animals in Research, the legal requirements of the country in which the work was carried out, and all institutional guidelines.

Three-spined stickleback

- Study organism

The three-spined stickleback is a widely distributed fish species in temperate and boreal regions of the northern hemisphere where it occupies a wide range of habitats, including marine coasts, brackish waters, and various freshwater environments. Beyond its varied habitat, the species also exhibits considerable phenotypic variation, from non-armoured, small fish in certain lake regions to large armoured marine populations (Bell & Foster 1994).

Due to its extensive phenotypic variation, the three-spined stickleback is a popular study organism in many fields of ecology, evolution, and behavioural biology. Its facultative shoaling behaviour outside of the breeding season and its competence in social learning make it a good study system to investigate animal personality in a social context (Atton et al. 2014).

- Animal capture and maintenance

Fish were caught in Tottle Brook, Nottingham (52°55’58.5’’N, 1°12’05.4’’W) with wire mesh minnow traps. Traps were set in the evening and collected the next morning. The fish were transported to the aquarium on the grounds of the University of Nottingham where they were distributed among ten 100L tanks (20 fish per tank, total n at the start of the experiments = 200). Ambient temperature was 15 – 16°C and the photoperiod was 8L:16D. Each morning a meal of frozen bloodworm (Chironomidae larvae) was provided by gently pouring it in at the top of the tank.

One week before the initiation of the experiments, each fish was given a unique Visible Implant Elastomer (VIE) tag code (Northwest Marine Technologies) in order to identify fish at an individual level throughout the experiment. We selected four injection sites (left side of the head above the eye, right side of the head above the eye, left below dorsal spines, and right below dorsal spines). To make the procedure as short as possible (approximately 30 – 45s per fish), fish were not anaesthetized (Fürtbauer et al. 2015). If no colour was injected at an injection site, a dummy injection was performed using a similar syringe as the one used for colour injections. The fish were allowed to recover for one week before the behavioural trials to

minimize the behavioural effects of the injections, which have previously been shown to have similar physiological effects as everyday life occurrences such as predation risk (Fürtbauer et al. 2015).

We measured standard body length (± 0.1mm) with callipers, and body weight (± 0.01gr) using a standard electronic balance. This was done during the tagging (i.e. one week prior to the behavioural trials) to reduce the number of times we needed to handle the fish.

- Boldness assays

To assess boldness, we moved an individual fish into an experimental tank (width * length * height = 0.2m*0.4m*0.3m), the sides of which were rendered opaque with white plastic, and allowed an acclimatization period of 3 minutes. Next, we quickly placed a large piece of cardboard on top of the tank for three seconds and removed it in a single smooth motion. Boldness was quantified as the time a fish took to return to normal movement (defined as a continuous horizontal displacement of more than one body length) (Chapman et al. 2010; Piyapong et al. 2010; De Winter et al. 2016). Based on pilot trials and previous experiments (De Winter et al. 2016) that showed that fish usually respond within 5 minutes, we used a maximum value of 300 seconds. In further analysis, the time to return to movement was subtracted from this ceiling value so that bolder fish were given a higher score.

Each individual was assessed three times with one week between each trial. Testing order was randomized throughout.

Following the group trials, we reassessed the participating fish for boldness one week after completion of the group experiments, allowing us to compare boldness before and after the group stage.

- Group composition

Since boldness was highly repeatable (see Results), we used mean boldness to categorize fish into personality categories (shy, medium, and bold) by selecting the lowest scoring 50 fish as 'shy', and the 50 highest scoring ones as 'bold' (we visually selected only females to prevent a 'sex effect', total n of individually tested females = 150, females selected for group trials: n = 100) (Dyer et al. 2009). Following this categorization, the fish were distributed into groups of four individuals. We established eight replicates each of bold groups (all individuals from the bold category), mixed groups (two bold and two shy individuals), and shy groups (all shy individuals) (total n of individual fish for group experiments = 96). All groups were composed out of individuals that did not share a holding tank during the boldness trials.

These groups were kept in six 100L tanks, with four groups per tank (n = 16 individuals per tank). Different groups in the tanks could be distinguished via the VIE tags. We chose to keep four groups per tank in order to create a slightly more natural social environment than provided by small, separated groups in smaller tanks. All groups were given a one-day acclimatization period, after which the group trials (see below) began. These trials were repeated weekly for four weeks (i.e. t_1 = 1 day, t_2 = 8 days, t_3 = 15 days and t_4 = 22 days). Since previous research suggests that guppies

(*Poecilia reticulata*) become familiar with each other when housed together for 12 days (Griffiths & Magurran 1997), this period covers the full familiarization process, from non-familiar (t_1) to fully familiar (t_4). Previous work also suggests that this period is sufficient to allow for spatial learning (in our case the location of food in the experimental tank), even though the speed of learning can differ between populations depending on environmental complexity of their living conditions (Odling-Smee & Braithwaite 2003; Odling-Smee et al. 2008). Since our experimental fish all came from the same source populations, this is not an issue here.

- Group trials

To test group performance, a group was introduced into a holding zone (w * l * h = 0.15m*0.1375m) in the experimental tank (w * l * h = 0.55m*0.45m*0.2m) filled with ± 0.15m of water (Fig. 5.1). The tank contained a centrally placed plastic plant and two petri dishes (0.10m diameter) hidden behind a rock (length = ± 0.15m, width = ± 0.05m). One of these petri dishes contained a supply of bloodworm (0.65 ± 0.01gr). The position of the food-containing petri dish was the same in all trials. This allowed us to compare the performance of all groups, regardless of a potential 'side bias'. Either there is a side bias, but it is not relevant for group comparisons since the petri dish was always on the same side, or there was no side bias, so the side was irrelevant. To standardize hunger levels, groups were not fed prior to the trials on the testing days. On non-testing days, food was provided in the same way as it was before the group trials.

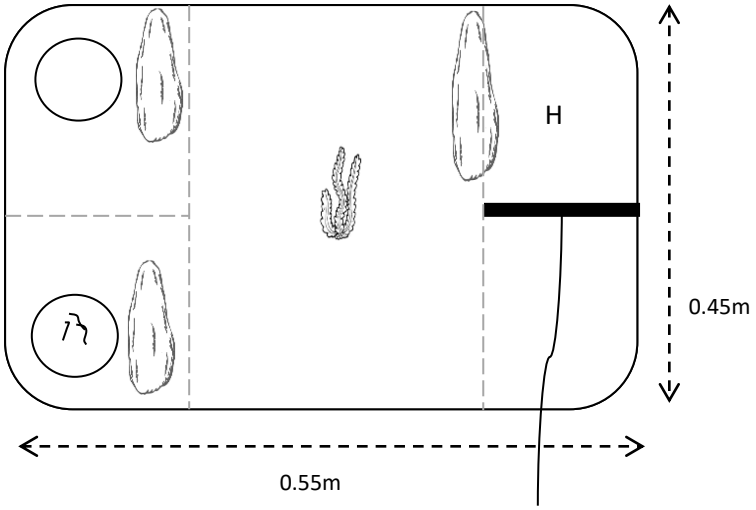


Figure 5.1: Experimental tank for the stickleback group trials with holding zone (H), removable rubber divider (horizontal bold black line), plastic plant, three small rocks, and two petri dishes, one of which (bottom) contains bloodworm. The dotted grey lines denote success (bottom left) or failure (top left) in the spatial learning assessment.

The fish were given a 3-minute acclimatization period after which the rubber divider between the holding area and the rest of the tank was removed via a string. During the following five minutes, the group was observed remotely via a Panasonic HX-A100 camera, mounted approximately 0.75m above the experimental tank.

During this period, we quantified the following parameters: 1) the time it took the first fish to locate the food source and start eating, 2) the number of fish that managed to feed before the food was depleted (if there was still food present after 300s, we noted the number of fish that had fed during this period), and 3) whether the first fish to pass the rocks would do so on the side of the food (success) or not (failure).

Additionally, a photograph was taken via the camera at 1-minute intervals, resulting in five photographs for each trial. We repeated this procedure at t_2, t_3 and t_4. During each trial day, groups were tested in a random order.

The photographs were then processed in ImageJ to obtain the distance between all possible fish pairs (for n individuals, this results in n(n-1)/2 distance values per photograph. Here 6 distance values were obtained for each photograph). These distances were then averaged for each time point (i.e. minute 1, 2, 3, 4, and 5). These five values were averaged again to obtain a single measure of group cohesion for each group trial, i.e. $\sum d_t / n_t$, with d_t the average distance for time point t, and n_t the total amount of sampled time points.

Social spider

- Study organism

Stegodyphus dumicola (Eresidae) is one of three permanently social species in its genus and it occurs in arid, shrubby, open bush land throughout southwestern Africa (World Spider Catalog 2017) . Social *Stegodyphus* spiders live in colonies that contain up to several hundred individuals. These species are characterized by a female biased sex ratio, reproductive skew, and lack of pre-mating dispersal which results in among-sibling mating and therefore a high level of inbreeding.

Females cooperate in all colony tasks such as prey capture, nest building and web maintenance.

Colonies build dense silken nests around branches of spiny bushes, surrounded by two-dimensional capture webs. Group members are mostly found hiding inside the nest, emerging only for prey capture and web maintenance. When a prey is caught in the catching web, a few individuals emerge, run towards to prey and attack it. Hereafter others may emerge to join the attack, and together they drag the prey to the closest nest entrance where yet other group members join in for feeding. *Stegodyphus dumicola* colonies appear to be composed by individuals with distinct, consistent behavioural types and behavioural variation among individuals was shown to be stable over time (Klange 2014). Additionally, individual personality has been shown to be linked to task differentiation and collective foraging in *S. dumicola* suggesting that behavioural variation can shape social organization as well as individual and group fitness in this species (Grinsted et al. 2013; Lichtenstein et al. 2016a).

- Animal capture and maintenance

Ten colonies of *Stegodyphus dumicola*, collected in Ezemvelo KwaZulu-Natal (South Africa) during October 2015, were kept in a climate-controlled room (27°C) at Aarhus University during the experiments. The colonies were watered every three days and fed ad libitum twice a week with either common green bottle flies (*Lucila sericata*) or two-spotted crickets (*Gryllus bimaculatus*).

We randomly selected 30 individuals (all subadult females) from each colony and measured prosoma width (± 0.01mm) with digital callipers (total n = 300) as a measure for body size. Each individual was transferred to a 68ml plastic vial that

contained wooden skewers to provide anchor points for the web. The individual spiders were watered and fed with a common green bottle fly twice a week, once following the personality test, and once four days later. This schedule ensured a standardization of hunger level.

- Boldness assays

Each individual was tested three times over the course of three weeks, with seven days between consecutive assessments. To assess 'boldness', we placed an individual spider in a petri dish (90mm diameter) that was covered with a clear lid. After 60s of acclimatization time, the lid was removed and two short bursts of air were delivered via an infant ear-cleaning bulb. Spiders respond to this stimulus by retracting their limbs and remaining motionless (i.e. 'huddling') (Fig. 5.2).



Figure 5.2: A typical huddle response by a *Stegodyphus dumicola* individual after being exposed to a burst of air.

As measure of boldness, we measured the time it took them to return to movement, which is highly variable between individuals. This is a well-established procedure to quantify boldness that has been shown to yield a repeatable behavioural measure in

S. dumicola (Keiser et al. 2014) and related species *S. sarasinorum* and *S. tentoriicola* (Grinsted et al. 2013; Pruitt et al. 2013; Settepani et al. 2015). Furthermore, in *S. sarasinorum*, this measure correlates with emergence and attack times during prey simulation events in group (Grinsted et al. 2013). We observed individuals for 5 minutes after the aversive stimulus and recorded the time it took them to return to movement, defined as continuous motion of more than one body length. In the following analyses, we subtracted scores from the ceiling value of 300s so that bold spiders have higher scores. After concluding all three trials of the individual assessment, prosoma width was measured again.

- Group composition

Following the individual trials, groups of six spiders were assembled based on the mean time it took individual spiders to return to movement (which we further refer to as 'boldness'). This measure for boldness was found to be highly repeatable (see results).

From each colony, the nine highest and nine lowest scoring individuals were selected for the group trials. Six randomly selected bold spiders were used to create an all-bold group. Six randomly selected low scoring spiders constituted an all-shy group. The remaining six spiders (three bold, three shy) made up a group of mixed behavioural composition (total n = 10 colonies x 3 groups per colony = 30 groups). All groups were housed in petri dishes (90mm diameter) with pierced lid that contained wooden skewers in a square. Each group was allowed one week of acclimatisation time before the group assessment trials. Groups were fed and

watered twice a week, once following the group trials, and once four days later. Food was standardized (five common green bottle flies each feeding period) to ensure similar hunger levels among the tested groups.

- Group trials

To obtain measures of group behaviour and performance, we weighed a single fly (± 0.001mg) and placed it in the middle of the square formed by the wooden skewers inside the petri dish. Next, we observed the colony for ten minutes. During this period we noted the time it took for the first spider to attack, and the number of spiders that bit the fly. After one hour, the prey item was gently removed and weighed again. As a measure of feeding efficiency, we calculated the percentage decrease in prey body mass.

This procedure was repeated three times, with one week between trials. After the final trial, prosoma width of all spiders in the group trials was measured again. We also noted whether or not all spiders survived the group trials.

Following this final measurement, all spiders were reunited with the rest of their kin in the original plastic containers.

Statistical analysis

The repeatability of our measure for boldness was calculated following Lessells & Boag 1987, with standard errors acquired via the method described in Nakagawa & Schielzeth 2010. To assess whether or not mean boldness (i.e. the mean of the three boldness trials prior to the group experiments) correlated with body length (stickleback), body size, or growth rate (social spiders), we used Spearman's rank correlations since the data were not normally distributed.

To test the effect of group composition and time on the different parameters measured during the foraging trials, we used generalized linear mixed models with composition, experience (in weeks), and their interaction as fixed effects and group ID as random effect. For each of the tested parameters, we tested the normality of the residuals with a Shapiro Wilks test. When the residuals were normally distributed (the time it took the first fish to locate the food in stickleback, time until attack and log-transformed feeding efficiency in social spiders), we proceeded with a linear mixed effect model (lme). For group cohesion in stickleback, we used a Gamma distribution with inverse link, for the number of fish that fed and the number of spiders that attacked we implemented a Poisson distribution with log link, and for spatial learning (stickleback) and we used a binomial distribution with logit link.

We proceeded with a stepwise refinement of the models, omitting the non-significant effects. A p-value of 0.05 was used as threshold of significance throughout. For the random effect in both study systems (group ID) we provide an estimate of the variance ± SEM.

Finally, we conducted post-hoc Tukey Honest Significance Difference (HSD) tests to investigate differences between the categories of factors that emerged as significant in the models.

For the social spiders, we additionally investigated potential correlations between the behavioural group parameters and group growth rate as well as group mortality via Spearman's rank correlation tests. Differences in growth rate and mortality between different group types of social spiders (i.e. all bold, all shy, or mixed) were analysed with Kruskal-Wallis rank sum tests.

All analyses were performed in R 3.2.3, and we used package 'lme4' for the implementation of the glmm's.

V.III. Results

Three-spined stickleback

- Individual boldness

We found that, over the three trials performed before the group experiments (n = 200), boldness was highly repeatable ($r = 0.712 \pm 0.031$, $p < 0.001$). Mean boldness did not correlate with body length ($\rho = 0.107$, $p = 0.168$), nor did boldness in each of the separate trials (trial 1: $\rho = 0.110$, $p = 0.155$; trial 2: $\rho = 0.098$, $p = 0.207$; trial 3: $\rho = 0.079$, $p = 0.309$). Comparing boldness before (i.e. mean boldness) and after the

group trials confirmed the high repeatability of our boldness quantification over a longer time period ($r = 0.857 \pm 0.018$, $p < 0.001$). Mean boldness before and boldness after the group trials did not differ significantly ($W = 4879$, $p = 0.4821$; mean before = 139.25 ± 9.53s, boldness after = 130.99 ± 9.53s).

- Group behaviour

Group composition significantly affected all measured parameters, except spatial learning, with bold groups being less cohesive and having fewer members fed. Shy groups took a longer time to find the food than mixed and bold groups (Table 5.1, n = 96 fish distributed into 24 groups). Experience likewise affected all parameters but one (the number of fish that fed during the trial), with group cohesion, the time for the first fish to find the food, and successful location of the food all improving over consecutive trials. However, the interaction between both factors was not significant in any case.

Table 5.1: Sources of variation for the measured parameters in the stickleback groups. Significant p-values in bold.

	Group cohesion		Time until finding food		Number of fish that fed		Successful location	
Error distribution	Gamma		Gaussian		Poisson		Binomial	
Fixed effects	χ^2_{df}	p	χ^2_{df}	p	χ^2_{df}	p	χ^2_{df}	p
Composition	14.754_2	**0.0006**	39.943_2	**<0.0001**	16.54_2	**0.0002**	5.4843_2	0.064
Experience	24.73_3	**<0.0001**	36.477_3	**<0.0001**	1.105_3	0.798	10.987_3	**0.012**
Composition * experience	6.6481_6	0.355	6.4404_6	0.376	0.431_6	0.998	2.907_6	0.820
Random effect	var	SEM	Var	SEM	Var	SEM	Var	SEM
Group ID	0.128	0.074	449.4	7.495	0.278	0.108	0.012	0.022

Regarding group composition, pairwise comparisons revealed that the mean distance between fish in bold groups was significantly larger than that in mixed and shy groups (i.e. bold groups were less 'cohesive', mean difference between bold and mixed groups: 0.063 ± 0.009m, $p < 0.001$, and between bold and shy groups: 0.062 ± 0.009m, $p < 0.001$; Fig. 5.3a). Both the time it took the first fish to locate the food and the number of fish that fed during the trial differed significantly between all three group compositions (Fig. 5.3b and c). In mixed groups, the first fish located the food faster than in bold groups (mean difference: 43.875 ± 8.145s, $p < 0.001$), in which the first fish found the food faster than in shy groups (mean difference: 67.75 ± 8.145s, $p < 0.001$). More fish fed in mixed groups than in shy and bold groups (mean difference mix-shy: 1.031 ± 0.218, $p < 0.001$, mean difference mix-bold: 1.5 ±

0.218, p< 0.001). Finally, in the spatial learning assessment, there was no significant difference between the groups of different composition (Fig. 5.3d).

Group cohesion improved with experience. More specifically, at t_1, fish swam farther away from each other than in the trials during the three following weeks (mean difference week 1 – 2: 0.044 ± 0.012m, p< 0.001, week 1 – 3: 0.039 ± 0.012m, p < 0.001, week 1 – 4: 0.047 ± 0.012m, p < 0.001; Fig. 5.3a). The time it took the first fish to find the food improved significantly between the first two and last two weeks (mean difference week 1 – 3: 28.25 ± 15.945s, p = 0.007; week 1 – 4: 33.667 ± 15.945s, p < 0.001, week 2 – 3: 23.875 ± 15.945s, p = 0.031, week 2 – 4: 29.291 ± 15.945s, p = 0.005; Fig. 5.3b). The performance on the spatial learning task showed significant improvements between the second and the last week (mean difference week 2 – 4: 0.375 ± 0.133%, p = 0.024; Fig. 5.3d). The difference between the first and last week was marginally non-significant (0.333 ± 0.340%, p = 0.056). For the number of fish that fed post-hoc tests did not reveal significant differences over time (Fig. 5.3c).

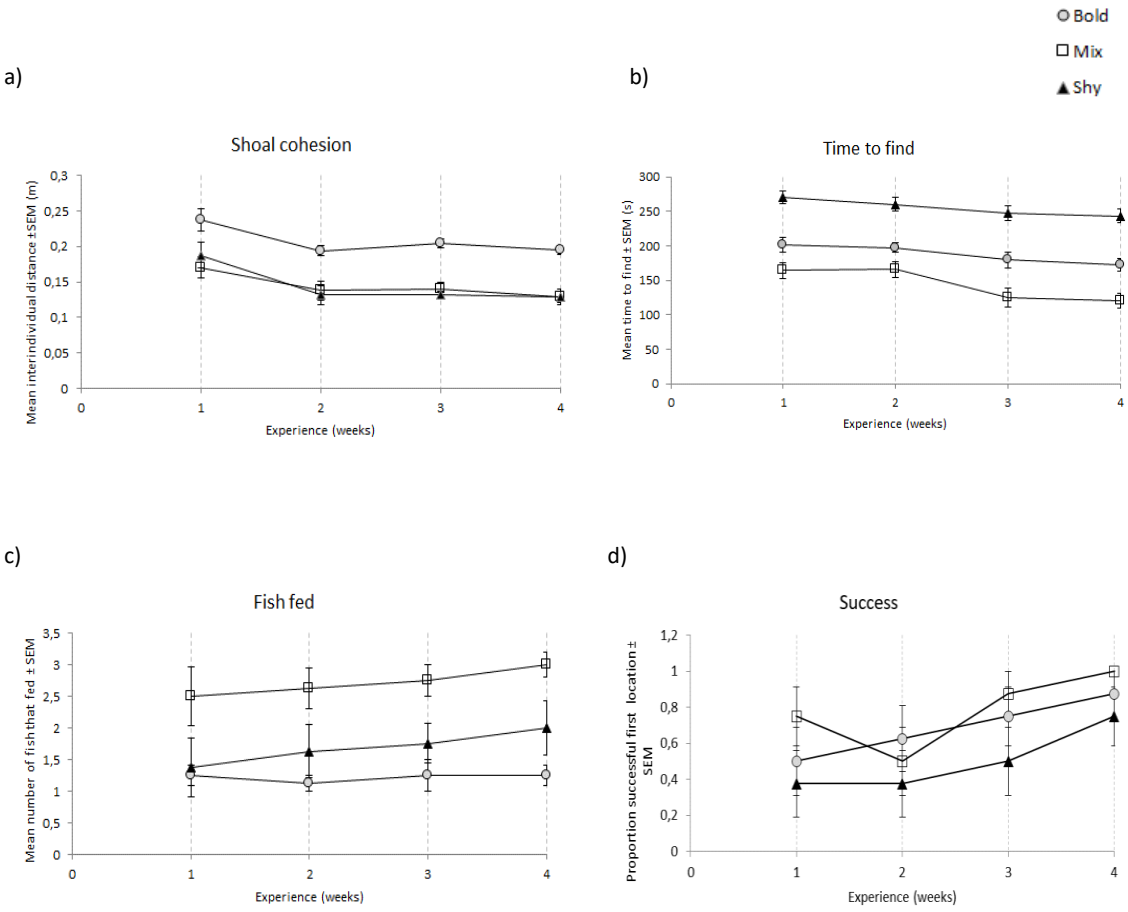


Figure 5.3: The effect of behavioural group composition and experience on a) group cohesion, b) time until the first fish found the food, c) the number of fish that fed, and d) whether or not the first fish successfully located the food at its first attempt. Black triangles denote shy group scores, grey circles bold group scores, and white squares mixed group scores.

Social spider

- Individual boldness

Individual boldness was significantly repeatable, both within and across colonies (overall repeatability: 0.653 ± 0.076, p<0.0001, see table 5.2).

Table 5.2: Repeatability of individual boldness within and across colonies of the social spider *Stegodyphus dumicola*, with standard errors and p-values (significant p-values in bold).

Colony	r	se	p
Overall	0.653	0.076	**<0,0001**
KZN01	0.775	0.061	**<0,0001**
KZN03	0.303	0.073	**0.005**
KZN06	0.705	0.068	**<0,0001**
KZN08	0.699	0.072	**<0,0001**
KZN09	0.811	0.050	**<0,0001**
KZN11	0.445	0.082	**<0,0001**
KZN12	0.723	0.066	**<0,0001**
KZN14	0.623	0.079	**<0,0001**
KZN15	0.620	0.076	**<0,0001**
KZN16	0.583	0.078	**<0,0001**

Boldness did not correlate with body size (prosoma width) as measured before (ρ = 0.0006, p = 0.991) and after (ρ = -0.015, p = 0.807) the individual personality trials. Growth rate (ρ = -0.075, p = 0.213) and web density (ρ = -0.091, p = 0.131) also did not correlate with boldness. The spiders from the original source colonies did not differ significantly with regards to mean individual boldness ($\chi^2_{df} = 8.659_9$, p = 0.469), but did show significant differences in growth rate ($\chi^2_{df} = 26.961_9$, p = 0.001) (Fig. 5.4). Finally, the distinct behavioural types (shy, average, and bold) did not differ in growth rate ($\chi^2_{df} = 3.988_2$, p = 0.136).

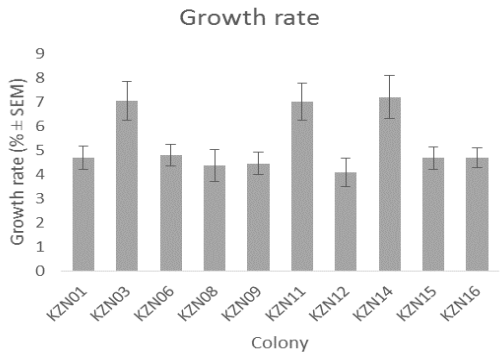


Figure 5.4: mean growth rate (% ± SEM) for the spiders from the original colonies.

- Group behaviour

Here, the behavioural composition of the groups also affected the measured parameters of group behaviour, i.e. time for the first spider to attack, the number of spiders attacking within ten minutes, and the feeding efficiency (Table 5.3). Experience affected only the feeding efficiency and the interaction between group composition and experience had no significant effect on any of the measures of group behaviour.

Table 5.3: Sources of variation for the measured parameters in the social spider groups. Significant p-values in bold.

	Time to attack		Number of spiders feeding		Feeding efficiency	
Error distribution	Gaussian		Poisson		Gaussian	
Fixed effects	χ^2_{df}	p	χ^2_{df}	p	χ^2_{df}	p
Composition	145.313_2	**<0.0001**	19.05_2	**<0.0001**	39.795_2	**<0.0001**
Experience	2.206_2	0.332	0.741_2	0.691	21.536_2	**<0.0001**
Composition * Experience	4.478_4	0.345	1.237_4	0.872	5.903_4	0.206
Random effect	Var	SEM	var	SEM	var	SEM
Group ID	464.5	6.906	0.028	0.053	0.200	0.141

Post-hoc tests showed that shy groups were significantly slower to attack than mixed and bold groups (mean difference shy-bold: 238.467 ± 15.78s, $p < 0.0001$; mean difference shy-mixed: 255.767 ± 15.78s, $p < 0.0001$) (Fig. 5.5a). The number of spiders feeding within the ten-minute observation window differed significantly between bold groups and the other two group compositions (mean difference bold-shy: 1.3 ± 0.181, $p < 0.0001$, mean difference bold-mixed: 1.467 ± 0.181, $p < 0.0001$) (Fig. 5.5b). Feeding efficiency was higher in mixed and shy groups than it was in bold groups (mean difference mix-bold: 1.541 ± 0.337%, $p < 0.0001$; mean difference shy-bold: 1.401 ± 0.337%, $p < 0.0001$) (Fig. 5.5c). Additionally, feeding efficiency was lower in the first week when compared to the last week (mean difference week 1-3: 1.159 ± 0.361%, $p = 0.001$) (Fig. 5.5c).

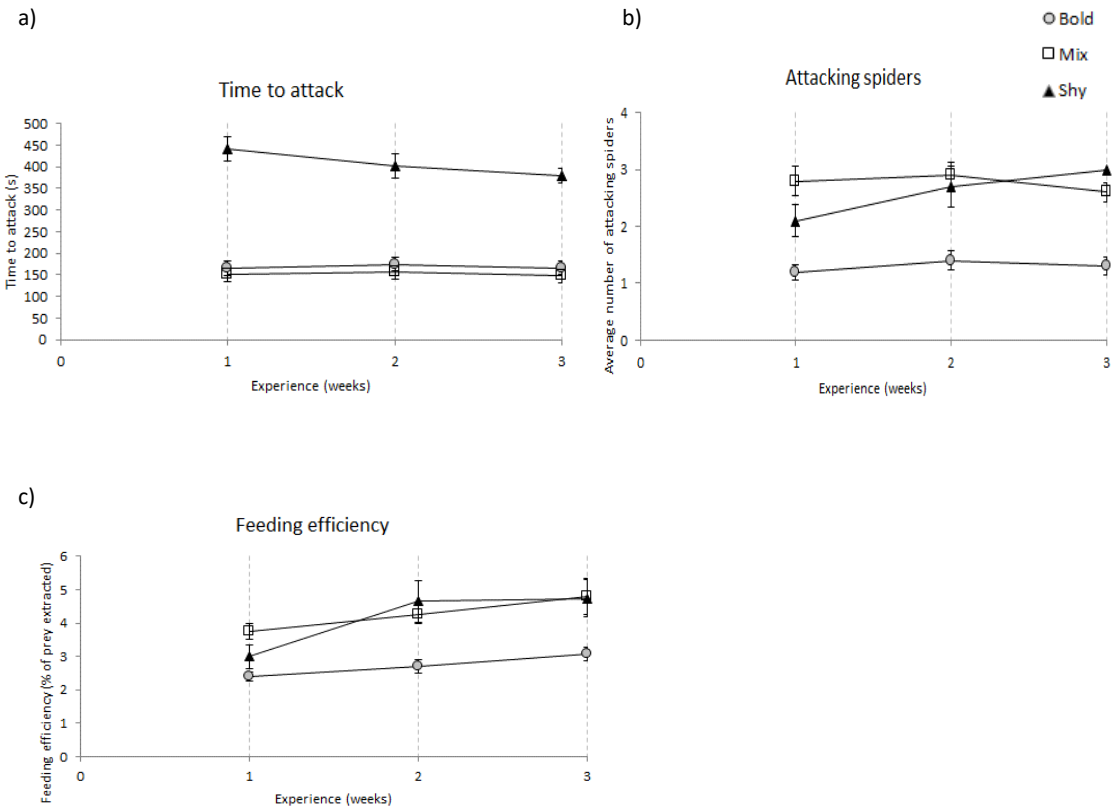


Figure 5.5: The effect of behavioural group composition and experience on a) time until the first spider attacks, b) the number of spiders feeding within ten minutes, and c) feeding efficiency as percentage of mass extracted from prey. Black triangles denote shy group scores, grey circles bold group scores, and white squares mixed group scores.

V.IV. Discussion

Our results suggest that groups of mixed behavioural phenotypes on the bold-shy axis perform better than homogenous groups in a collective foraging task. Mixed groups seem to possess the best of both worlds: the speed of finding food and feeding efficiency of bold groups, and the cohesion and sharing of shy groups.

Experience also improved performance for these parameters, but, importantly, much less so in a highly social species. We found no significant interaction between group composition and time for any of the quantified parameters in either species.

Composition

With the exception of the spatial learning task in stickleback, group composition influenced all aspects of group behaviour measured in both study systems. More specifically, mixed groups either outperformed both bold and shy ones, or they performed as well as the best performing groups of the two other possible compositions (i.e. all bold or all shy).

To explain the composition effect on group behaviour, producer-scrounger dynamics tend to be invoked (Dyer et al. 2009; Kurvers et al. 2010). Shy 'scroungers', so the reasoning goes, follow bold 'producers'. Shy individuals that follow bold ones are, according to this hypothesis, more likely to discover a new food patch. However, this rests on the assumption that, unlike shy individuals, bold ones are less likely to follow others of the same behavioural type. This does not necessarily seem to be the case in stickleback as both bold and shy fish prefer to associate with bold fish (Harcourt et al. 2009). A recent study, however, provides evidence that shy fish choose to follow other shy fish when given the option (Nakayama et al. 2016). As such, the support for the producer-scrounger hypothesis based on following preferences is ambiguous, but not only shoaling preference, might differ for different behavioural types, shoaling *tendency* might as well. Bold fish have been found to be less inclined to shoal than shy ones (Ward et al. 2004), and bolder fish, or those paired with a bolder

partner, are less likely to approach their partner (Jolles et al. 2014). Furthermore, in guppies shy fish tend to have more and stronger network connections (Croft et al. 2009). In stickleback, bold fish show a more uniform distribution of interactions whereas shy fish interact more, but these interactions are also more skewed towards a preferential association with a limited number of conspecifics (Pike et al. 2008). Furthermore, since boldness and aggression are often (but not always) linked in both stickleback (Huntingford 1976; Bell & Sih 2007) and the social spider *Stegodyphus sarasinorum,* a closely related species of our study species (Grinsted et al. 2013), bold individuals might be more likely to chase away unwanted followers. Based on our results, we suggest that mixed groups could have a network structure similar to the one found among shy individuals (see also Pinter-Wollman et al. 2016). In other words, we expect to see groups within the overall network with bold individuals at the centre of these within-network groups. Suggestively, in great tit flocks, groups of mixed behavioural composition do indeed show within-patch movement (a trait of exploratory individuals) while maintaining high group cohesion (a trait of less exploratory individuals) (Aplin et al. 2014)

Finally, in social systems where there is some degree of task differentiation, the link between individual personality and task participation or task aptitude can contribute to the success of mixed-behaviour groups (Pruitt & Riechert 2011; Wright et al. 2014). In *Stegodyphus sarasinorum*, for example, individual boldness is positively correlated with participation in prey capture (Grinsted et al. 2013). However, in *Stegodyphus dumicola* such a correlation has not been found (Keiser et al. 2014), but even in this species it has been found that bold individuals were more likely to attack prey for the first few days after colony establishment. This pattern, though, tapers off later (Pruitt & Keiser 2014). This result has been replicated, and early attackers

tend to gain more mass too, but bolder spiders lose mass faster and perish from starvation more quickly (Lichtenstein et al. 2016a; Lichtenstein et al. 2016b). The personality composition of a colony, specifically the number of bold individuals, is a relevant predictor for the colony's latency to attack prey and the number of attackers (Keiser & Pruitt 2014; Wright et al. 2015). This contrasts with our findings as fewer spiders attacked in bold groups in our study. There are, however, complex interactions with body condition, group size, and field versus laboratory conditions to take into account (Wright et al. 2015). .

Experience

In three-spined stickleback, all measures of group behaviour, apart from the number of fish that fed, were influenced by experience. More specifically, all affected aspects of group behaviour improved or stayed the same as individuals grew more experienced with each other and their environment. In social spiders only feeding efficiency improved over time. This, however, could reflect increased hunger levels due to a change in feeding regimen (see methods).

The effect of experience on group behaviour and performance is generally attributed to the facilitated use of social or environmental information. Earlier work has indeed uncovered that, as time passes and individuals get more familiar with each other, the efficiency of social learning and information acquisition increases. Guppies, for example, learn more efficiently from familiar demonstrators (Swaney et al. 2001) and prefer to follow familiar individuals (Laland & Williams 1997). Distinguishing types of available information has shown that three-spined stickleback primarily use social

cues and local enhancement, not public information, as sources of information gleaned from their environment (Harcourt et al. 2010). A promising avenue for future research is therefore to assess whether the transfer of each of these types of information is affected in the same way by increasing experience. Complementary to the more efficient use of information, experience can additionally exert its effect via the formation of a stable social network. In other words, after being introduced to previously unknown conspecifics, individuals might 'settle down' into a certain network position or social role. In the cooperatively breeding cichlid *Neolamprologus pulcher*, for example, behavioural type (in interaction with body length) affects the establishment of social ties and settlement into a certain social status (Schürch et al. 2010). In the same species, indirect phenotypic effects of an individual's behavioural type have been shown to affect social interactions that the focal individual does not partake in (Hamilton & Ligocki 2012). Finally, a reduced perception of risk, or a reduction in aggressive behaviour, could also contribute to the effect of experience (Utne-Palm & Hart 2000; Morrell et al. 2008).

Our results (the lack of experience effect in social spiders) suggest that the effect of experience could mainly be mediated through learning ability or familiarity. Whether the effect of familiarity is achieved via learned recognition or self-referent matching (see Atton et al. 2014), an initial phase could involve the establishment of a social network. Following this line of thought leads us to suggest that, at first, the social information present in a group of individuals previously unknown to each other is used to shape a social network. Later, the mechanisms used to acquire social information can be turned to other tasks such as finding food or following others. To further investigate this hypothesis, one could examine whether the time to achieve familiarity covaries with specific social systems. One could expect, for example, that

systems with a rigid structure (i.e. strong dominance hierarchy) achieve familiarity more rapidly. This could be due to the relatively quick establishment of such a social network, which also tends to remain stable for significant parts of the individuals' lives (McDonald & Shizuka 2013). This idea can be extended across evolutionary time, which would suggest that in certain social systems (e.g. eusocial colonies) the effect of familiarity on the behaviour and performance of that group becomes negligible (Berger-Tal et al. 2015) since in such system relatedness can act as an *a priori* form of familiarity.

Interaction and sociality

In contrast to our stated hypothesis that experience might be more influential in groups of mixed behavioural phenotypes, we found no evidence for interaction effects between behavioural composition and experience for any of the parameters in either study system. This strongly suggests that group composition and experience each affect group behaviour via different, independent routes. As such, our results deliver experimental evidence that the hypothesized mechanisms underlying the effect of group composition (i.e. producer-scrounger dynamics (Dyer et al. 2009) and, potentially, task differentiation) and the effect of experience (i.e. learning, see Atton et al. 2014) influence group behaviour independently. It also suggests that if social niche specialization takes place, it does so independently of behavioural group composition, which would provide support for a substantial role of behavioural plasticity of personality traits in group context (Laskowski & Bell 2014). And while behavioural consistency can be individually beneficial during collective foraging

(Ioannou & Dall 2016), social conditions have been shown to affect the repeatability of bold behaviour, with individually housed sticklebacks displaying more repeatable behaviour than those housed in groups (Jolles et al. 2016). In our case, boldness before and after the group trials was found to be repeatable, but this does not allow us to evaluate possible individual plasticity during the group trials. Therefore, future work could investigate the possibility of group-induced plasticity by testing individuals during the group phase of the experiments.

Behavioural composition is highly relevant in both our study systems, which suggests that, even though evolution might reduce the importance of experience, and specifically familiarity, for group behaviour in a foraging context (Berger-Tal et al. 2015), this does not affect the importance of behavioural variation for group behaviour. This, in turn, suggests that one evolutionary foundation of individual behavioural variation (or 'animal personality') could be its relevance for living in groups (authors' note: this does not imply group selection per se, but for more discussion on the topic, see e.g. Pruitt & Goodnight 2014; Gardner 2015; Grinsted et al. 2015; Biernaskie et al. 2016). If an individual benefits from being in a productive group, then it might be advantageous not to be the most common behavioural type since this could negatively affect group efficiency, and concomitantly, individual fitness. Such dynamics would lead to frequency-dependent selection, which has been suggested as cause for individual behavioural variation (Wolf & Weissing 2010; Wolf & McNamara 2012). It is important to note, though, that this requires a certain degree of plasticity. This seems inconsistent with the concept of animal personality, a key element of which is temporal consistency (Dingemanse & Réale 2005; Réale et al. 2007). However, personality and plasticity need not be at odds with each other. In fact, predictability in behaviour, or the degree of behavioural plasticity, has been

proposed as personality trait in itself (Biro & Adriaenssens 2013). This idea has been elaborated with the development of behavioural reaction norms, which view the behavioural response of an individual over an environmental gradient as the trait that should be considered in an evolutionary context (Dingemanse et al. 2010). Therefore, while some behavioural types might be more fixed in their behaviour (e.g. bold stickleback tend to be behaviourally consistent during collective foraging, see Ioannou & Dall 2016), behavioural plasticity may be induced in others through an environmental factor such as group living (Jolles et al. 2016). Such plasticity (perhaps only of a portion) of group members may lead to a more optimal behavioural composition of the group, possibly via a social niche mechanism (Bergmüller & Taborsky 2010). In the social spider *Anelosimus studiosus* optimal group composition does seem to be site-dependent (Pruitt & Goodnight 2014). Once this behavioural variation within a group is established, the potential costs of plasticity could lead to the 'entrenchment' of the associated behaviour, maybe via genetic accommodation, the process through which phentotypic variants that are initially environmentally induced become genetically determined via selection (Renn & Schumer 2013; Schlichting & Wund 2014). The possibility of genetic accommodation depends on the stability of the groups and their environment. In highly stable conditions, genetic accommodation is more likely to occur if plasticity is costly, whereas highly variable conditions could offset this cost, maintaining substantial plasticity in (part of) the group. Of course, this line of thought only holds for social species and suggests only one potential evolutionary mechanism underlying the occurrence of consistent inter-individual variation. In both non-social and social species, the phenomenon of animal personality likely has multiple intertwined evolutionary roots.

To address the decreased effect of experience (and probably familiarity, see earlier) on group behaviour in highly social groups, we propose the following scenario: organisms that have stepped onto the evolutionary path from solitary living towards (eu)sociality, through whatever mechanism it occurs, become more 'attuned' to living in group. Therefore, the effect familiarity has on several group-level processes diminishes. However, since groups of mixed behavioural types perform better than behaviourally homogenous groups, regardless of social system, behavioural variation is maintained, even in situations of high genetic kinship or cross-generational familiarity (e.g. communal breeding). We hypothesize that increasing sociality correlates with a decrease in the effect of familiarity on group performance since high relatedness among group members negates the potential effect of familiarity (Berger-Tal et al. 2015). This is a tentative hypothesis which requires further research. Experiments along the lines of those we performed in this study using (non-model) species with different social systems can prove useful. Likewise, similar behavioural experiments on species that display high within-species variation in social system, ranging from burying beetles (*Nicrophorus vespilloides*) , to house mice (*Mus musculus*) (for an overview of variation in intraspecific social systems, see Schradin 2013) would also be illuminating. Our study provides experimental evidence that group composition and experience are in fact two independent mechanisms that can both improve group performance, but the effect of familiarity is diminished in a highly social species. The use of two substantially different study systems allows us to hypothesize that these mechanisms are likely to be evolutionarily decoupled. Finally, our findings suggest a substantial role for behavioural plasticity in the evolution of animal personality and thus caution against using a point estimate of the repeatability of personality traits to understand the evolution of consistent inter-individual behavioural variation and its relation to group composition.

VI. Conceptual explorations:*

Bacteria, welfare, and mutualisms

* A part of this chapter (VI.I.) has been published as:

De Winter, G.; Stratford, J.P. & Chapman, B.B. (2015). Using bacteria to study consistent variation in individual behavior. *Behavioral Ecology.* **26**(6): 1465 – 1469.

VI.I. Using bacteria to study consistent variation in individual behaviour

Abstract

Two recent observations in behavioural biology have sparked great interest and have already yielded many novel and intriguing insights. Bacteria appear to live lives of unforeseen behavioural complexity, and the consistent behavioural variation among individual animals is often not 'noise' but turns out to be a highly relevant ecological and evolutionary feature in itself.

Research covering these two phenomena has proceeded largely in isolation and the rich behavioural lives of bacteria have not yet been studied with consistent interindividual behavioural differences in mind. Yet, the parallels between animal and bacterial behaviour that are increasingly being uncovered, as well as the particular characteristics of bacteria, point towards a new approach in the study of consistent individual variation in behaviour. Using bacteria can bring fruitful opportunities to the field and allows researchers to address questions that are very difficult to pursue using animal model systems.

Notwithstanding a few challenges, bacteria can provide an alternative study system that may elucidate several evolutionary and ecological aspects of consistent individual behavioural variation.

Introduction: Bacteria and behaviour

The study of consistent individual variation in behaviour, often referred to as animal personality, has blossomed in recent years. Individuals of the same species are often found to differ consistently in their behaviour, both within and between populations. To understand the evolutionary and ecological causes and consequences of this variation, the main study subjects are, perhaps unsurprisingly, vertebrates. These, however, are not the only organisms that exhibit variation in a range of behaviours. In addition to the recent call to include more invertebrate taxa in the study of animal personality (Kralj-Fišer & Schuett 2014), it is being increasingly recognized that bacteria and plants can show suites of complicated behaviours (Crespi 2001; West et al. 2007; Karban 2008).

There are tantalizing hints that bacteria provide a tractable study system to examine behavioural variation that can be integrated with animal behavioural ecology more readily than often assumed. Recent work on the intricate social lives of bacteria shows that there is a variety of behaviours that can be investigated (Crespi 2001; West et al. 2007). Additionally, high levels of within and between strain phenotypic diversity have been noted (e.g. Darch et al. 2015). In most cases, the behaviour of individual bacterial cells is pooled into the average behaviour of groups known as cheaters, persisters, etc. There are, however, some historical precedents in the study of phenotypic variation in bacteria at the level of individual cells (Kelly & Rahn 1932; Powell 1955; Spudich & Koshland 1976).

Behaviour in bacteria and animals

Interestingly, the focus of two of the earliest studies on individual bacterial phenotypic variation was growth rate (Kelly & Rahn 1932; Powell 1955). Along with recent work that suggests a connection between bacterial behaviour and growth rate or metabolism (Taylor & Zhulin 1998; Li et al. 2014) this offers a potential link with animal behaviour studies that tie individual behavioural variation to growth rate (Stamps 2007) and metabolism (Careau et al. 2008; Réale et al. 2010).

And there are more parallels. Environmental variability and food availability have been implicated as relevant actors in the development and maintenance of animal personality (Sih & Bell 2008; Luttbeg & Sih 2010; Wolf & Weissing 2010). Similar ideas have been proposed to understand phenotypic, and more specifically behavioural, variation among bacteria, whether the focus lies on chemotaxis (Frankel et al. 2014) or social behaviour (Park et al. 2003). More generally, resource partitioning, temporal variation and spatial structure all play a role in the emergence and preservation of bacterial phenotypic variation (Rainey et al. 2000).

An animal's or bacteria's environment is not composed solely out of abiotic ingredients. The presence of other organisms moulds behavioural variation as well. Antagonistic interactions, such as predation and parasitism, are associated with the rise and maintenance of animal personality (Dall et al. 2004; Dingemanse & Réale 2005; Bell & Sih 2007; Barber & Dingemanse 2010; Kortet et al. 2010). In the bacterial realm, corresponding findings concerning the effects of predation (Morgan et al. 2010) and phage infection (Bohannan & Lenski 2000; Buckling & Rainey 2002; Webb et al. 2004) on phenotypic variation have surfaced.

Organisms also interact with members of their own species. The social environment formed by these interactions can impact the presence, absence, and pattern of consistent individual behavioural variation. The social niche hypothesis stipulates that, whether through choice or coercion, individuals in a population adopt a social role, and, in doing so, influence the behavioural variation present (Bergmüller & Taborsky 2010). The social lives of microbes have been getting much attention lately (Crespi 2001; West et al. 2007; Xavier 2011) and behaviours such as cooperation and cheating are the focus of substantial research efforts, as are the circumstances that dictate when these behaviours occur and what affects their prevalence. Through manipulating group composition, one can test the social niche hypothesis (e.g. Laskowski & Bell 2014; Laskowski & Pruitt 2014). Bacteria provide a useful system to do this. For example, introducing a mutant strain that does not contribute to the production of common goods (e.g. siderophores in *Pseudomonas spp*.) establishes a proportion of 'forced' cheaters in a population. Non-cheaters have been shown to partially compensate for the resulting loss of common goods (Harrison 2013). Does this compensation alter their behaviour? Vice versa, does it affect the cheaters' behaviour?

A specific aspect of social behaviour involves group behaviour. In bacteria, quorum sensing and biofilm formation are well-studied examples. Group behaviour, and especially its relation to the behaviour of the constituent individuals, has only recently gained traction in research covering animal behavioural variation. Most animal studies relating group and individual behaviour have been done in eusocial insects and social spiders (e.g. Chapman et al. 2011; Hui & Pinter-Wollman 2014; Jandt et al. 2014; Keiser et al. 2014; Pruitt & Keiser 2014). Recently, however, phenomena such as shoaling and gregariousness have been studied in other species

as well (e.g. Magnhagen & Bunnefeld 2009; Jolles et al. 2014; Planas-sitja et al. 2015). In contrast, among bacteria the group rather than the individual is the level that attracts most attention.

Bacterial personality?

Variation is the bedrock of natural selection, and, as such, we expect to find it throughout life's kingdoms. Seeing behavioural variation in animals and bacteria should not be surprising. However, the mystery of 'animal personality' lies in the consistent interindividual differences. To maximize fitness in the wide variety of environmental conditions that many organisms experience during their lifetime, a high degree of behavioural flexibility would be very useful. Instead, individual animals tend to display a rather limited plasticity in behaviour and the behavioural differences among individuals are often conserved across contexts (Dingemanse & Réale 2005). Do we find this in bacteria as well? If so, can we use this to explore the phenomenon in ways that are difficult or unfeasible in animal systems?

Individual phenotypic variation in bacteria has not yet received a lot of attention, despite a few historical precedents (Kelly & Rahn 1932; Powell 1955; Spudich & Koshland 1976). Nevertheless, the occurrence of phenotypic heterogeneity and behavioural plasticity in bacterial populations is increasingly being recognized (Harrison 2013; Frankel et al. 2014), as is its importance in phenomena such as quorum sensing, biofilm formation and antibiotic resistance (for more details, see e.g. Drenkard & Ausubel 2002; Balaban et al. 2004; Cárcamo-Oyarce et al. 2015).

Despite parallels and complementary efforts, we should not be blind to the differences between animal and bacterial study systems. Animals are not bacteria. To infer that what holds for one also holds for the other is, at best, premature. A first step to explore the extent of the commonality between both is to address aspects of behavioural variation that have been studied well in one, but less in another system: Group-level behaviour and its relation to the behavioural variation among the constituent individuals in animals on the one hand, and consistent individual behavioural variation in bacteria on the other.

Studying behavioural variation implies selecting one, or several, behaviour(s) to assess. Rather than artificially trying to develop similar assays for animals and bacteria, a more fruitful approach might be to ask which behavioural axes are most relevant for the taxon being studied and design assays that can capture variation in these axes under different ecological conditions. For example, the previously mentioned common goods production by *Pseudomonas spp*. provides an opportunity to investigate the cooperative production of a resource in different social regimes (i.e. more or less cheaters). Alternatively, cooperatively hunting bacteria such as *Myxococcus spp.* (see e.g. Crespi 2001; Morgan et al. 2010) can be used to investigate prey capture efficiency at different prey availability rates and how this affects individual and group behaviour, as well as the link between both.

Challenges…

To investigate consistent individual behavioural variation, bacteria can provide an alternative, complementary framework. Bacteria are quite different from the animals often used in studies of consistent individual variation in behaviour, and yet, research in the past few decades has shown that several aspects of animal behaviour have analogues in the bacterial realm, especially social behaviours such as biofilm formation, cooperative foraging, division of labour, and cooperative reproduction (e.g. Crespi 2001, but see Dunny et al. 2008 for a cautionary note on interpreting and extrapolating these behaviours).

However, using bacteria to investigate individual variation in behaviour is not without its challenges. A key component in the definition of animal personality is consistency across time and contexts (Dall et al. 2004; Sih et al. 2004; Dingemanse & Réale 2005). Thus, a sturdy parallel with animal behavioural variation requires some degree of consistency in the observed variation in bacterial behaviour. To establish this, a specific behaviour needs to be quantified on several separate occasions. The short generation time of most individual bacterial cells renders this challenging. Alternatively, if one chooses the genotype as marker of individuality (Janzen 1977), this brevity of generation time can lead to a rapid accumulation of mutations, causing the starting genotype and the genotype tested on subsequent times to diverge. It is difficult and somewhat arbitrary to determine when this results in comparing two distinct individuals rather than one individual over time.

Studying individual behavioural variation in wild populations is challenging, even in animal systems (Dingemanse & Réale 2005). The relative lack of knowledge

concerning, and difficulty of observing, natural microbial populations make this even harder in bacteria.

…And opportunities

However, bacteria also present opportunities for behavioural ecologists, in some ways mirroring and extending the advantages of studying individual behavioural variation in invertebrates (see Kralj-Fišer & Schuett 2014) (Fig. 6.1). By taking advantage of the peculiarities of bacterial study systems, questions concerning the evolutionary and ecological dynamics of consistent individual behavioural variation that are hard to tackle in animal study systems can be addressed.

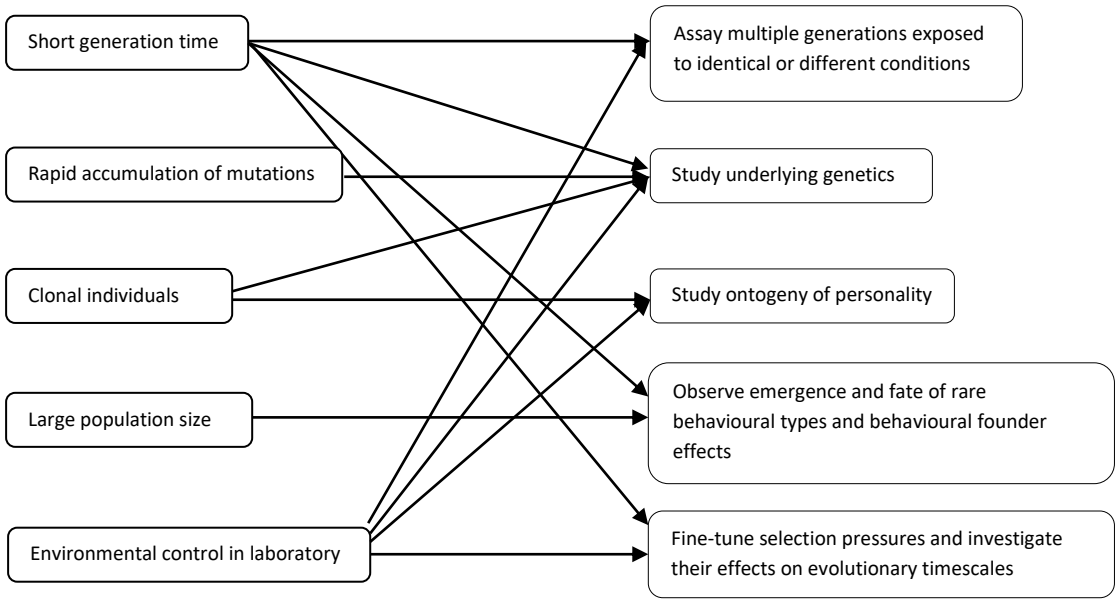


Figure 6.1: The peculiarities of bacterial study systems (left) can provide unique opportunities (right) for the study of individual behavioural variation.

The short generation time provides a prospect to assay behaviour across a number of generations that is rarely feasible in animals. Relatedly, the rapid accumulation of mutations could, if certain mutations can be coupled to behavioural differentiation, elucidate some of the genetic foundations of behavioural variation (for links between molecular mechanisms and phenotypic variation in bacteria, see e.g. Korobkova et al. 2004; Smits et al. 2006). These aspects of bacterial systems could be very helpful in addressing questions concerning the fitness consequences of behavioural variation. One could, for example, start with a bacterial population composed out of bacteria of similar behavioural type and observe whether or not variation among individuals increases in subsequent generations. If so, this provides a strong indication of the adaptive value (or at least of the absence of negative fitness consequences) of behavioural variation within a population, even when the individuals are exposed to identical conditions (see van Vliet & Ackermann 2015). This also provides the opportunity to establish whether or not a plateau is reached in the amount of behavioural variation present in a population, and, if so, how long it takes to achieve this plateau and how stable it is. The genetic tools and genomic information available for many bacteria offer the additional chance to investigate and manipulate the genetic roots of the observed changes.

Additionally, using bacteria allows researchers to establish and study populations of a size that is unachievable in animal systems. Observing the emergence and ultimate fate of rare behavioural types is more likely to occur in larger populations and being able to witness evolution in real time in large populations brings questions concerning the evolutionary dynamics of individual behavioural variation into view. Such large populations enable researchers to, for example, take several behaviourally similar and dissimilar, or behaviourally common and rare, samples of a

population and introduce these to a new environment. By letting these offshoots develop, the phenomenon of behavioural founder effects can be studied in greater detail than possible in animal systems. Is the duration of these effects different for different behavioural types? Are the effects identical for behaviourally similar founders? How do they influence the behavioural variation present after many generations?

Furthermore, the ability to establish a substantial number of clonal individuals can circumvent the challenge of studying developmental influences on individual behavioural variation in animals. After all, each individual animal has a unique developmental history (see the replicate individual design in Stamps & Groothuis 2010). Studying the ontogeny of personality differences can benefit greatly from having many clonal individuals at one's disposal. The ability to assess the behavioural evolution of individuals that possess the same genes and are exposed to the same tightly controlled environment allows researchers to address questions concerning both the behavioural development of individuals and of the behavioural variation in populations. Starting with many replicate individuals and allowing these to develop and reproduce asexually in identical and different environments is an excellent starting point to address questions such as: Do clones raised in identical environments undergo the exact same behavioural development (i.e. how significant is the contribution of 'molecular noise' to non-genetic individuality in homogenous conditions, see e.g. Korobkova et al. 2004)? Do clonal founder cells give rise to populations that display similar behavioural variation, if any, among their constituents? At which point in (evolutionary) time does behavioural divergence start? The same questions can be asked for clones in different environments, providing a way to way separate the relative genetic and environmental

contributions to the evolution of behavioural variation. The degree of environmental control and amount of clonal individuals required make bacteria a particularly suited study system to tackle these issues.

Finally, while studying bacterial behaviour in natural populations is difficult, the degree of environmental control possible in a lab setting is very high, which can reduce ecosystem complexity to a point where singling out and manipulating specific contributing factors becomes a viable option (Rainey et al. 2000). This means that in the previous experimental proposals the ecological context can be manipulated and controlled to a degree (and for evolutionary timescales) seldom achievable in animal study systems. As such, the context-dependency of the ecological and evolutionary development and maintenance of consistent individual variation in behaviour can in itself become a major and highly controllable topic of study.

Additionally, taking the study of individual behavioural variation beyond the paradigm study system, i.e. animals (and specifically vertebrates), is useful to discern whether or not there are general evolutionary and ecological principles at work (see also Kralj-Fišer & Schuett 2014). Are certain influences relevant only in some taxa but not in others? What's the role of organismal complexity, if any? How do specific characteristics of species affect the origin, maintenance, and evolution of behavioural variation? These are questions that are best addressed when data is available for taxa spread across the web of life.

Conclusion

Exploring novel study systems to investigate consistent variation in individual behaviour can prove beneficial and allows a broad perspective on this perplexing phenomenon. Bacteria are an underexplored study system in this field, but their short generation time, the genetic and environmental control, and the possibility of establishing large and clonal populations are all aspects of their biology that can help researchers address a suite of exciting questions.

VI.II. Animal personality and its implications for animal welfare

Abstract

The widespread discovery of consistent behavioural differences between individual animals has led to the development of the concept of 'animal personality'. Recently, this phenomenon has attracted substantial research attention and the influence of many environmental factors has been investigated in order to understand the evolutionary origin and maintenance of consistent variation in individual behaviour.

Furthermore, an animal's personality can affect aspects of its life, such as susceptibility to infection or the response to social stressors, which in turn impact its welfare. Here, farms and zoos are used to illustrate how consistent individual behavioural differences can affect animal welfare in these settings. Furthermore, the interplay between individual behaviour and growth, reproduction, the social environment, and human-animal interactions, can have repercussions for animal welfare. These repercussions are discussed and lessons and challenges for a 'personality-based' conception of animal welfare are outlined.

Finally, through the use of an integrated personality-welfare approach, new research directions are identified and some possible routes forward are proposed.

Introduction

The existence of individual variation in animal behaviour is a booming topic in behavioural ecology. It is becoming clear that the individual variability in behavioural expression by organisms is not necessarily a maladaptive deviation from a hypothetical optimal strategy, but rather a feature of interest in itself for those who seek to understand behavioural variation and its importance in ecology and evolution (Dall et al. 2004; Dingemanse & Réale 2005; Réale et al. 2007). An impressive body of research that investigates many aspects of individual behavioural variation (also known as 'animal personality') has been developed. Numerous features of an organism's existence have been found to play a role in maintaining and determining the specific behavioural pattern of animal personality, both at an individual and population level..

Little work, however, has explicitly looked at the importance of animal personality for animal welfare issues (for some exceptions, see e.g. Stoinski et al. 2012; Tetley & O'Hara 2016; Koolhaas & Reenen 2016). There are nevertheless reasons to think that individual behavioural variation should not be ignored in the practice of animal welfare. Here, I draw upon recent work in animal behaviour and welfare studies to briefly outline why the recognition of on animal's distinct combination of behaviours (i.e. its behavioural type (BT)) is important for animal welfare. Acknowledging that the needs of animals can vary both within and between species leads to the need for an individual-based conception of animal welfare.

There are many environmental factors that affect individual behavioural variation, both via evolutionary mechanisms and plasticity, such as predation, parasitism,

resource availability, and social aspects of life (Barber & Dingemanse 2010; Bergmüller & Taborsky 2010; Kortet et al. 2010; Luttbeg & Sih 2010; Réale et al. 2010). Shifting the focus to captive animals (i.e. in farms and zoos) negates the influence of many of these, as predation is virtually absent, parasitism monitored and controlled, resources standardized and usually plentiful, and several forms of competition less stringent. However, life in captivity comes with its own challenges, including drastically reduced ranges, limited interaction between and within species, increased exposure to human beings, crowded conditions, antibiotic use, etc. Many, if not all, of these can induce substantial amounts of stress in the animals under consideration. Recent research has shown interactions between BT and stress response (Carere et al. 2010; Montiglio et al. 2012; Koolhaas & Reenen 2016). A proactive coping style in lab mice and rats, for example, is often linked to higher aggression and lower behavioural flexibility in comparison to individuals that display a reactive coping style (Koolhaas et al. 1999; Carere et al. 2010; Koolhaas et al. 2010). Furthermore, the interaction between environmental cues and BT also affects stress response. For example, in three-spined sticklebacks (*Gasterosteus aculeatus*), aggressive and bold individuals respond to a predator stressor by breathing more rapidly than non-aggressive, shy individuals (Bell et al. 2010).

Farm and zoo animals are exposed to unique conditions (e.g. extensive interaction with humans or artificially-composed groups) that induce stress. Importantly, different BTs may respond differently to stressors. Therefore, it seems timely and relevant to apply an 'animal personality perspective' to the welfare of captive animals. Farms and zoos will form the main focus in what follows, but there are other areas in which similar considerations might apply (e.g. the pet industry,

laboratory animals, wildlife refuges, etc.), each of which has its own idiosyncrasies that should be taken into account.

First, I will explore how animal personality correlates with traits that are relevant in farm and zoo settings, such as growth, reproduction, and living in groups. Based on this, a framework for integrating animal personality into animal welfare consideration will be outlined, along with opportunities and challenges.

Reproduction

Reproduction is a factor of substantial relevance for a farmer, and personality can play a role here. Farmed mink (*Mustela vison*) that have been bred to be 'confident', 'timid', or without specific behavioural consideration in mind, show differences in their reproductive process. In general, confident mink reproduce faster and have the shortest gestation time. Mink bred without a specific behavioural goal have a longer gestation time, but lower kit mortality (Malmkvist et al. 1997). Again, there are some hints from more contemporary research that something similar holds beyond the farm. In zebra finches (*Taeniopygia guttata*), highly exploratory males perform more mating attempts, but this did not translate into increased reproductive success (Mccowan et al. 2015). Even when personality is linked to reproductive success, such as in great tits (*Parus major*) and common eiders (*Somateria mollissima*), the exact nature of this link is fluid and depends on environmental fluctuations in space and food for the former, where fast-exploring females and slow-exploring males have greater reproductive success in years following a harsh winter, while a reverse

pattern was observed following mild winters (Dingemanse et al. 2004). For eiders, the link between personality and reproductive success depends on predation. Nests of female eiders that rapidly formed breeding coalitions were predated more when predation pressure was relatively low, but nests of females that were slower in forming social ties more often lost nestlings when predation pressure was high (Öst & Jaatinen 2015).

In zoos too, the recognition of individual behavioural variation has a research pedigree that can be traced back to a time before animal personality became a widespread topic of enquiry in ecology and evolution. Perhaps not surprisingly, early efforts to assess the personality dimensions of zoo animals focused on great apes, specifically chimpanzees (Crawford 1938; Yerkes 1939). For a long time, these primates were the main study subjects in non-human personality research. Lately, however, the scope of such assessments has moved beyond primates, and has additionally begun to address potential ties with other aspects of animal life.

It is in considering breeding programs that factors affecting reproduction become particularly important. Behavioural parameters can be used as initial indicators for breeding status in cheetahs (*Acinonyx jubatus*), for example, where animals ready to breed score lower on a tense-fearful measure (Wielebnowski 1999). Reproductive success in itself can also correlate with behavioural traits. In captive black rhinoceroses (*Diceros bicornis*), dominant behaviour has a negative effect on breeding success for males, but a positive one for females (Carlstead et al. 1999). This sex-dependent effect has implications for the formation of breeding pairs. This is illustrated by the increase in reproductive success following behaviourally disassortative mating in captive cockatiels (*Nymphicus hollandicus*) (Fox & Millam 2014). Assortative mating based on personality traits related to anxiety has been

noted in mound-building mice (*Mus spicilegus*) (Rangassamy et al. 2015). Such personality-based mating and differential mating success has been proposed as a facilitator for reproductive isolation (Ingley & Johnson 2014).

Domestication and translocation

The previously-mentioned studies highlight that conditions on the farm and in the zoo are different from those in the wild, which can affect not only the development and maintenance of individual behavioural variation, but also its potential fitness effects, insofar as this applies in artificial conditions. Another lesson here is that an important factor in the consideration of captive animal personality, by definition, does not really have an analogue in wild populations: domestication (although urbanization could be considered as a limited analogue).

Charles Darwin noted that the process of domestication tends to result in a suite of traits that are often convergent among different domesticated species, a phenomenon that is known as the 'domestication syndrome' (Wilkins et al. 2014). Included in this syndrome are several behavioural traits, such as reduced aggression and increased social behaviour (Kaiser et al. 2015). Pleiotropy and other genetic interactions mean that the artificial selection pressures that constitute domestication can also engender trait correlations (Careau et al. 2010; Wiener & Wilkinson 2011). This is, of course, not limited to behavioural trait correlations, but can include links between behaviour, morphology, life history, and physiology (Careau et al. 2010). In zebrafish (*Danio rerio*) that have been selectively bred for

several generations to be ‘bold’ or ‘shy’, the bolder ones had a higher fast-start swimming performance and more elongated body shapes with higher caudal regions (Kern et al. 2016). Behavioural syndromes are widespread in nature as well (Sih et al. 2004; Dingemanse et al. 2007; Adriaenssens & Johnsson 2013), even at different levels of biological organization (Chapman et al. 2011b). The tangled genetic roots of these syndromes, both in wild and domesticated lineages, could act as constraints on possible trait correlations (Dochtermann & Dingemanse 2013). Selection for one trait can, in other words, affect other traits, which offers a potential explanation for the occurrence of the domestication syndrome.

Breeding programs are often initiated not just to ensure viable zoo populations, but also to create the opportunity for reintroduction in the wild. The success of a reintroduction may also depend on personality traits of the translocated individual. In captive-bred swift foxes (*Vulpes velox*), for example, boldness in captivity correlates with an increased chance of early death following reintroduction (Bremner-Harrison et al. 2004). However, it should be noted that relying on correlations between BTs and individual reintroduction success in one species to consider reintroduction efforts in another one might not always work. For example, boldness towards novelty correlated positively with survival after reintroduction in captive-raised Tasmanian devils (*Sarcophilus harrisii*) (Sinn et al. 2014). Even in wild individuals that are translocated, personality traits can predict survival after translocation. Common brushtail possums (*Trichosurus vulpecula*) that were more fearful during capture and handling were more likely to survive five months after being relocated (May et al. 2016). Similarly, personality traits often correlate with dispersal (e.g. Dingemanse et al. 2003; Duckworth and Kruuk 2009; Cote et al. 2010;

Myles-Gonzalez et al. 2015) or migratory tendency (e.g. Chapman et al. 2011a; Found & St. Clair 2016) in wild animals, as well as the subsequent settling success.

Social environment and group composition

Early work in the assessment of animal personalities on the farm was mainly focused on the link between the behaviour of individual animals and productivity. Pigs, for example, can be classified into resisting or non-resisting behavioural types (Hessing et al. 1994). This has consequences for productivity as pigs that are placed into groups of mixed behavioural composition gain weight more quickly and are less prone to develop pleurisy, a painful condition wherein the lungs become attached to the ribcage (Hessing et al. 1994). The finding that groups of mixed behavioural composition appear to convey benefits to the individuals in those groups when compared to animals in groups composed out of behaviourally more homogenous individuals has only recently been corroborated by work in behavioural ecology on the guppy *Poecilia reticulata* (Dyer et al. 2009) and the social spider *Stegodyphus dumicola* (Keiser & Pruitt 2014; Wright et al. 2015).

On many farms, animals are kept in highly-crowded conditions that could facilitate the spread of pathogens. Since behavioural traits often correlate with life history traits such as growth, metabolism, and immunity (Biro & Stamps 2008; Careau et al. 2008; Réale et al. 2010), individual behavioural variation can be tied to both susceptibility to infection (Koolhaas 2008; Barber & Dingemanse 2010; Hoverman & Searle 2016) and subsequent infectiousness (Araujo et al. 2016). This correlation

between behaviour and pathogen susceptibility or infectiousness can be based on both a direct (e.g. exploratory animals that are more likely to encounter pathogens or social animals that are more likely to spread disease) and an indirect (e.g. hormonal or genetic factors that influence both behaviour and immune competence) link (Koolhaas 2008; Barber & Dingemanse 2010). An interesting interaction with welfare is that positive affective states (i.e. the subjective experience of positive emotions) can boost immunity via, for example, interactions between the HPA-axis and the immune system (Boissy et al. 2007; Whitham & Wielebnowski 2013).

Group behaviour is an aspect of animal life that is also particularly important in a zoo setting, since animal groups there are often created artificially. In order to establish all-male gorilla (*Gorilla gorilla*) groups, for example, taking into account individual differences in aggression can mean the difference between harmonious peace when sociable non-aggressive males form bachelor groups, or unmanageable violence when males scoring high on aggression are grouped together (Gold & Maple 1994; Stoinski et al. 2004). An interesting point of consideration for wild-caught zoo animals from those species that display a well-developed culture, such as various primates and cetaceans, is that mixing animals from different cultures might lead to initial periods of acclimation difficulties. There is, however, very little research on what happens when animals from different cultural groups are put together in captive conditions, which provides an interesting avenue for further research.

Human-animal interaction

A final point to address, when considering behavioural variation among individual farm and zoo animals, is animal-human interaction. Whether it be caretakers, veterinarians, or visitors, captive animals are exposed to human beings to a degree that would rarely, if ever, be achieved in nature (although again urbanization could be a viable competitor, see the above discussion about domestication). Behavioural assessments of black rhinoceroses have resulted in a personality trait dimension termed 'friendly to keeper', which, unsurprisingly, was highest in captive-bred animals, and correlated with a faster approach towards a novel object (Carlstead et al. 1999). However, it is not just the captive animals that we should consider, but the one on the other side of the enclosure as well. The keeper's personality can also affect the interaction between a zoo animal and its caretakers. Keeper scores on traits such as conscientiousness and neuroticism affected their interaction with Bengal tigers (*Panthera tigris tigris*) (Phillips & Peck 2007). Including the visitors is relevant too. The size of the visiting crowd affected several behaviours in lowland gorillas. Larger crowds increased contact aggression in males, but not females (Stoinski et al. 2012). Interestingly, the response to variation in crowd size depended not only on sex, but also on the individual personalities of the gorillas. Male gorillas were more likely to display contact aggression when there were large crowds of visitors, and individuals of both sexes that scored high on the 'dominance' trait were more likely to feed under low crowd conditions in contrast to the apes with a low dominance score that were more likely to feed in higher crowd conditions.

Lessons and challenges for animal welfare

"The greatness of a nation and its moral progress can be judged by the way its animals are treated."

Mohandas Gandhi

The behavioural traits that compose animal personalities affect several facets of an animal's life, many of which can impact welfare. This realization has led to recent calls to consider animal personality in welfare assessments (Whitham & Wielebnowski 2009; Tetley & O'Hara 2012; Whitman & Wielebnowski 2013; Boissy & Erhard 2014).

However, assessing animal personality or animal welfare is not the most straightforward of tasks. Which behaviours to measure depends not only on what is logistically feasible (certainly when considering zoo animals of a certain size or in a specific habitat), but also on why the behaviour is measured in the first place (e.g. as a breeding predictor, or for reintroduction in the wild, or to ensure positive within-group interaction). Furthermore, behaviours that are termed the same (i.e. 'boldness') can be measured in numerous ways. How comparable are these, both within and across species (Carter et al. 2013)? Measuring multiple behaviours seems to be a good way to get a more complete view of an animal's personality, but this adds the complication of testing order (Bell 2013). What if measuring one behaviour affects the measurement of another one? How does one decide when to measure

which behaviour? On top of this, anthropomorphism and subjectivity are common challenges when trying to interpret animal behaviour or welfare (Broom 2007).

Nevertheless, there are foundations upon which we can build an integrated approach that encompasses animal personality and animal welfare. As the examples given above show, there are many behaviours that consistently differ among individual animals. Often, these correlate with other relevant biological factors for animals on farms or in zoos. With regards to welfare, there are some tell-tale signs that can aid in assessing whether an animal experiences something akin to pleasure (for a 'positive' conception of welfare), such as play or affiliative behaviour, or is suffering or stressed (for a 'negative' conception of welfare), such as stereotypical repetitive behaviour (Ijichi et al. 2013). Of course, animal welfare should pay attention to both these types of cues in order to promote the former and diminish the latter (Boissy et al. 2007; Boissy & Erhard 2014). Beyond avoiding injury, disease, stress, and aggression (i.e. reducing the negative dimension), which has been the focus for most welfare-related work this far, enhancing the positive dimension can also contribute significantly to the overall welfare of zoo and farm animals. A beneficial social environment and environmental enrichment are tools that seem particularly efficient in achieving this (Boissy et al. 2007). Since animal personality correlates with both social interactions (Gold & Maple 1994; Stoinski et al. 2004) and the response to environmental enrichment (Powell & Stoke 2008; Powell & Gartner 2011), accounting for consistent variation in individual behaviour can contribute not only to avoiding negative aspects of animal welfare, but also to enhancing the positive ones.

The effects of individual behavioural variation on various aspects of an animal's life that affect its well-being and are relevant in farm or zoo conditions give us a

powerful tool to take steps towards a more humane existence for animals. Some proposed general considerations are summarized in table 6.1, and a hypothetical example for both a farm and zoo animal is worked out in figure 6.2.

Table 6.1: Considerations for animal welfare that take into account animal personality.

Reproduction
Farms Promote mass gain, fast reproduction Zoos Breeding programs, genetic diversity, reintroduction
Welfare considerations
- Behaviourally (dis)assortative mating and subsequent reproductive success - Behaviour-growth and behaviour-reproduction trait correlations - Individual responses to crowding / stress / group composition that affect mating, reproduction, and growth
Social environment / group composition
Farms Increased group size, crowding, disease transmission Zoos Artificial groups of (sometimes) wild-caught animals, clash of cultures (?)
Welfare considerations
- Behaviour-immunity correlations - Individual response to group size and crowding effects - Different BTs may occupy different social niches / hierarchical positions - Resource / space monopolization by specific BTs - BT-specific competitive interactions - Amount of food sharing / grooming / alloparental care may depend on behavioural group composition
Human-animal interaction
Farms Farmers or modern robotic equivalents, visitors, equipment Zoos Keepers, visitors, veterinary checks
Welfare considerations
- Individual differences in fear / neophobia / stress response - Habituation (both its speed and degree) may depend on BT - Origin (wild-caught vs. captive-bred) can affect BT and correlated interactive behaviours and tendencies - Individual differences in learning capability

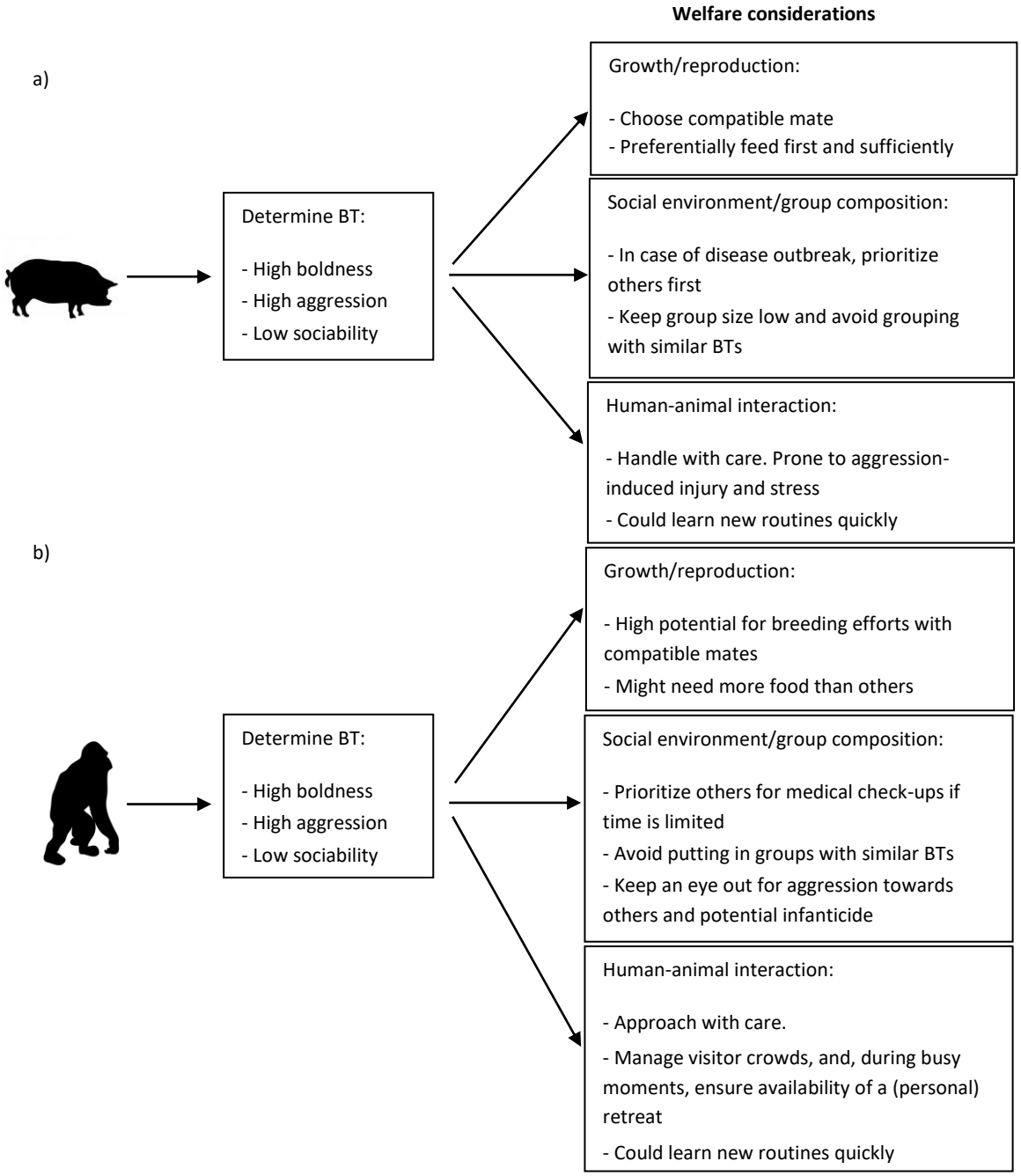


Figure 6.2: A hypothetical example from the farm (a) and the zoo (b) of how animal personality could be used in the consideration of animal welfare of both a focal animal and the animals it lives with. After assessing an animal for preferably multiple behaviours, the correlation of BT with several traits that can affect welfare allows farmers/zookeepers to implement measures to strive for optimal welfare of the focal (and other) animals. Note that this is hypothetical and that the development of real guidelines is likely species- and context-specific and requires additional research (see text for more detail).

To take full advantage of the opportunities animal personality provides for the improvement of animal welfare, there are several research avenues that deserve more attention. A proper personality assessment of an individual animal requires multiple measurements of multiple behaviour, repeated over time. For zoo animals this might be more achievable than on large scale farms. Still, developing easily implementable behavioural rating scales, and perhaps (partially) automated approaches, is a pursuit that should prove useful for a wide range of conditions. Advances made in a controlled zoo setting might eventually make their way into evolutionary and ecological studies of animal personality in wild populations. Alongside, some thought ought to be given to the standardization of behavioural measurements, certainly within species, in order to ensure comparability. Between-species differences should not be ignored, however. Both BTs and correlations between behavioural and other traits can be species-specific, as earlier examples indicate.

Carefully-maintained breeding records and well-established pedigrees are often quite useful as a starting point to unravel the genetic basis for behavioural traits and the relative contribution of plasticity (Dingemanse et al. 2010; Dochtermann & Roff 2010; Dingemanse et al. 2012). As such, farms and especially zoos could be a hidden treasure trove of unexplored information for evolutionary biologists, certainly when combined with rigorous behavioural assessments. Of course, rearing environment and conditions will have to be taken into account in order to paint an accurate picture of the genetics underlying animal personality. But here too, farms and zoos offer considerable amounts of possible control. Also, zoos allow researchers to study animal personality in animals that are hard to keep in the lab without having to deal with the logistical difficulties of doing it in the wild. Farms are prime locations to

study how domestication affects behaviour and how selection for specific non-behaviour traits can influence behavioural traits, trait correlations and the formation or disassembly of behavioural syndromes.

Conclusion

While the term 'personality' comes loaded with connotations and anthropomorphism, recent work has demonstrated that inter-individual behavioural variation is an important feature of animal life. Since the bulk of the research performed on the phenomenon of animal personality is fairly recent, the full extent of its potential has yet to be revealed. It is already clear that variation in individual behaviour is a pervasive feature that spans many taxa and is relevant for numerous biological processes ranging across space and time.

Animal personality correlates with several aspects of animal life that affect welfare considerations for captive animals. This suggests that adopting an 'animal personality perspective' could lead to new insights and progress. Here, I have briefly discussed how individual behavioural variation is linked with factors such as reproduction, social interactions, and human-animal interactions. All of these are pertinent in farm and zoo settings, where animal welfare is (or should be) a prime concern. Next, I have outlined challenges and lessons for animal welfare emerging from this animal personality view.

Overall, the conclusion is clear: the welfare of individual animals depends not only on their circumstances, but on their personality as well. Different animals within a

species react differently to the conditions they find themselves in, which means that an accurate assessment of an animal's welfare is not exclusively a function of its circumstances, but includes its personality as well.

VI.III. Animal personality in mutualistic interactions

The long reach of animal personality

The concept of animal personality, or the existence of consistent individual variation in behaviour, has quickly become a topic of great interest in behavioural ecology (Réale et al. 2007). The majority of studies in this area focus on explaining the origin and maintenance of this phenomenon. Lately, however, its large-scale evolutionary and ecological consequences have also attracted attention (Wolf & Weissing 2012). Processes that shape patterns of biodiversity, such as dispersal (Cote et al. 2010; Myles-Gonzalez et al. 2015) and speciation (Ingley & Johnson 2014), are influenced by the personality composition of animal populations. Following these findings, proposals have been made to integrate the concept of animal personality in other fields of biology, such as speciation (Ingley & Johnson 2014), biogeography (Canestrelli et al. 2015), and food web ecology (Toscano et al. 2016). Yet, despite the growing interest and increasing number of empirical studies, as well as theoretical and conceptual work in the field, some potentially relevant aspects have not been getting much attention thus far.

Whilst antagonistic interspecific interactions such as predation and parasitism are often studied in an animal personality context (Bell & Sih 2007; Dingemanse et al. 2009; Barber & Dingemanse 2010; Kortet et al. 2010; Pettorelli et al. 2011; Poulin 2013; Kekäläinen et al. 2014), the 'personalities' under consideration are virtually always those of the predated or parasitized individuals (for some rare exceptions,

see Pruitt et al. 2012; Mcghee et al. 2014). Animals, however, are embedded in a matrix of interactions, both within- and between-species. While within-species interactions such as intraspecific competition and mate choice have found their way into research concerning animal personality (Bergmüller & Taborsky 2010; Ingley & Johnson 2014), an important subset of between-species interactions, facultative mutualisms, have been neglected thus far.

The power of others

The 'social niche hypothesis' exemplifies how conspecifics influence an animal's personality and population-level patterns of consistent individual variation. The hypothesis postulates that the social roles animals perform in a population can be a key element in both the ontogenetic development and evolutionary origins and maintenance of animal personality (Bergmüller & Taborsky 2010).

Competition for food, resources, mates, and so on, can lead animals to choose, or be pushed into, specific social niches. This can then affect the behaviour of individual animals. Additionally, animals of a certain behavioural type could be more prone to occupy certain social niches. As this suggests, determining the causal relationship is difficult. Does the social niche underlie consistent individual behaviour (i.e. personality), or is it the behaviour that drives the choice of niche (or settlement into a forced niche)? Support for both options exists. In some cases social interactions appear to induce variation in behavioural types (Laskowski & Pruitt 2014; Modlmeier et al. 2014), whereas in others behavioural type predicts social behaviour (Laskowski

& Bell 2014). It is interesting to note that the behavioural type of individuals that are not directly involved in the interaction that is taking place can also affect the social behaviour of the interactors. An example of this is how the personality of a sub-adult male can shape the interactions between dominant males and females in the cichlid *Neolamprologus pulcher* (Hamilton & Ligocki 2012).

When adopting an interspecific perspective, antagonistic interactions such as parasitism and predation have been shown to affect the development and maintenance of consistent individual behavioural variation.

To increase the odds of transmission, parasites are often able to manipulate the behaviour of their host(s) (Poulin 2013). However, the behaviour of potential hosts can also affect which parasites, and how many of them, it encounters. Active birds and exploratory primates, for example, carry a larger parasite burden than their less active and less exploratory fellows (Garcia-Longoria et al. 2014; Mccabe et al. 2015).

Behavioural traits can be linked in population-level behavioural syndromes (Sih et al. 2004). Parasite infection may affect several different behaviours and can thus lead to the development of novel syndromes. Existing syndromes could also be 'decoupled' if parasites do not equally affect all behaviours incorporated in the syndrome (Barber & Dingemanse 2010; Poulin 2013). Finally, acting as parasite host influences other aspects of an organism's life history, such as growth rate, fecundity, and immune function. Each of these has, in itself, been proposed to affect behavioural traits (Barber & Dingemanse 2010; Kortet et al. 2010). All this illustrates the complexity of the interaction between parasitism and animal personality.

Predation is another antagonistic interspecific interaction that is often studied in the context of animal personality's ecology and evolution. If predation risk is present, it is

generally perceived as a substantial contributing factor in the occurrence and patterning of behavioural types and syndromes in the prey population (Dall et al. 2004; Dingemanse & Réale 2005; Pettorelli et al. 2011).

Like parasitism, predation can influence the occurrence and specifics of behavioural syndromes. Predation risk induces a consistent population-level correlation between boldness and aggression, and boldness and activity, in three-spined stickleback (*Gasterosteus aculeatus*) for example (Bell & Sih 2007; Brydges et al. 2008).

At this point, however, there is already an omission to note: studying animal personality in the context of predation (or parasitism) does so almost exclusively from the prey's (or host's) perspective, but the predator-prey interaction does not exclusively flow in that direction (Sih et al. 2012). Frequency-dependent selection is often invoked as possible factor in the evolutionary maintenance of personalities in animal populations (Wolf & Weissing 2010; Wolf & McNamara 2012). Since predator-prey relationships tend to display frequency-dependent dynamics, it is not unreasonable to suggest that the presence, density, and type of prey could affect the behavioural variation in predator populations (similar considerations apply for host-parasite interactions). Experimental support for these two-way personality dynamics, however, is very scarce. One recent study has indeed found behavioural reciprocity between three-spined sticklebacks and one of their predators, the northern pike (*Esox lucius*), where the time a predator needed to first notice the prey affected the prey's response, which, in turn, affected the predator's attack strategy (Mcghee et al. 2014). Further research that investigates both sides of the predator-prey or host-parasite interaction could be very enlightening (Lima 2002; Pruitt et al. 2012).

Expanding the scope

Beyond uncovering the predator's or parasite's perspective, there is a whole set of interspecific interactions that is being ignored, namely those that are not antagonistic. Facultative mutualisms are pervasive throughout the tree of life and important to understand many aspects of biodiversity and biogeography (Bronstein 2001). Very few studies have addressed how the animal personalities on both sides of such non-antagonistic interspecific interactions influence these interactions (for a rare example, see Wilson et al. 2014).

Since antagonistic interactions such as predation and parasitism are already known to affect, and be affected by, the personality of their participants, interspecific interaction is a known influence on animal personality. Taken together with the finding that individual variation in intraspecific cooperative behaviour (e.g. cooperative breeding or hunting) is often linked to consistent individual variation in other behavioural dimensions (for an overview and examples, see Bergmüller et al. 2010), this provides tentative support for the hypothesis that facultative interspecific mutualistic relationships can be relevant influences on consistent individual variation in behaviour.

An additional point of consideration is that mutualistic relationships can open up new niches for their participants through, for example, allowing the exploitation of previously unattainable resources, or an increased tolerance for certain environmental parameters. As such, non-antagonistic interspecific relationships can directly impact biogeographical patterns (Louthan et al. 2015). For example, the presence of mutualistic fungal endophytes can mitigate the effect of drought stress

on the grass *Bromus laevipes*, and, in doing so, can expand the range of the grass for several thousand kilometres into drier territory (Afkhami et al. 2014). A more indirect effect is that some personality types could be more or less likely to engage in such interactions. This, in turn, would mean that, due to differential engagement in interspecific interactions, different personality types of both interacting species can show different biogeographical distributions. Such effects on large scales of space and time highlight the relevance of including non-antagonistic interspecific relationships in the recent developments towards an integration of animal personality with other fields of biological enquiry such as biogeography (Canestrelli et al. 2015) and food web ecology (Toscano et al. 2016).

Finally, integrating non-antagonistic interspecific interactions in animal personality research might elucidate how consistent behavioural variation is maintained in certain contexts. Frequency-dependent selection is one mechanism that is proposed to maintain variation in behavioural types in a population (Wolf & McNamara 2012). Yet again, it is in the context of antagonistic interactions, such as predation, parasitism, and competition, that this mechanism tends to be invoked. A recent study, however, shows that a balancing polymorphism can be maintained as a result of a symbiotic relationship (Watanabe et al. 2016). In this investigation, Watanabe et al. demonstrate that a colour polymorphism in the mugworth aphid (*Macrosiphoniella yomogicola*) is maintained through the attendance of the ant *Lasius japonicas*. Through quantifying the correlation between ant attendance and aphid colour morph proportion, they showed that ants strongly favour aphid colonies with intermediate proportions of the two colour morphs. Though the exact mechanism still requires elucidation, perhaps a similar frequency-dependent process can maintain a polymorphism in consistent behaviour in one or more non-

antagonistically interacting populations of different species. In other words, the interaction between populations of potential symbiotic partners could be a factor that evolutionarily sustains individually consistent behavioural polymorphisms via frequency-dependent mechanisms (Wolf & McNamara 2012).

Studying interacting personalities in a changing world

Mutualistic interactions are often relevant aspects in ecosystems. However, environmental changes can put pressure on these interactions (Toby Kiers et al. 2010; Fortuna et al. 2013; Revilla et al. 2015). Global warming, for example, can unhinge existing partnerships and promote novel species assemblages (Toby Kiers et al. 2010; Ordonez et al. 2016) with the potential for new mutualistic interactions. Vice versa, biotic interactions can affect how species respond to climate change and alter existing species interactions (Alexander et al. 2016). Studying how different behavioural types (or 'personalities') affect these interactions is therefore a timely pursuit.

One approach is to extend and amend the experimental macroecology approach advocated by Alexander at al. (2016), based on testing the effects of different potential competitors on functional traits and integrating these findings in predictive demographic range models, beyond competitive plant interactions, as the authors themselves suggest. An appropriate choice of study system, such as short-lived vertebrates or invertebrates, would allow an experiment that, after establishing whether or not personality traits correlate with aspects of the mutualistic

relationship (i.e. duration, willingness to engage,…), relocates part of a population that usually develops a symbiotic relation with another species, thereby separating these individuals from their potential partners. In their new habitat, they would then be exposed to new potential interspecific partners. Then, comparing the subpopulations in the old and new locale could help to assess not only the possible consequences of climate change, but also begin to resolve queries regarding the contribution of old and new partner species to the survival and reproduction of the target species, how easy or hard it is for the target species to engage in non-antagonistic interactions with a novel species, and whether the response to losing and/or gaining partners is tied to behavioural type. By designing appropriate control conditions, one could separate the effects of personality from those of local adaptation. (figure 6.3 provides a decision tree to guide experimental design, and figure 6.4 a hypothetical lab-based example based on an ant-aphid mutualism that incorporates several possible pathways illustrated in figure 6.3).

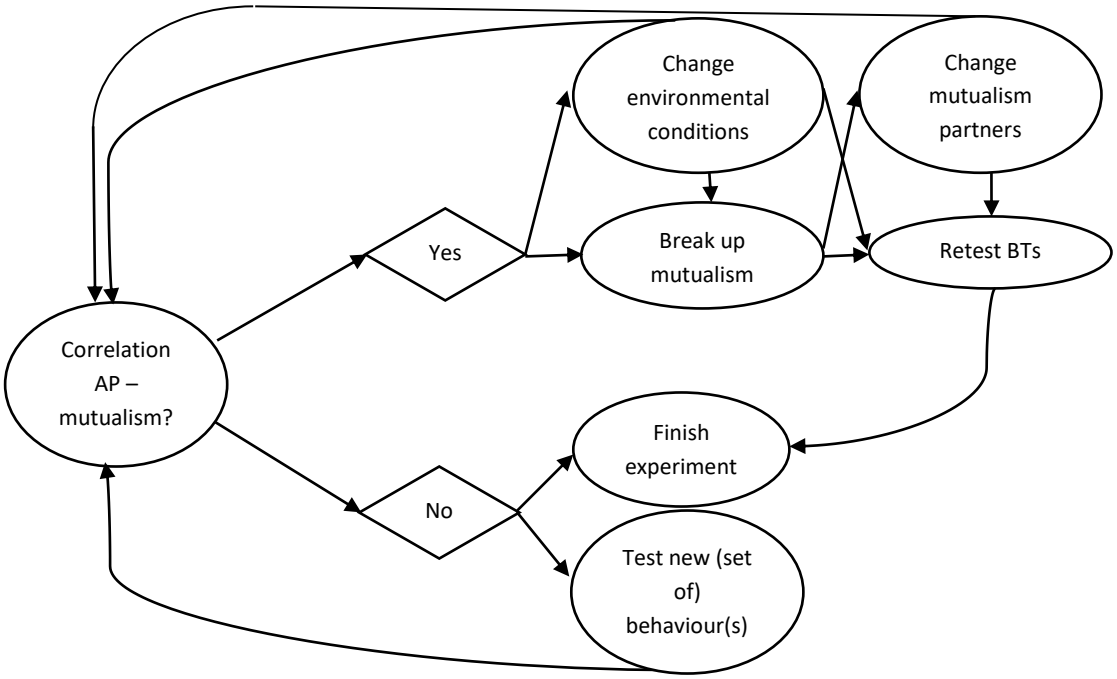


Figure 6.3: Potential experimental pathways to study animal personality in the context of non-obligatory mutualisms (AP: animal personality; BT: behavioural type).

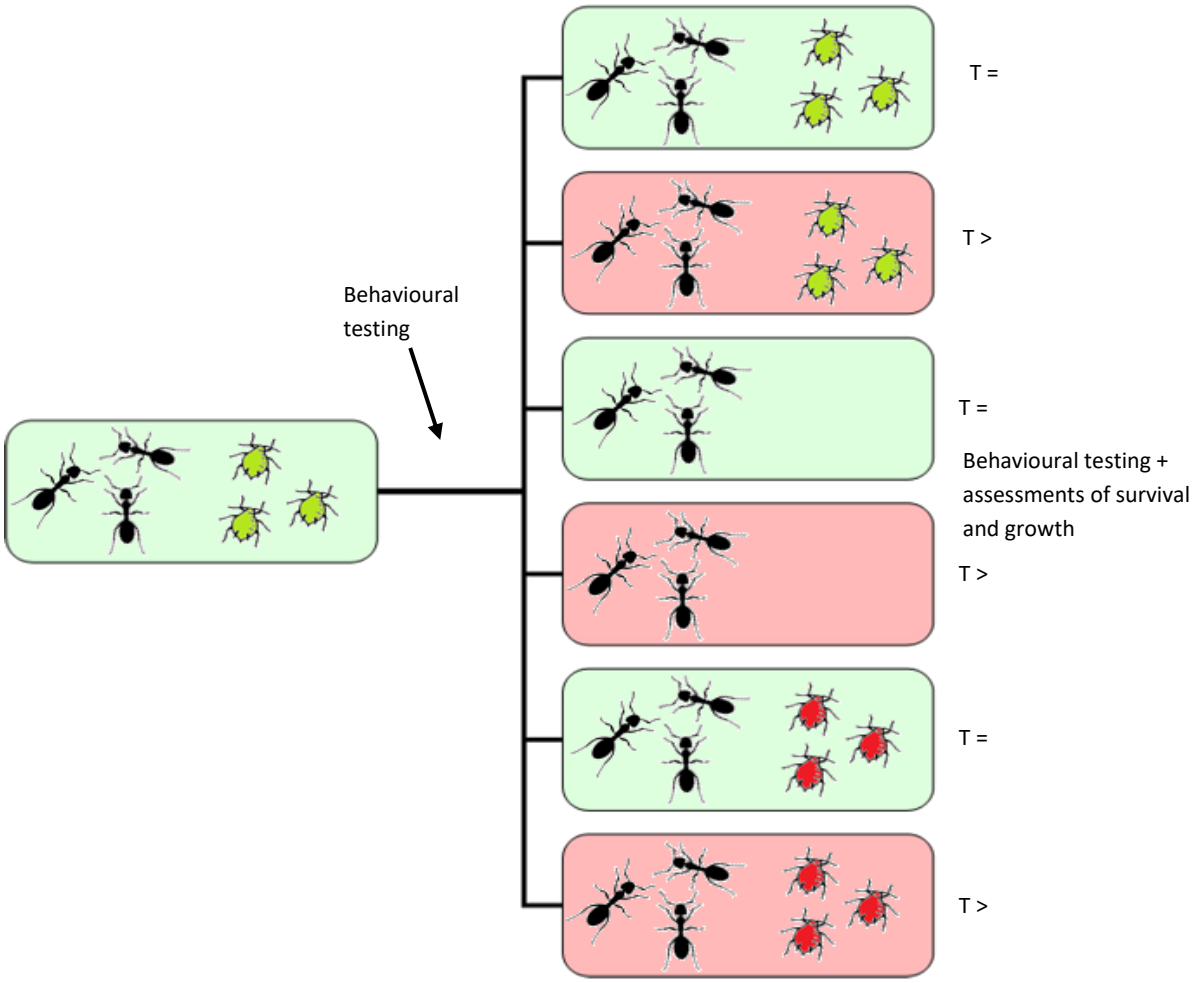


Figure 6.4: Hypothetical experimental set-up. The starting condition is a known mutualism (left). After behavioural testing, at individual and/or colony level, individuals from the colonies, are subjected to novel conditions (right). From top to bottom (green aphids: same mutualism, red aphids: potential new mutualism; green background: same temperature; red background: increased temperature): 1) same mutualism, same temperature (i.e. control); 2) same mutualism, increased temperature; 3) no mutualism, same temperature; 4) no mutualism, increased temperature; 5) new mutualism, same temperature; 6) new mutualism, increased temperature. After, and perhaps during, these new conditions, colonies can be assessed for, for example, survival, growth, and behaviour. This allows testing the effect of temperature on a known mutualism, on the loss of a mutualism, and the potential gain of a novel mutualism, but, importantly, by taking into account behavioural types and composition, the effect of behaviour and its interaction with temperature change can also be assessed.

Another approach would be to use natural laboratories (Woolbright et al. 2014) where, due to e.g. a recent radiation or habitat partitioning caused by human or environmental factors, populations of interacting species have been separated into sub-populations with different proportions of potential mutualistic partners of both species . Alternatively, the use of mesocosms can provide a means to achieve this set-up in semi-natural conditions. Performing behavioural and environmental surveys of the subpopulations involved could then illuminate some of the unknowns concerning the environmental and behavioural factors that influence, and are influenced by, non-antagonistic interspecific interactions. For example, a recent study used mesocosms to investigate the effect of habitat choice (surface vs. burrow) on a cleaning mutualism between crayfish (*Procambarus clarkii*) and an annelid (*Cambarincola barbarae*), where crayfish in burrows lost most of their mutualistic annelid partners (Ames et al. 2015). Including assessments of consistent individual behaviour in a similar set-up could provide information on the effect of personality on mutualistic interactions, as well as the potential effect of environmental factors.

Whether performing lab-based experiments, translocations in (semi-) natural conditions, or observations in natural laboratories, there are some key considerations to take into account, such as the specifics of the study system, the behaviours to assess, potential threshold effects, and the time-scale being used (see Box 1 for a summary).

Box 1: Key considerations in the study of interspecific non-antagonistic interactions in an animal personality context.

- *Study system specifics*: This covers a wide range of characteristics that can affect the behaviours measured and how the experimental individuals respond to the imposed changes. In an invertebrate or ectothermic study system, for example, a rise in temperature is likely to elicit a change in activity level, which, in turn, can affect both intra- and interspecific social behaviour, such as the chance of meeting a new mutualistic partner or the cost of this relationship for one or both partners (in the context of the example, perhaps more active ants are more demanding resource-wise on their aphid partners). Such environmental co-variables, which are much less controllable in natural conditions, may affect different study systems in different ways and therefore require the inclusion of appropriate control conditions whenever possible.

- *Behaviours to assess*: Ideally, a suite of behaviours would be tested. However, this is not always possible due to time or logistical constraints. Well-known study systems may have a strong literature record detailing prior work that could be used as a guideline. In other cases, behavioural experimentation may be required to find repeatable behaviours that display consistent individual variation and are relevant in the context of interspecific interactions. Furthermore, depending on the study system, one should take into consideration the biological level at which behaviour is quantified (e.g. individual vs. colony, see (Chapman, Thain, et al. 2011; Pinter-Wollman 2012; Scharf et al. 2012; Jandt et al. 2014)).

- *Threshold effects*: Certain aspects of individual behaviour, group behaviour, and interspecific interactions can be considered as threshold traits where a specific value denotes the limit between two different states. Group size, population density, population behavioural composition, and exposure to environmental factors during development can all affect individual and group behaviour via threshold dynamics (Careau et al. 2008; Modlmeier, Keiser, et al. 2014; Farine & Montiglio 2015; Westneat et al. 2014). Furthermore, the presence or absence of mutualistic interactions itself can depend on the population density of potential partners (Holland et al. 2004). Follow-up or complementary experiments could vary the population density of one or both partner species to ascertain the presence or absence of population density thresholds, which could then provide valuable feedback for future work.

- *Time-scale*: Since population dynamics, by definition, play out over multiple generations, it is worthwhile to set up long-term experiments. One strategy to achieve this is through designing and securing funding for experiments of a (very) long duration, ideally backed by several research groups. Another strategy is to carefully select the study system and opt for organisms with a short lifespan and rapid reproductive cycle, and perhaps even bacterial systems which may elucidate long-term evolutionary dynamics in behavioural variation (De Winter et al. 2015).

Including both partners of symbiotic relationships in animal personality research can reveal not only how behavioural types and syndromes affect non-antagonistic interspecific interactions, but also how these interactions feed back on the behavioural types of the organisms involved. This opens up a new suite of questions that can greatly extend our knowledge concerning animal personality (see Outstanding questions).

Outstanding questions

- Which personality type is more likely to begin, or engage in, a mutualistic interactions?
- Is loyalty to an interspecific partner tied to behavioural type?
- In the case of loyal, exclusive partnerships, do the interactions themselves show consistent differences between sets of interactors, i.e. in frequency or duration?
- In the case of non-loyal or non-exclusive partnerships, does the personality match or mismatch between partners affect aspects of the relationship (e.g. cheating, see Wilson et al. 2014)?
- Does being in a mutualistic relation change an animal's personality? If there is a change, is it similar in both partners? Is it similar for different initial personality types?
- What happens to an animal's behavioural type when a partnership ends?
- How do the effects of mutualistic interactions and environmental cues on consistent variation in individual behaviour interact? Also, how do environmental effects, consistent individual variation in behaviour, and their potential interaction, affect mutualisms?

Conclusion: Embracing variation for a better view

Consistent variation in individual behaviour has recently established itself as a widely pursued research topic at the forefront of behavioural ecology. Both its complex underpinnings and far-reaching consequences are subjected to ongoing exploration. As these investigations proceed, we are beginning to realize just how pervasive the

phenomenon is. Not only does consistent individual variation in behaviour occur in disparate taxa, it might be one of the factors that influences the growth and pruning of the tree of life. More and more, we are starting to see just how interwoven the branches in this tree (or web?) (Keeling & Palmer 2008; Soucy et al. 2015) are. What affects one branch is therefore likely to affect others. Broadening the research scope of animal personality to include multiple branches via the study of non-antagonistic interspecific interactions can only benefit our understanding of its causes and consequences. The effects of animal personality transcend species borders and antagonism, they are part of the interwoven web of life.

VII. General conclusion:

Complexity, summary, and routes ahead

VII.I. Webs, loops, and interactions

Animal personality, or consistent variation in individual behaviour, is a complex and multi-faceted phenomenon. Many potential causes and effects of its occurrence have been identified. However, truly establishing causality is particularly difficult since causal and consequential factors often interact. Consistent individual variation in behaviour is embedded in a complicated web of ecological, evolutionary, and other biological facets that can affect, and be affected by, the behavioural variation present within and among populations (Réale et al. 2007; Réale al. 2010). Elements within this web are furthermore not static elements, and feedback loops are likely to exist (Dingemanse & Wolf 2010; Sih et al. 2015).

To investigate the interactive biological web surrounding individually consistent variation in behaviour, the work in this thesis focused on observable patterns of behavioural variation and its potential biological correlates. Through avoiding the restriction that comes with investigating specific proximate mechanisms, and via studying observable patterns without necessarily establishing causality, potential confusion concerning levels of explanation can be avoided (Dall et al. 2012).

VII.II. Studying selected strands: Early life, morphology, ecology, and social behaviour

In this project, a few of the strands in the web encircling animal personality were subjected to closer scrutiny, chosen based on a literature review and subsequent identification of areas that warranted further enquiry and were tractable within the scope of a PhD thesis.

Early life

A first study looked at the occurrence of 'personality' in the early life of lab-bred three-spined stickleback with parents from two distinct wild populations. It has been suggested that developmental processes can have substantial impacts on behavioural consistency and correlations between behaviours (Stamps & Groothuis 2010a; Stamps & Groothuis 2010b). However, most research is performed using adult individuals. To truly gain insight into the influence of developmental processes, early stages of life have to be investigated as well. Recent work has begun to address this omission. In the project here (Chapter 2), no evidence suggested the presence of repeatable personality traits in lab-reared juvenile stickleback. This finding has since been corroborated by work in e.g. tadpoles (*Rana dalmatina*) that were not exposed to predator cues (Urszán et al. 2015) and Eastern mosquitofish (*Gambusia holbrooki*) that displayed 'personality' only after reaching sexual maturation (Polverino et al. 2016). In young, lab-reared convict cichlids, on the other hand, repeatable

differences in boldness and exploratory behaviour have been reported (Mazue et al. 2015).

The juvenile sticklebacks used in this project, however, did display body length-behaviour correlations that differed between the offspring of the two different parental populations. In one population body length correlated negatively with exploration, in the other one, body length correlated negatively with social behaviour. Different predation pressures in the parental population might be one of the contributing factors for these findings, since predation has been shown to affect personality traits in three-spined sticklebacks (Bell & Sih 2007; Brydges et al. 2008). The presence of these early life correlations suggests a(n) (epi)genetic basis for the differences between the two study populations, and provides tentative support for an adaptive hypothesis (Bell & Stamps 2004).

This relevance of body length hints at a role of significance for morphological features in affecting behavioural variation, an aspect that was subsequently further explored.

Morphology

The stickleback radiation on North Uist has given rise to substantial morphological divergence among populations. As such, this study system provided an excellent opportunity to investigate the potential correlation between morphological and behavioural variation. One of the aspects of morphology that has been explored in this context concerns defensive traits, such as body depth in crucian carp (*Carassius*

carassius) (Hulthén et al. 2013) and shell shape in aquatic snails (*Radix baltica*) (Ahlgren et al. 2015). The link between defensive morphology and individual boldness has led to the development of the 'phenotypic compensation hypothesis', which proposes that bold individuals compensate for their risky behaviour by expressing a more defensive morphology.

By behaviourally testing sticklebacks from seven wild populations and quantifying their armour characteristics, this project sought to test this hypothesis (Chapter 3). In contrast to expectations, substantially armoured stickleback were found to be significantly less bold than those with a less armoured phenotype. Additionally, armour correlated positively with activity, contradicting our stated hypothesis that armour is energetically costly and might reduce movement.

Of course, both behaviour and morphology are multivariate phenotypic aspects. Therefore, the phenotypic compensation and/or energetic cost hypothesis could find support when considering different traits. Work in convict cichlids (*Amatitlania siquia*), for example, points towards a correlation between behavioural type and coloration (Schweitzer et al. 2015), an aspect that future work could explore in stickleback nuptial coloration as well.

Furthermore, only a few within-population correlations between behaviour and morphology were found in this PhD project, which suggests that the ecological differences among populations might be a main cause for the differences in among-population behaviour. This led to the consideration of various ecological factors and their potential correlation with variation in consistent individual behaviour in the same study system.

Ecology

Several ecological variables are known to affect the occurrence and maintenance of behavioural types and syndromes in a population. By gathering data on four potential ecological influences on animal personality to extend the work in North Uist, this part of the overall PhD project explored the ecology of personality (Chapter 4). The four ecological factors taken into account included two reasonably well-studied ones, predation pressure (e.g. Bell and Sih 2007; Brydges et al. 2008; Dingemanse et al. 2009; Kortet et al. 2010; Kortet et al. 2015) and parasitism (e.g. Barber & Dingemanse 2010; Kortet et al. 2010; Poulin 2013; Kortet et al. 2015; Barber et al., 2016). The other two ecological factors have, so far, been less intensively examined, namely resource availability (Luttbeg & Sih 2010; Simon et al. 2016) and interspecific competition (Webster et al. 2009). Furthermore, studies concerning the ecology of personality often focus on a single factor, or, exceptionally, two (Kortet et al. 2015).

Here, I assessed the influence of four ecological factors upon personality variation in seven populations of wild stickleback on North Uist. The presence of predators correlated with decreased activity. Medium parasitism was linked with high boldness, and high parasitism with reduced exploratory behaviour. Resource availability correlated positively with boldness and activity. The presence of interspecific competitors had a negative effect on activity and exploration. Two interesting findings should be noted. First, no single factor affected all measured behaviours. Second, factors less widely addressed in the broader literature (in this

case, resource availability and interspecific competition) can have a significant influence on the behavioural variation present in a population.

This illustrates the complexity of animal personality in an ecological context, and how the phenomenon is thoroughly integrated in a web of interacting variables. This intricacy poses substantial challenges for studies seeking to uncover the ecology of personality, especially in natural populations. Nevertheless, more and more the integral nature of animal personality in ecology and evolution is being recognized in e.g. community ecology (Bolnick et al. 2011), food web ecology (Toscano et al. 2016), biogeography (Canestrelli et al. 2015), speciation (Ingley & Johnson 2014), and the fields of ecology and evolution in a broad sense (Wolf & Weissing 2012).

Another important aspect of an animal's ecology is the presence of conspecifics. Living in group and social interaction have been proposed as relevant influences on animal personality (Bergmüller & Taborsky 2010). Therefore, the next part of this PhD involved a study of animal personality and its relation with group behaviour and social system.

Social behaviour

Following the development of the 'social niche specialisation hypothesis', the links between social behaviour and consistent individual differences in behaviour have become a major topic of study in the field of animal personality. This hypothesis proposes that the social interactions between individuals drive behavioural differences between these individuals (Bergmüller & Taborsky 2010). With the

potential importance of social behaviour in mind, various social behaviours and their interactions with animal personality have been scrutinized (e.g Carter et al. 2014; Jacoby et al. 2014; Jolles et al. 2014a; Jolles et al. 2014b; Keiser et al. 2014; Laskowski & Pruitt 2014; Modlmeier et al. 2014; Keiser et al. 2016; Laskowski et al. 2016).

One of the insights these investigations have turned up is that the composition of behavioural types in a group affects aspects of collective behaviour (e.g. Eskridge & Schlupp 2007; Dyer et al. 2009; Keiser & Pruitt 2014; Pruitt & Keiser 2014; Farine and Montiglio 2015; Planas-Sitja et al. 2015; Wright et al. 2016). Beyond behavioural group composition, experience, including familiarity, also affects group behaviour (Utne-Palm & Hart 2000; Swaney et al. 2001; Atton et al. 2014; Laskowski & Pruitt 2014; Laskowski et al. 2016).

As part of this PhD project, the interaction between these two factors, group composition and experience, was explored in two study taxa with a different social system (shoaling stickleback vs. social spiders) (Chapter 5). By repeatedly assessing group foraging behaviour over a period of time, it was found that behavioural group composition had a strong effect on collective foraging in both systems, but that the effect of experience was stronger in stickleback. Furthermore, the interaction between group composition and experience was not significant in either system for any of the measured behaviours. This led to the suggestion that the two investigated factors (composition and experience) exert their effects through a different mechanism. A further hypothesis is that the effect of experience, especially via familiarity, decreases in systems with a more developed sociality.

This study highlights the value of broad, cross-taxonomic views on animal personality. The final part of this PhD project therefore focused on exploring the animal personality concept in other fields of biology.

Zooming out: Conceptual forays into new fields

Animal personality is a term that is often used as a synonym for consistent variation in individual behaviour. Is this phenomenon, however, really restricted to the animal kingdom? A first conceptual exploration of this project (Chapter 6.1.) investigates this question in the micro-world. This investigation builds on work highlighting the behavioural complexity in bacterial populations (Crespi 2001; West et al. 2007) and the recognition of phenotypic heterogeneity among bacteria (Harrison 2013; Frankel et al. 2014). It is argued that bacteria, despite some challenges, can present opportunities for the study of 'animal (?) personality'. It is interesting to note that recently, there have been a few similar explorations into 'plant personality' (Karban 2008; Simon et al. 2016). Consistent variation in individual behaviour could thus be a biological phenomenon that extends well beyond the animal realm.

A second piece of conceptual work investigates how consistent individual behavioural variation can be leveraged in the field of animal welfare (Chapter 6.2.). Here, the finding of differential responses to stress of individual animals and its connection to behavioural type (Koolhaas et al. 1999; Carere et al. 2010; Montiglio et al. 2012) is combined with the recognition of personalities in zoo and farm animals (Gold & Maple 1994; Hessing et al. 1994; Malmkvist et al. 1997; Weiss et al. 2006;

Gartner & Powell 2011). This results in a call for the consideration of animal personality in animal welfare issues, and a brief example is outlined, accompanied by a tentative set of guidelines. In combination with the few other works in this area (Boissy & Erhard 2014; Koolhaas & Reenen 2016), this might lead to the development of a applicable framework that might improve animal welfare in various settings.

Finally, the large-scale and interactive reach of consistent variation in individual variation is explored by focusing on a neglected set of organismal interactions in this context: facultative interspecific mutualisms (Chapter 6.3.). While intraspecific social behaviour (Bergmüller & Taborsky 2010; Webster & Ward 2011; Hamilton & Ligocki 2012; Wolf & Krause 2014) and antagonistic interspecific interactions such as predation and parasitism (Kortet et al. 2010) have been studied in the context of animal personality, non-antagonistic interspecific interactions have garnered little attention this far (one notable exception is Wilson et al., 2014). It is suggested that personality differences in interacting species can shape mutualistic interactions and can affect large-scale ecological and evolutionary processes. This long reach of animal personality has recently been acknowledged and proposals to integrate the phenomenon in diverse fields of enquiry such as speciation (Ingley & Johnson 2014), biogeography (Canestrelli et al. 2015), and food web ecology (Toscano et al. 2016) have been developed. Indeed, the more we learn about animal personality, the more it seems to permeate ecology and evolution. Notwithstanding the recent burst of studies in the field, this means that there remain many regions left to explore.

VII.III. The road ahead

Ongoing research continues to uncover novel aspects of the ecology and evolution of consistent individual behavioural variation that warrant further enquiry. Animal personality thus remains a fertile ground for future research. The complexity of the phenomenon additionally implies that many other facets of biology, not necessarily limited to animal behaviour (see e.g. Chapter 6), can be included in these investigations.

The ontogeny of animal personality over an individual's lifetime is one point of investigation that seems to be a fruitful avenue for future research. A recent study in eastern mosquitofish (*Gambusia holbrooki*), for example, showed that personality emerged even in absence of extrinsic factors that are often proposed to affect behavioural differentiation in a population. Furthermore, personality was not detectable in juveniles (see Chapter 2) and behavioural plasticity diminished as individuals aged (Polverino et al. 2016). Species that undergo metamorphosis additionally present a unique opportunity to study the persistence of personality over a life-history event of unparalleled 'remodelling' (Wilson & Krause 2012a). Yet, some studies suggest that personality traits are conserved over metamorphosis (Hedrick & Kortet 2012; Wilson & Krause 2012b) although there appear to be sex difference in the conservation of repeatability in field crickets (*Gryllus integer*) where boldness in females, but not in males, was repeatable across metamorphosis (Hedrick & Kortet 2012).

Many ecological factors (e.g. predator presence, parasite prevalence, resource availability, habitat stability, etc.) are purported influences on the behavioural

variation present in a population. However, studying more than one of these in natural conditions presents a challenge. Only now the first studies looking at multiple of these factors are beginning to appear (Kortet et al. 2015) (see also Chapter 4). To gain a more complete understanding of how ecology and personality interact several traits and several ecological variables ought to be included. To achieve this, numerous developments, from selecting suitable study systems, over standardizing behavioural assessments, to technological advances, could be pursued. Many opportunities to involve ecologists, computer scientists (for e.g. robust tracking software development), animal biologists, and field specialists await here.

Since animals rarely live in isolation of their conspecifics, the social environment has become a major topic of interest in animal personality studies, spurred on by the social niche specialisation hypothesis (Bergmüller & Taborsky 2010). However, the diversity and variation in social systems found in the animal kingdom ensures that there is still a lot to explore (see Chapter 5). Some recent promising enquiries look at, for example, social network theory (Krause et al. 2010; Jacoby et al. 2014; Kleinhappel et al. 2014), eusocial species (Grinsted et al. 2013; Pruitt et al 2013; Settepani et al. 2013 Wright et al. 2015), and personality at multiple scales, such as at the level of the individual and the colony (Chapman et al. 2011b; Way et al. 2011; Scharf et al. 2012; Modlmeier et al. 2014b; Wright et al. 2016). Comparative studies where the variation in social system (either within or between species) is correlated to personality characteristics also seems a worthwhile direction for future research (see Chapter 5). Social complexity and cognition are often thought to be linked (Dunbar 1998). Cognition and its potential link with personality is another frontier where substantial exploration remains to be done in the study of consistent variation in individual behaviour (Carere & Locurto 2011; Sih & Del Giudice 2012; Griffin et al.

2015) and the first studies tackling this have recently been appearing (Trompf & Brown 2014; Feng et al. 2015; Gibelli & Dubois 2016; Nawrith et al. 2017).

A final area of further study that will be mentioned here is of a more conceptual nature. It involves probing the full extent of individually consistent behavioural variation in ecology, evolution, and biology in a more general sense (Wolf & Weissing 2012). The phenomenon has been proposed as factor of relevance in, for example, speciation (Ingley & Johnson 2014), dispersal (Cote et al. 2010), and even in the understanding of biogeographic patterns (Canestrelli et al. 2015) and food web structure (Toscano et al. 2016). Furthermore, 'extent' here is not limited to the different aspects of biology that can be affected by consistent variation in individual behaviour. Investigating how far across the tree of life it spreads is also a question to be addressed. Do plants (Simon et al. 2016) and micro-organisms (De Winter et al. 2015), for example, display such variation as well? And if so, how (dis)similar is it from its occurrence in their animal (evolutionary) relatives?

Overall, consistent variation in individual behaviour is embedded in an intricately tangled web of biological factors and interactions. There are many nodes and strands in this web that require further study. Additionally, the full extent of this web is still to be determined. Keeping true to perhaps the most pervasive of scientific adages: More research is needed.

As it should be.

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