ANIMAL PERSONALITIES ON THE MOVE

Aparajitha Ramesh

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Aparajitha Ramesh

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Promotores
Prof. dr. F.J. Weissing
Prof. dr. A.G.G Groothuis

Co-promotor
Dr. M. Nicolaus

Beoordelingscommissie
Prof. dr. A.M. Bell
Prof. dr. K. van Oers
Prof. dr. M.E. Maan

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Introduction

Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less.

-Marie Curie

Animal personality, defined as consistent individual differences in behavioural tendencies, is a ubiquitous phenomenon, occurring in most taxa. Animal personality research and more generally, ‘personality-thinking’ has shifted the focus of behavioural studies from viewing behaviours as independent entities to a more integrated view of behavioural structure or behavioural organisation. Thus individual variation is being considered more and more as an important factor for understanding behaviour rather than noise. In this thesis, I am broadly concerned with how movement, be it long distance migration or small-scale foraging movements, is related to personality differences. Differences in movement tendencies are seen within and between populations and are often associated with whole suites of behavioural traits. In my thesis, I take an integrative approach, combining ecological (‘eco’), evolutionary (‘evo’), and developmental (‘devo’) perspectives, to study the link between movement and personality. To this end, I use a combination of empirical (using three-spined sticklebacks, *Gasterosteus aculeatus*) and theoretical modelling studies. Here, I first introduce the concept of ‘animal personality’, sketching the history of the concept and its link to human personality. Second, I briefly review various approaches to animal personalities (eco, evo, devo) and the need for an integrative approach. Third, I focus on the causes and consequences of movement-related personality differences that form the core of my thesis. Finally, I briefly outline the various chapters of my thesis.

Why study individual variation?

What can we hope to achieve by probing the why’s and how’s of individual differences? Consider the following (adapted) analogy from John and Robins (2022). If Martians were to come to Earth and observe the nature of how transportation systems, particularly cars, in human societies work, they would be looking at two similar yet profoundly different questions. First, they would set out to unravel what characteristics comprise the general essence and nature of a car (presence of wheels, engine that makes the wheels rotate, a seat for a human etc.). Second, they would need to find a way to describe the variation we see in cars (to determine if there is an optimal car) including but not limited to whether these variation change in some way the aspect of transportation (functional) or not (noise, unrelated to transportation). However, knowing that the facets of variation can be immense - colour, size, weight, colour of internal wiring, the fuel requirement, the speed, to name a few, it is important to classify and meaningfully understand the nature of such variation, according to the interest of the researcher. Variation in speed and colour need not be correlated but speed and type of fuel are necessarily correlated, and perhaps also connected to the shape of the car. For example, racing cars may come in a variety of colours while tractors do not. Is this variation interesting? Perhaps, but it may not be related directly to its functioning.

However, the shape of a racing car is designed to be light, aerodynamic, to fit one person and to move at immense speeds whilst that of a tractor is to handle heavy loads and be robust, not necessarily to move at high speeds. It is of some importance to understand that a perfect car, moving at great speeds and being robust to heavy loads, cannot be achieved (in case of organisms, such perfect organisms, freed from all trade-offs and constrains, are called 'Darwinian demons'). This information is integral to understanding the functioning of cars, especially in revealing the non-independence of parts and how they function with the overall environment that they are used in.

As outlined above, structured variation is the raw material for uncovering links and underlying architectures, which are essential for a complete understanding of car transportation. It would be a mistake to consider such structured variation as 'noise', at it is the very thing that gives us extra information about the system and the trade-offs in a top-down approach. An alternative (bottom-up) approach to understand the structure and functioning of a car would be to disrupt certain components and observe the implications of such a disruption. Such an approach is, for example, used in Molecular Genetics, where genes are systematically knocked out to reveal their function ('loss-of-function' mutations). In the behavioural sciences, where information on the system under scrutiny is much more coarse-grained and complex, a corresponding bottom-up approach does not seem realistic. The essence of 'personality thinking' lies in making use of the information embedded in structured 'noise' and understanding behaviours as integrated and co-adapted traits. With this, I hope to have sufficiently convinced the reader of the importance of delving into the intricacies of individual differences and the interesting puzzle it both creates and solves.

A brief history of animal personality

Individual phenotypic differences within animal populations are a widespread phenomenon that has been observed and studied for a long time. Famously, Darwin acknowledged the importance of phenotypic variation and considered it as valuable raw material for natural selection to act upon. Phenotypic variation not only comprises morphological but also behavioural variation. However, since behaviour was almost always considered more flexible than morphological traits, differences in behaviours did not attract extensive studies. Although biologists were always aware of individual variation in behaviour, it was for long considered as 'noise' around a population's 'true' mean. Only in the last decades, it is being realised that individuals exhibit consistent behavioural differences even in the absence of other obvious differences such as sex, size, morphology etc. This raised questions about its causes and adaptive value (e.g. in physiology, Bennett 1987; ecology and evolution, Bolnick et al. 2003, Réale et al. 2007; epidemiology, Lloyd-Smith et al. 2005, Breckling et al. 2006).

Systematic consideration of individual behavioural differences started with human psychology research, wherein the term personality was used to describe behavioural aspects that were consistently different between individuals. In human psychology, the currently widely accepted model describing the structure of behavioural variation and personality differences is the five-factor model (Costa et al. 2001; Gosling 2001). Human subjects are classified along five ‘independent and orthogonal’ axes (McAdams 1992), that are named extraversion, agreeableness, conscientiousness, neuroticism and openness. These axes comprise a large number of subtle individual traits themselves, but the idea behind the five-factor model is to distil these unique differences into broader categories. These classifications are then used to either ask how these traits came to exist (development and stability of personalities), or to predict, for example, individual differences in socio-economic status or susceptibility to a certain illness.

More recently, the concept of personality has been extrapolated to animals (Gosling and John 1999), upon repeatedly finding that individuals within a population differ consistently and systematically from each other in their behavioural tendencies. ‘Animal personality’ is often defined as individual differences in behavioural tendencies that are consistent over time and contexts (or a variation of that definition, Dall et al. 2004; Stamps and Groothuis 2010a; Sih et al. 2015; Kaiser and Müller 2021). Correlations between functionally different behavioural tendencies, such as ‘aggressiveness’ (the tendency to show agonistic behaviour toward conspecifics) and ‘boldness’ (the tendency to exhibit risk-prone behaviour in a potentially dangerous situation), are referred to as ‘behavioural syndromes’ (sensu Sih et al. 2004) or ‘coping styles’ (Koolhaas et al. 1999). The concept and the methodology used in ‘animal personality’, is markedly different from those used in human personality studies. Although some have classified animal personalities into five axes (exploration, activity, aggressiveness, sociability, and boldness), such a classification is completely different from the five-factor model of human personality. Moreover, there are many more ’animal personality traits’ than the five listed above, including responsiveness to environmental change, and the tendency to move to another environment (Réale et al. 2007).

Implications of animal personality

Personality differences may be underpinned by genetic variation, (Van Oers et al. 2010); developmental plasticity (Stamps and Groothuis 2010b); physiological and metabolic states (Careau et al. 2008); via social environment (Bergmüller and Taborsky 2010; Fischer et al. 2017); parental effects (Potticary and Duckworth 2020), and so on. Irrespective of these different underlying mechanisms leading to personality differences, there is accumulating evidence these differences are linked to variation in survival and reproductive success in many cases (e.g. Cote and Clobert 2007; Boon et al. 2008; Nicolaus et al. 2012; Mouchet et al. 2021). These can have potential implications at the population, up to the ecosystem levels (Wolf and Weissing 2012).

Not only do animal personalities affect population dynamics, for example, by increasing carrying capacity due to specialisation and competition avoidance, animal personality can also affect intra- and interspecific interactions via non-random distribution of personality types over space (an extensive account of ecological and evolutionary consequences is reviewed in Wolf and Weissing 2012). In many cases, these may be cascading effects, due to complex feedback from individual to population to community level dynamics. Therefore, animal personalities, that are ubiquitous in nature have widespread consequences extending to populations and ecosystems (Svanback and Bolnick 2007;

Dall et al. 2012; Wolf and Weissing 2012).

An integrative approach to studying animal personality

My PhD project was based on a research proposal that was entitled 'Eco-evo-devo of migration syndromes'. The aim was to study personality differences related to migration from an ecological, evolutionary, and developmental perspective, and to also consider links between these perspectives. Such links are important, because the environment (eco) can play a selective (evo) and an inductive role (devo) for phenotypic variation and the three components need to be studied on equal footing to understand the complex feedback among them.

Evolutionary theory predicts that consistent individual variation and its development reflect the signature of the environment. Indeed, environment (eco) is the driver of genotypic and phenotypic variation and covariation, while development (devo) acts as a regulator by orchestrating various genetic components and physiological modules leading to systematic phenotypic variation, thereby mediating interactions between genes and environment. Individual variation can correspond to fitness differences leading to selection on phenotypes (evo) that are particularly suited to the environment. Eco-evo-devo as a field recognises that organisms are continually subjected to changing environment and development plays a mediating role in the interaction of genes and ecology, thus feeds back on ecosystem level and these themselves may be subject to evolution, thus necessitating an integrative approach (Beldade et al. 2011; Abouheif et al. 2014). Combining this with our knowledge potential implication of personalities (mentioned above), it seems necessary to take such an integrated approach in studying animal personalities.

Links between movement and personality

Animal movement takes vastly different forms; from the scales of movement (local foraging to trans-oceanic migrations), to medium of migrations (land, water or air); either solitary or in social groups of hundreds and spanning different periods of times. An interesting aspect of movement is that individuals within a population commonly vary in their movement tendencies. Individuals differ consistently from one another in various aspects, including speed and duration of movement, and the nature and amount of local information used for movement decisions (Shaw 2020).

There are a several good reasons to think that movement and personality are intimately related: (1) Even if the local conditions are favourable over longer duration, it is often selectively advantageous to produce not only philopatric offspring, but also some offspring that move on to other habitats. The production of mixed movement types may be viewed as a 'bet-hedging strategy', which prevents that all descendants are lost if the current conditions change or if the current location is struck by disaster.

This general principle explains why different movement types coexist throughout the animal kingdom and why such differences can even be found among siblings or in clonal organisms. (2) Individuals differing in movement strategies are exposed to very different challenges and to different environments. From an adaptive perspective, it is therefore to be expected that differences in movement are associated with many other phenotypic differences, including differences in morphology (e.g. body size, movement apparatus, armament), physiology (e.g. metabolic rate), and behaviour (e.g. responsiveness to environmental cues, exploration tendency, boldness).

Indeed, it has been recognised that different movement tendencies co-vary with several morphological (e.g. body size, muscle density) and behavioural traits (e.g. aggressiveness, exploratory tendency, neophilia), thus forming a syndrome in many cases. In the context of dispersal or migration, moving individuals differ from residents in suites of traits that either make movement more effective and efficient or reduce the costs associated with movement. The functional integration between movement tendencies and other phenotypic traits is termed 'dispersal syndrome' or 'migration syndrome' (Dingle 2006). One well-studied example of 'dispersal syndromes' is the case of western bluebirds (*Sialia mexicana*), where more aggressive males are more successful in dispersing to and colonizing new habitats and out-competing heterospecifics. Therefore, mean aggression level is typically high in newly established populations and rapidly decreases across generations through negative density-dependent selection on aggression (Duckworth and Badyaev 2007; Duckworth et al. 2015). Even when movement takes place in the context of foraging or mating, variation in movement tendencies can also be associated with personality variation. For example, in a foraging context, whenever there are spatiotemporally varying resources, animals employ different movement strategies to deal with environmental changes - whilst some are responsive to environmental change and track the resource peaks, others are unresponsive and benefit mostly from the movement of responsive individuals (in mallard ducks, Harper 1982; in pigs, Bolhuis et al. 2004). These differences can be maintained by frequency-dependent selection on responsiveness in a changing environment (Wolf et al. 2008). Furthermore, these differences can be associated with different sensory and movement abilities to track environmental change, metabolic needs and stress response to changes, thus forming a link to also proactive-reactive personality types (Koolhaas et al. 2010).

Implications of individual variation in movement tendencies

Movement forms a link between an organism and its environment, thus having consequences for meta-population dynamics, spatial distribution, and range expansion. It is integral for species persistence in many cases (e.g. presence of dispersers and philopatric individuals is necessary for meta-population persistence in the western bluebirds, Duckworth and Badyaev 2007). These personality-dependent movements can result in different clustering of behavioural types in heterogeneous habitats (Spiegel et al. 2017). Small variations in movement and space use can create a positive feedback between ecological conditions and evolution of individual differences in movement strategies themselves. For example, variation in dispersal and movement tendencies can lead to spatial sorting of dispersers at the range front, which in turn increases variation leading to a positive feedback loop (Shine et al. 2011). Movement is thus affected by several ecological processes and has far-reaching effects on population dynamics and spatial distribution of organisms. This subsequently affects inter-specific interaction and ecosystem dynamics themselves and has the potential to create eco-evolutionary feedback (Shaw 2020). Thus, it is imperative to take an integrative approach, as mentioned above, to study movement tendencies and personality.

Outline of this thesis

In this thesis, I have taken a two-pronged approach (empirical studies and theoretical modelling) to study movement and personality in an integrative manner.

I used the three-spined stickleback system of migratory and resident populations to investigate the following:

1. Do individual movement tendencies (migration tendencies) co-vary with other behavioural traits forming a migration syndrome? (eco of movement and personality; descriptive study in **Chapter 2**)
2. What mechanisms underlie the behavioural divergence observed between resident and migratory populations? (devo of movement and personality; common garden experiment in the lab in **Chapter 3**)
3. How stable are individual- and population-level behavioural differences across social and ecological contexts? (eco-evo of movement and personality; experiments in a semi-natural mesocosm in **Chapters 4, 5**)

In addition to empirical work, I participated in two modelling studies that aim to answer the questions,

1. When and how do spatio-temporal fluctuations of the environment lead to the evolutionary emergence of individual differences in resource competition strategies? (eco-evo of movement and personality, **Chapter 6**)

Empirical study system

All empirical work reported in this thesis has been conducted with three-spined sticklebacks. For this thesis, we used sticklebacks because: (1) They form populations of migrants and residents in the same region, thus allowing comparison of movement types and related differences and drawing conclusions regarding the impact of human-induced habitat fragmentation over short times (also see below); (2) They have been shown to have different personality types and syndromes within and between populations (e.g. Bell and Stamps 2004; Dingemanse et al. 2007) thus having a foundation for

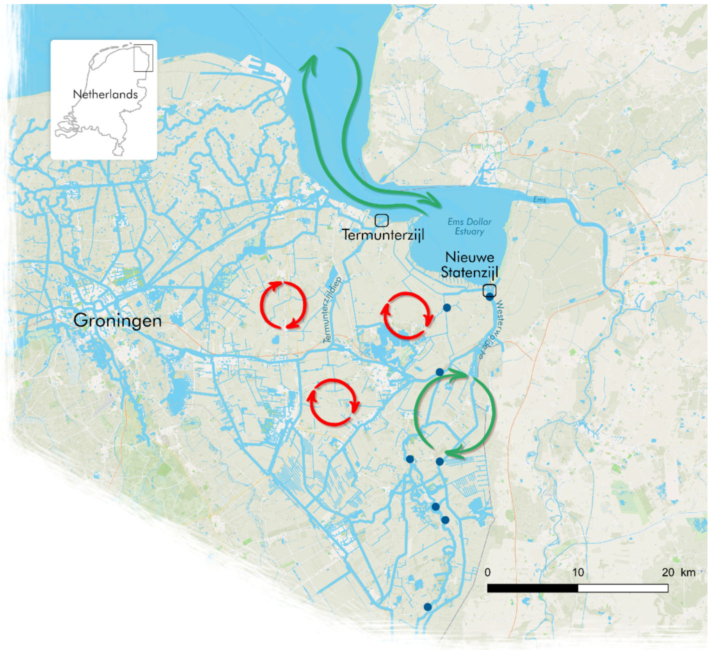


Figure 1.1 – ***The waterways of Groningen, in the north of the Netherlands.*** *The river inlets are shown. Sticklebacks migrate from the sea from the Ems-Dollard estuary into freshwater river systems in spring to breed and return to the sea for autumn (Green arrows). Most or all of the side arms and smaller channels of the river are blocked off from the main river and hence connection to the sea is lost, creating several replicate populations of land-locked sticklebacks that spend their whole life cycle in freshwater (red). This field system is also equipped with large PIT (Passive integrative transponder) stations, to monitor tagged fish as they pass through the rivers (seen as blue dots). Picture credits: Mavromatika.*

hypotheses on movement related personality differences; (3) Multiple mechanisms underlie personality variation in this system, including genetics (Greenwood et al. 2016), developmental plasticity (Dingemanse et al. 2009; Langenhof and Komdeur 2018) and parental effects (Stein et al. 2018).

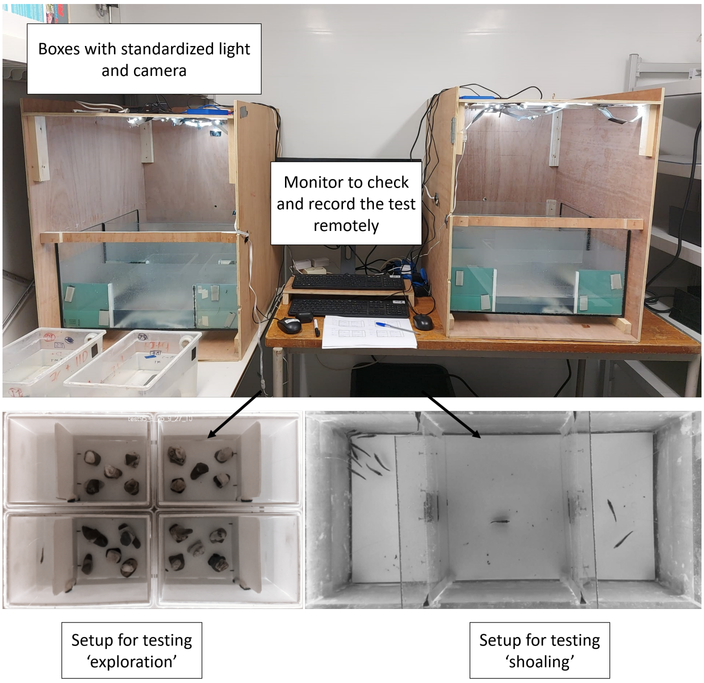
Field studies – migrant and resident sticklebacks

In the last 50 years in the Netherlands, man-made barriers (such as pumping stations and sluices) have been extensively built in rivers to maintain water levels below sea-level, with the consequence that some of the side water drainages are cut-off from the main river channel. This created an unintended natural field experiment, wherein several populations of anadromous three-spined sticklebacks ('*migrants*') have become land-locked ('*residents*') in some of these replicate side-arms of the river. Over contemporary timescales, we expect resident populations of sticklebacks to have experienced very different selection pressures by completing their life-cycle entirely in freshwater as opposed to the ancestral migrants, that spend a significant part of juvenile growth

Figure 1.2 – ***Migrant and resident stickleback with scale.*** *Adult residents are, on average, almost half the size of adult migrants in the wild populations. Photo credit: Ben Kawam*

at the sea, during winter. We used this field system to study whether resident and migrant stickleback populations exhibit consistent phenotypic differences (morphology and personality), as a result of this recent human-driven change. To establish the diversity in behavioural types, we conducted extensive field work around the year along different drainages in the province of Groningen, the Netherlands. Wild sticklebacks of migratory origin were caught at the mouth of the rivers while resident sticklebacks were caught from land-locked river inlets (Fig. 1.1).

The migrant and marine populations of sticklebacks have a wide geographic distribution and show near uniform morphologies throughout the Northern Hemisphere (Walker and Bell 2000). These stickleback populations have changed very little over the past 7 – 12 million years (Bell and Foster 1994). This is partly due to high levels of inter-connectedness between oceanic populations, leading to gene flow (Taylor and Donald McPhail 2000), suggesting that little genetic drift is likely to have occurred in them over the last few decades. Hence, they can be considered ‘true’ ancestors to infer phenotypic divergence in resident sticklebacks.



Boxes with standardized light and camera

Monitor to check and record the test remotely

Setup for testing 'exploration'

Setup for testing 'shoaling'

Figure 1.3 – ***Studying behaviours in the lab.*** *Lab behavioural assays are conducted in standardized setup and most importantly tested focal individuals in isolation, giving fine-scaled resolution into behaviours of individuals. Here we show the system of boxes fitted with light and camera to record behaviours across trials. Different setups are used for testing different behaviours in the lab.*

Lab studies – personality tests

In the lab, we conducted fine-scale monitoring of behaviour of individuals under controlled conditions for a thorough analysis and understanding of differences in migration syndrome between the land locked and migrating populations. These include exploration and boldness in a novel environment, anti-predator strategies against predatory fish and social behaviour with conspecifics (aggression, shoaling). These behaviours are functionally different and are crucial during the whole life cycle of individuals.

In **Chapter 2**, I ask if there are differences in phenotypes – morphology and behaviour, in residents compared to migrants after ~ 50 years of isolation in freshwater due to barriers to migration (size differences shown in Fig. 1.2). To this end, I repeatedly sampled and characterised the phenotypic traits of individual resident and migrant sticklebacks over two study years in lab-based assays.

In **Chapter 3**, continuing on the results from **Chapter 2**, I investigated whether the phenotypic differences between residents and migrants were underlined by genetic differentiation using a common-garden study design. This was carried out by systematically comparing F1 juveniles of various crosses raised in a controlled lab environment in freshwater. Using this study design, we were able to address if the divergence caused by

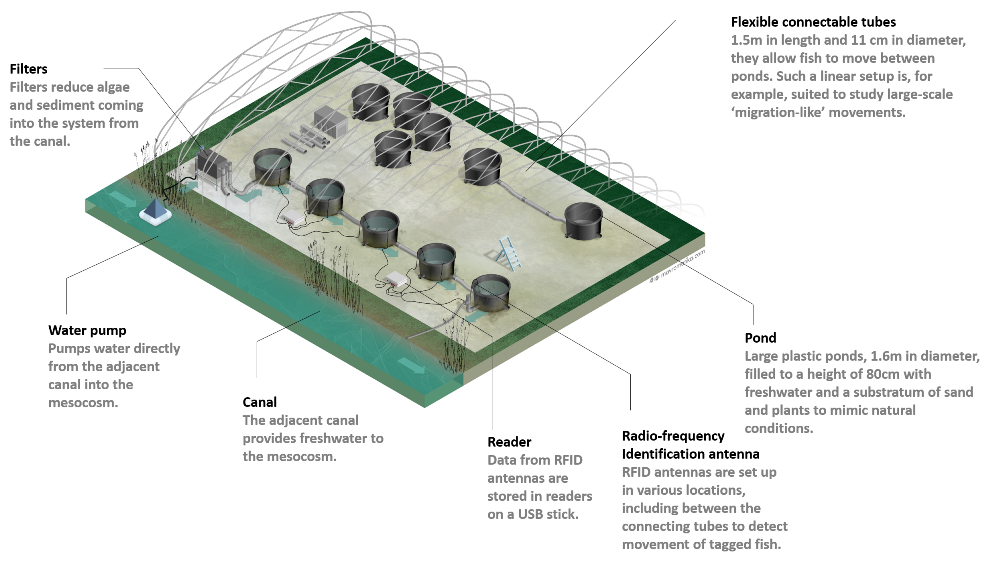


Figure 1.4 – ***The mesocosm system at the University of Groningen.*** *The mesocosm system, a set of connected semi-natural ponds, was established to overcome some of the drawbacks of lab-based testing of sticklebacks. These enable us to monitor tagged stickleback remotely, in groups, over large distances and longer durations of time. Picture credit: Mavromatika*

~50 generations of isolation was enough to also elicit underlying genetic differentiation.

These lab-based behavioural assays were performed in highly standardized and controlled environments, including testing in small tanks in isolation (Fig 1.3). Although these were very useful in reducing the influence of unwanted external variables, we often found counter-intuitive results regarding the differences between migrants and residents, particularly in movement-related behaviours.

Mesocosm studies – experiments under semi-natural conditions

To bridge the gap between the situation in the lab and in the field, we set up a semi-natural mesocosm system that mimics the natural environment as closely as possible while still allowing to follow individuals for extended periods of time. The mesocosm system consists of a modular system of ponds and corridors that can be assembled in different ways, depending on the requirements of the experiments (Fig. 1.4). The mesocosm system is supplied with water from an adjacent natural freshwater ditch, with a possibility of creating a flow with a pump, thus mimicking the situation in the wild. These provide a semi-natural environment for fish, along with some control over the configuration of ponds, spatial scales by connecting more ponds, addition of enrichment / predator cue in specific ponds, etc. The other aspect of the mesocosm system is the remote monitoring of fish using Radio Frequency Identification (RFID) system in their natural social groups, over longer time periods, which is almost impossible to obtain in the lab. Individual fish are injected with a Passive Integrative Transponder

(PIT tag) encoded with a unique identity code and the system is fitted with antennas which detect and record the presence of tagged fish in their vicinity. This technique is an effective well-proven tracking system without the need of recapture and handling. By making use of different types of antennas in different orientations (e.g. circular antennas across corridors to track movement between ponds; flat-bed antennas fitted with a shelter placed within a pond to assess time duration of sheltering), we created an unique facility to gather fine-scale data on individual differences in movement strategies, habitat preference, territory takeovers etc.

In **Chapter 4**, we asked whether the residents and migrants in our system exhibited differences in movement tendencies in the predicted direction (i.e., residents moving less than migrants) and at two spatial scales (within vs. between-ponds). We also tested if population differences were maintained across manipulated ecological and social contexts. In order to answer these questions, we used a linear setup of the mesocosm with five connected ponds, spanning a distance of ~ 15 m and tested individual migrants and residents in their own social groups (with varying group sizes and flow conditions).

In sticklebacks, shoals are highly dynamic and individuals may need to alter their behaviours in order to perform optimally in the shoal. Furthermore, conservation efforts are currently underway to reconnect migratory and resident populations. Hence, in **Chapter 5**, we investigated if and how individual migratory tendencies were affected by the social environment. We thus manipulated group compositions (proportion of migrants or residents in mixed groups) to test whether individual sticklebacks modulate their movement tendencies in response to changes in their social groups and whether social modulation is stronger in residents or migrants.

Theoretical studies

Theoretical modelling studies have been very important in ecology and evolution, especially in the field of animal personality to lay a conceptual foundation and for creating testable predictions (e.g. Wolf et al. 2007, 2008). Individual-based models (IBMs) especially are well-suited for studying the implications of personality differences and the spatial distribution of individuals. A unique feature of individual-based modelling is that it operates at the smallest level of individuals, incorporating structure-function relationship that are obtained in ecological studies and not on higher abstraction levels, thus making it highly attractive to study emergent properties. The disadvantages of an individual-based approach (e.g. that analytical solutions cannot be derived) are more than compensated by the possibility of making realistic assumptions on individual-level behaviour. Individual based models are highly informative for mimicking biological processes because, we can let selection act on the level of individual phenotype, leading to changes in the genotype frequencies and keep track of these genotype frequencies within and across generations easily (DeAngelis and Mooij 2005). IBMs incorporate survival and reproduction in a natural way, without the necessity of deriving a fitness function and to make assumptions on how fitness differences translate into evolutionary change. Moreover, IBMs reveal non-equilibrium dynamics as well as equilibrium outcomes (e.g. Baldauf et al. 2014). Last, but not least, they reveal the evolutionary emergence of polymorphisms, while many other methods (such as selection gradient techniques) assume from the start that individual differences are not relevant.

Modelling the implications of variation in movement strategies

A recent theoretical study by DiNuzzo and Griffen (2020) investigates the implications of personality differences on the spatial distribution of animals. In an **intermezzo** chapter, we first point out some serious shortcomings of that study and ways to model the interplay of personality and movement in a more coherent manner. **Chapter 6** shows how individual-based modelling can shed new light on the role of individual differences in the context of foraging. From an evolutionary perspective, it is hard to explain the persistence of individual differences in foraging behaviour because it is expected that an optimal foraging strategy, with associated traits such as optimal competitiveness exists, which would lead to a monomorphic population. In the simplest case, ecological models use 'ideal free distribution' (IFD) to model the distribution of foragers and assume that all individuals are identical. The next set of ecological models incorporating individual differences assume that they are maintained by other factors that are indirectly related to foraging. However, if movement and related behaviours affect intakes, they would be under selection, in a foraging context. This requires a systematic analysis of conditions favouring polymorphisms in a foraging context. Thus, in **Chapter 6**, we ask if the dynamics of patch-choice in a spatiotemporally varying environment can itself drive the evolution of personality types and what underlying mechanism drives the emergence and maintenance of polymorphisms in competitive abilities of foragers, in an eco-evolutionary individual-based simulation.

2

Habitat fragmentation induces rapid divergence of migratory and isolated sticklebacks

A. Ramesh
T.G.G. Groothuis
F.J. Weissing
& M.Nicolaus

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Abstract

The adaptive capacity of many organisms is seriously challenged by human-imposed environmental change, which currently happens at unprecedented rates and magnitudes. For migratory fish, habitat fragmentation is a major challenge that can compromise their survival and reproduction. Therefore, it is important to study if fish populations can adapt to such modifications of their habitat. Here, we study whether originally anadromous three-spined stickleback populations (*Gasterosteus aculeatus*; 'migrants') changed in behaviour and morphology in response to human-induced isolation. We made use of a natural field-experiment, where the construction of pumping stations and sluices in the 1970s unintendedly created replicates of land-locked stickleback populations ('resident') in the Netherlands. For two years, we systematically tested populations of residents and migrants for differences in morphology and behavioural traits (activity, aggressiveness, exploration, boldness and shoaling) in lab-based assays. We detected differences between migrant and resident populations in virtually all phenotypic traits studied: compared to the ancestral migrants, residents were smaller in size, had fewer and smaller plates and were significantly more active, aggressive, exploratory and bolder and shoaled less. Despite large ecological differences between 2018 and 2019, results were largely consistent across the two years. Our study shows that human-induced environmental change has led to the rapid and consistent morphological and behavioural divergence of stickleback populations in only ∼50 generations. Such changes may be adaptive but this remains to be tested.

Introduction

Humans induce unprecedented fast changes in many habitats, thereby imposing new selective pressures to animal populations (so-called 'human-induced rapid environmental change', sensu Sih 2013). Animals thus need to implement quick adaptive responses to these changes to maintain their ability to survive and reproduce. One of the first responses to these challenges is often behavioural as behaviour directly mediates how individuals interact with their environment. Consequently, it is expected that animal populations will respond to human-induced changes through behavioural modifications as a first step, which then may pave way for other morphological and/or physiological adaptations (Sih et al. 2011; Tuomainen and Candolin 2011; Wong and Candolin 2015). Animal personalities are behavioural traits that are consistent across time or contexts and are often correlated to form "behavioral syndromes" (Stamps and Groothuis 2010a). Animal personalities presumably have significant consequences for the speed and the outcome of adaptation processes to changing environments (Bolnick et al. 2011; Dall et al. 2012; Sih et al. 2012; Wolf and Weissing 2012). For example, personality variation may slow-down or speed-up rate of microevolution depending on whether personality structure retards adaptive evolution (Dochtermann and Dingemanse 2013) or provides 'pre-adapted' phenotypes, which drive faster adaptation in multiple dimensions (Wagner and Altenberg 1996; Barrett et al. 2008; Wolf and Weissing 2012; Van Gestel and Weissing 2018). Furthermore, existence of personalities and mechanisms maintaining such intraspecific variation within populations can have an immense effect on the adaptive potential of these populations in response to environmental change (Réale et al. 2007; Bolnick et al. 2011; Dall et al. 2012; Sih et al. 2012; Wolf and Weissing 2012; Moran et al. 2016).

Human-driven changes have disproportionately affected freshwater species, which have suffered the largest declines of 84% on average (WWF living planet report 2020*). One of the greatest threats is habitat fragmentation that decreases habitat size and functional connectivity between habitats (Legrand et al. 2017). Migratory fish species, in particular, rely upon moving between sea and freshwater or between other habitats to reach spawning and nursery habitats (Fullerton et al. 2010). Hence, blocking access to these habitats can compromise the reproduction and survival of such migratory species (Hutchings 2002). The important questions that connect the fields of animal personality, conservation, ecology, and evolution are whether and how migratory fish can adapt to the sudden isolation. Our study system in the north of the Netherlands is ideally suited to address such questions: In the last 50 years, man-made barriers (such as pumping stations and sluices) have been extensively built in rivers to maintain water levels below sea-level, with the consequence that it has blocked some of the side arms of main river channels. This created an unintended natural field experiment, wherein several populations of anadromous three-spined sticklebacks ('migrants') have become land-locked ('residents') in some of these replicate side-arms of the river. Over contemporary timescales, we expect resident populations of sticklebacks to have experienced very different selection pressures by completing their life-cycle entirely in freshwater as opposed to their ancestral migrants, that spend a significant part of juvenile growth at the sea, during winter. We used this opportunity to study whether resident populations exhibit consistent phenotypic differences (morphology and personality) compared to ancestral anadromous sticklebacks, as a result of this recent human-driven change.

Three-spined sticklebacks have become a model system for studying rapid phenotypic divergence because populations generally harbor high phenotypic variation which enables them to adapt to a multitude of environments and through various proximate mechanisms (genetic, hormones, developmental plasticity, parental effects; see review for freshwater colonization in Table 2.1). Likewise, it is repeatedly found that phenotypic differences can occur among populations with and without exposure to predation (Bell and Sih 2007; Dingemanse et al. 2007; Stein et al. 2018; Dingemanse et al. 2020). Yet, little is known about population phenotypic divergence following habitat fragmentation over shorter timescales. To fill this knowledge gap, we sampled resident and migrant stickleback populations over two years and quantified differences in morphology and in behavioural traits involved in movements and anti-predator strategies: activity, aggressiveness, exploration, boldness and sociability (Magurran and Seghers 1994; Wolf et al. 2008, 2011; Cote et al. 2010, 2013; Chapman et al. 2011; Trompf and Brown 2014; Sommer-Trembo et al. 2017). In this species, these behaviours are repeatable and can be phenotypically integrated (Bell and Stamps 2004; Bell 2005; Bell and Sih 2007; Dingemanse et al. 2007, 2020). Our field system provides an excellent opportunity to answer (1) whether ~50 years of isolation have been sufficient to induce

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[*] WWF (2020) Living Planet Report 2020 -Bending the curve of biodiversity loss. Almond, R.E.A., Grooten M. and Petersen, T. (Eds). WWF, Gland, Switzerland.

morphological and behavioural differences between resident and migrant populations; (2) if the observed differences remained consistent over the two study years. Based on the synthesized literature on freshwater adaptation in this species (Table 2.1, we expect that individuals in resident populations should exhibit smaller body size with less armature as well as decreased levels of activity, exploration, boldness and shoaling compared to the ancestral migratory population).

Table 2.1 – **Overview of freshwater adaptations from marine and migratory three-spined sticklebacks.** Sticklebacks can adapt to freshwater via a multitude of proximate mechanisms. This table provides a non-exhaustive overview of these mechanisms that may be at play in our migrant-resident study system.

Trait	**Change following freshwater adaptation**	**Mechanism**	**Reference**
Life history	Younger and smaller at maturity, Lower growth rates, association between growth rate and plate morphology in freshwater (low-plated morphs grow faster in freshwater compared to high-plated morphs)	Genetic and/or developmental plasticity	Snyder (1991); Marchinko and Schluter (2007); Robinson (2013)
Morphology	Reduction in number and size of lateral plates, reduction in size of dorsal spines, diminution or absence of pelvic spines	Genetic and/or developmental plasticity	Bell et al. (1993); Colosimo (2005)
Physiology	Lower thyroid levels, lower metabolic rates, osmoregulation, tolerance to freshwater	Genetic, Developmental plasticity, Transgenerational plasticity, Parental effects	Lam and Hoar (1967); Kitano et al. (2010, 2012); Kitano and Lema (2013); Kusakabe et al. (2017)
Swimming ability / Buoyancy	Lower swimming endurance, interaction between plates and size of swim-bladder (reduce tissue density / lateral plates or increase size of swim-bladder)	Genetic	Tudorache et al. (2007); Dalziel et al. (2012)
Behavior	Decrease in schooling, shoaling, anti-predator behaviour toward freshwater predators, parental care	Genetic, Developmental plasticity, Transgenerational plasticity, Parental effects	Wark et al. (2011a); Di-Poi et al. (2014); Stein and Bell (2015, 2019); McGhee et al. (2015)

Methods

Study populations and data collection

Our study sites were located along two main rivers, Termunterzijldiep and Westerwoldse Aa originating from the Ems Dollard estuary in the province of Groningen, the Netherlands (Fig. 2.A1). We caught incoming migrants at the two sea locks (“TER”; 53°18′7.24″, 7°2′17.11″ and “NSTZ”; 53°13′54.49″, 7012′30.99″), whereas resident sticklebacks were caught in two adjacent land-locked polders (“LL-A”; 53°17′56.14″, 7°2′1.28″ and “LL-B”; 53°17′16.52″, 7°2′26.46″). To prevent sampling biases, we used lift-, hand- and fyke nets in resident populations and lift netting for incoming migrants directly at the fish passages at the sea locks, ensuring that all fish were caught.

All individuals were transported to the laboratory within two hours of capture in aerated bags. After acclimatization to the laboratory conditions (temperature and 1% salinity water) for one hour, we took the following morphological measurements of all individuals: total length (the length from the tip of the snout to the end of the tail), standard length (the length from the tip of the snout to the base of the tail), body mass, category of lateral plating (fully-plated, partially-plated and low-plated forms; Bell and Foster 1994) and clipped fins and/or spine of individuals for unique individual identification. We used standard length as proxy for size in all analyses because this measure is highly correlated with the two other measures namely, total length and body mass (Supplementary Table. 2.A1) and was less error prone than total length. Thereafter, we placed each fish in an individual “home tank” (L × W × H: 30 cm × 16 cm × 18 cm) that was visually isolated from others and enriched with one artificial plant. Fish were fed frozen blood worms and brine shrimps (3F Frozen Fish Food bv.), ad libitum. On the following day, the fish were allowed to acclimatize to the new environment and laboratory conditions (day 0). From day 1 to day 4, fish were subjected to a range of behavioural tests (Fig. 2.1). On day 6 or 7 fish were released in the wild at their site of origin or kept in the lab for further breeding experiments. The laboratory conditions were set to mimic the natural conditions in terms of temperature (range 5°C to 20°C, depending on season) and photoperiod (range 10:14 L:D to 16:8 L:D, cycled with natural levels).

Data collection occurred between March – May in the years 2018 and 2019. These years were drastically different in terms of the weather conditions of the summer and winter of the previous years (see Supplementary Fig. 2.A2). Compared to winter of 2017, the winter of 2018 was particularly cold with frozen ditches and main canals until March and the following summer was in contrast very warm and dry leading to small ditches partly drying up (Maximum daily temperature (2017 vs 2018): 29.9°C vs. 35.7°C; and mean annual precipitation (2017 vs. 2018): 25.9 cm vs. 17.88 cm; data from Royal Netherlands Meteorological Institute). In 2018, a total of 251 fish were caught (189 migrants and 62 residents) and in 2019, 74 fish were caught (38 migrants and 36 residents). It is noted that in 2019, we were successful in capturing migrants from only one population (“NSTZ”). Our sample size was determined by the number of fish we could successfully catch, while ensuring that batches were caught at different time intervals to avoid confounding effects of season.

Wild animals were sampled using a fishing permit from Rijksdienst voor Ondernemend Nederland (The Netherlands) and an angling permit from the Hengelsportfederatie Groningen-Drenthe. Housing and testing of behaviours were in adherence to the project permit from the Centrale Commissie Dierproeven (The Netherlands) under license number AVD1050020174084.

Behavioral assays

Five behaviours were scored for both migrants and residents: general activity in home tank, aggression towards a conspecific, exploration of a novel environment, boldness in a predator inspection trial and shoaling tendency (Fig.2.1). Activity, aggression and exploration were live-scored by five observers whereas the boldness tests and shoaling assays were filmed and subsequently scored using the software BORIS v.6.2.4. (Friard and Gamba 2016). Details of each assay are given below. The tests were performed during the light period (usually between 9 am and 6 pm). The sequence of fish to be tested was drawn at random. It was not possible to be blind to the status of fish, as migrants and residents exhibited large size differences.

In the behavioural assays (except shoaling), we measured several variables. As justified below, we used one of these variables as a proxy of the behaviour of interest. In addition, we performed principal component analyses (PCA) including all measured variables per behaviour under study and used the first principal component PC1 (explaining most of the variance) as a proxy for the behaviour (Supplementary Table 2.A1). The PCA-based results (not reported), did not differ from the results on the single variables.

Activity

The general activity level of each individual was recorded in their home tank using a grid at the bottom of the tank (Fig. 2.1). Each individual was observed for a period of 60 s and its position in a 10 × 6 square grid space was recorded every 5 s. With the recorded position the following values were calculated: unique squares visited, number of square changes and total distance travelled (adapted from Dingemanse et al. 2007). In the analyses reported in the main text, we used number of square changes as a proxy for ‘activity’.

Aggression

Immediately after the activity test, we introduced an empty transparent glass in one corner of the home tank, in order to acclimatize the focal fish to the new object (120 s). Subsequently, the empty glass was replaced with a similar one containing a smaller conspecific from the same population (“intruder”). During the following 120 s, we scored the position of the focal individual and its response towards the intruder (bites, spine-up display) every 10 s (Fig. 2.1). The mean and minimum distance to the intruder to the intruder and the total number of bites were then calculated (adapted from Bell Stamps, 2004). Spine-up threat display was hard to notice for residents because of their smaller spines and subsequently dropped from observations. We re-used intruders for a maximum of five different trials and controlled for intruder identity in the later analyses. To disentangle aggression from sociability, we used the total number of bites as a proxy for ‘aggression’, rather than the time spent near the intruder.

Exploration

For studying exploration in a novel environment, the focal fish was placed into an opaque acclimatization compartment (4 cm × 6 cm) within a tank of size equal to the home tank, a water level of 5 cm, and with a 10 × 6 square grid at the bottom. The tank included five stones that extend to the top of the water surface to block the view and force the fish to swim around them to gather information about the environment (Fig. 2.1). After an acclimatization period of 120 s, the compartment was gently removed, releasing the fish into the arena and the subject started the exploration test, lasting for 300 s. During this period, the position of the focal fish was recorded every 5 s. With the recorded position the following values were calculated: unique squares visited, number of square changes and total distance travelled (adapted from Dingemanse et al. 2007). In the analyses reported in the main text, we used number of square changes as a proxy for ‘exploration’.

Boldness

In the boldness tests, we measured the responses of the focal fish toward a model of a predator, European perch (*Perca fluviatilis*), with joined soft body that moves realistically when moved remotely using a thread disturbed (Kozak and Boughman 2012). European perches naturally occur in our field sites are considered one of the primary predators of sticklebacks. The focal fish was moved from its home-tank into a bigger, novel tank (60 cm × 30 cm × 30 cm) with three compartments, filled with 10 cm of water. Of the three compartments, the predator model was presented in the left compartment while the focal fish was released from the right compartment. The space between the ‘fish’ compartment and ‘predator’ compartment was divided into eight equally spaced grids with one fish-distance (6 cm) between the subsequent grids (Fig. 2.1). The focal fish was first placed into the fish compartment of the tank and the opaque barrier was removed. Subsequently, the focal fish could explore the novel tank for a period of 120 s without the predator being visible. After that period, the focal fish was gently pushed back into the fish compartment and the opaque barrier was replaced. Meanwhile the opaque barrier to the predator model was removed and replaced by a transparent barrier. The barrier of the fish compartment was removed again and the boldness trial of 300 s was recorded in a camera. In the subsequent video-scoring, the latency to exit the fish compartment, the number of inspection bouts (i.e. directed swimming towards the predator crossing at least one square and ending when the fish swam back into the opposite direction), the total duration of inspection bouts, the number of predator visits (i.e. visiting the last grid next to the predator compartment, < 6 cm), the total duration spent near the predator compartment and the minimum distance to the predator compartment were recorded. If a fish did not exit, its latency to exit amounted to the maximum or 300 s and all other values were recorded as NA (adapted from Wilson and Godin 2009). At least half of the water was replaced after

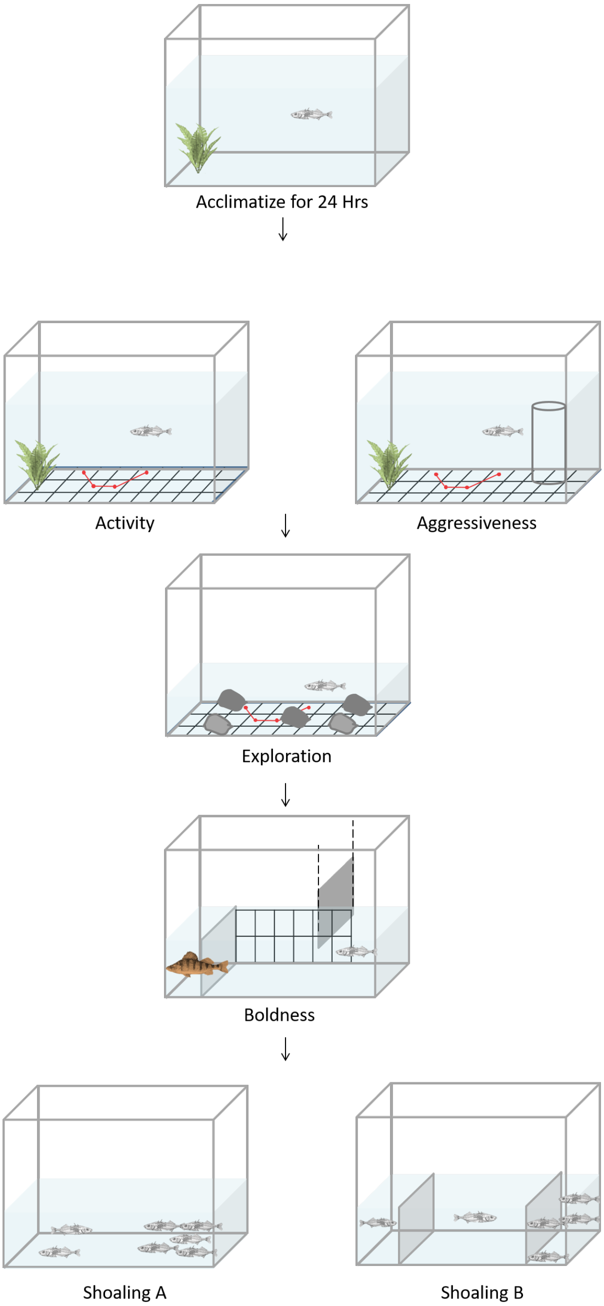


Figure 2.1 – ***Behavioral assays***. *The flow chart represents the order in which assays were performed along with illustration of different behavioural assays and the placement of grids used for extracting different parameters.*

testing 10 fish in the arena. We used the number of inspection bouts towards the predator (number of inspection bouts performed in the first minute after the focal fish entered the arena) as a proxy for ‘boldness’. This measure is preferable to latency to exit, as it is less related to activity, and to time spent near predator, as it takes into account the total time the fish spent in the test arena.

Shoaling - A

In 2018, individual shoaling tendency was scored in a group of ten fish. Fish that were captured on the same day and within the same population were placed into a larger tank (60 cm × 30 cm × 30 cm) filled with 10 cm of water where they could interact freely with each other (Fig. 2.1). After 120 s, all shoaling fish and then all non- shoaling fish were caught, identified and shoal composition noted. Fish were considered to shoal if they associated with another fish within one-fish distance (< 6 cm) at the end of the test. The procedure was repeated three times to calculate a shoaling score or ratio (1.0 is when individual was found to be associated with the shoal in all three trials, adapted from Wark et al. 2011b)

Shoaling - B

The shoaling assay conducted in 2018 adopted a setting where individuals were able to interact with one another. However, this captured very little among-individuals differences. Hence, we readjusted this test in 2019 by assaying individual shoaling in a large tank divided into three compartments: a central testing arena where the focal fish was released and two end compartments containing the stimulus shoal ($n = 5$ unfamiliar conspecifics) and two distractor fish ($n = 2$ unfamiliar conspecifics) (Fig. 2.1; adapted from Wark et al., 2011). The stimulus shoal and distractor fish comprised of migrants if the focal fish was migrant and residents otherwise. The stimulus shoal and distractor fish compartments were switched in sides to prevent a place or side bias and the fish were replaced with new stimulus shoal and distractor fish after five trials. At the start of the test, the focal fish was allowed to acclimatize for 120 s in the central arena without viewing the ends compartments that were covered with opaque barriers. The focal fish was returned to its home-tank momentarily and the opaque barriers were replaced with transparent barriers. The focal fish was then reintroduced to the center of the focal arena to record its shoaling behaviour for 300 s (shoaling time, spending one fish-distance (< 6 cm) from the shoal). The water was partially replaced after testing 10 fish in the arena.

Statistical analyses

To test whether resident and migrant fish differ in the proportions of the three common lateral plate morphs, we used a Chi-squared test for each year separately. We then analyzed variation in standard length and all the behavioural traits measured (activity, aggression, exploration, standardized scores for predator inspection and shoaling) in Linear Mixed Models (LMMs) with Gaussian errors. Status (resident vs. migrant, with migrant being the reference category), year (2018 vs. 2019, 2019 being the reference category) and status × year interactions were included as fixed effects. Date and time of testing were not significant and were thus removed from the models. In all models, observer ID and the combination of population ID-year (four populations in 2018 and three populations in 2019, giving seven levels) were fitted as random effects. We did not detect any sex differences in any of the behaviours (Supplementary Table. 2.A1), and thus decided to pool data from both sexes. Fish that became sick or parasitised were removed from the analyses ($n = 65$). All GLMMs were constructed in R v. 3.6.1 R Core Team (2021) using the lmer function of the 'lme4' package (Bates et al. 2015). The statistical significance of fixed effects was assessed based on the 95% confidence interval (CI): an effect was considered significant when its 95% CI did not include zero. The sample sizes slightly varied between tests due to missing data and are reported with the outcome of each statistical test.

To establish the existence and structure of behavioural syndromes in migrants and residents, we ran multivariate mixed models that estimate covariances and correlations among all traits. However, due to lack of model convergence, covariances could not be estimated this way. Other advocated methods (e.g. structural equation modelling (SEM); Dingemanse et al. 2010) could not be applied due to limited sample sizes. Hence, we estimated syndromes based on pairwise Spearman correlation with sequential Benjamini and Hochberg correction for multiple testing (Benjamini and Hochberg 1995). Data were zero-inflated in some behaviours (Aggression in residents; Activity and Aggression in migrants). We discarded these behaviours from the correlational analyses to prevent spuriously high correlation coefficients. Correlation analyses focus on pairwise relationships between traits, thus ignoring higher-order effects (Dingemanse et al. 2010). To overcome this, we also compared the results of a PCA approach to summarize the structure of relationship between all the behaviours within categories of migrants and residents between the years, which did not yield qualitatively different results (not shown).

Results

Morphological differentiation

Residents had more low-plated forms compared to migrants in 2018, but not in 2019 (Fig. 2.2), although the difference between migrants and residents seem to display a similar pattern in both years. This is confirmed by chi-square test on the relative proportions of lateral plate morphs between residents and migrants (Proportions of fully, partial and low plated morphs in 2018 = 0.44, 0.47, 0.09 in migrants and 0.15, 0.30, 0.56 in residents respectively; $\chi^2(df = 2, N = 247) = 64.536, p < 0.01$ and in 2019 = 0.68, 0.18, 0.15 in migrants and 0.56, 0.16, 0.28 in residents respectively; $\chi^2(df = 2, N = 66) = 1.785, p = 0.410$). Residents were significantly smaller than migrants in both years. All fish were also larger in 2019 compared to 2018 (Table 2.2; Fig. 2.2).

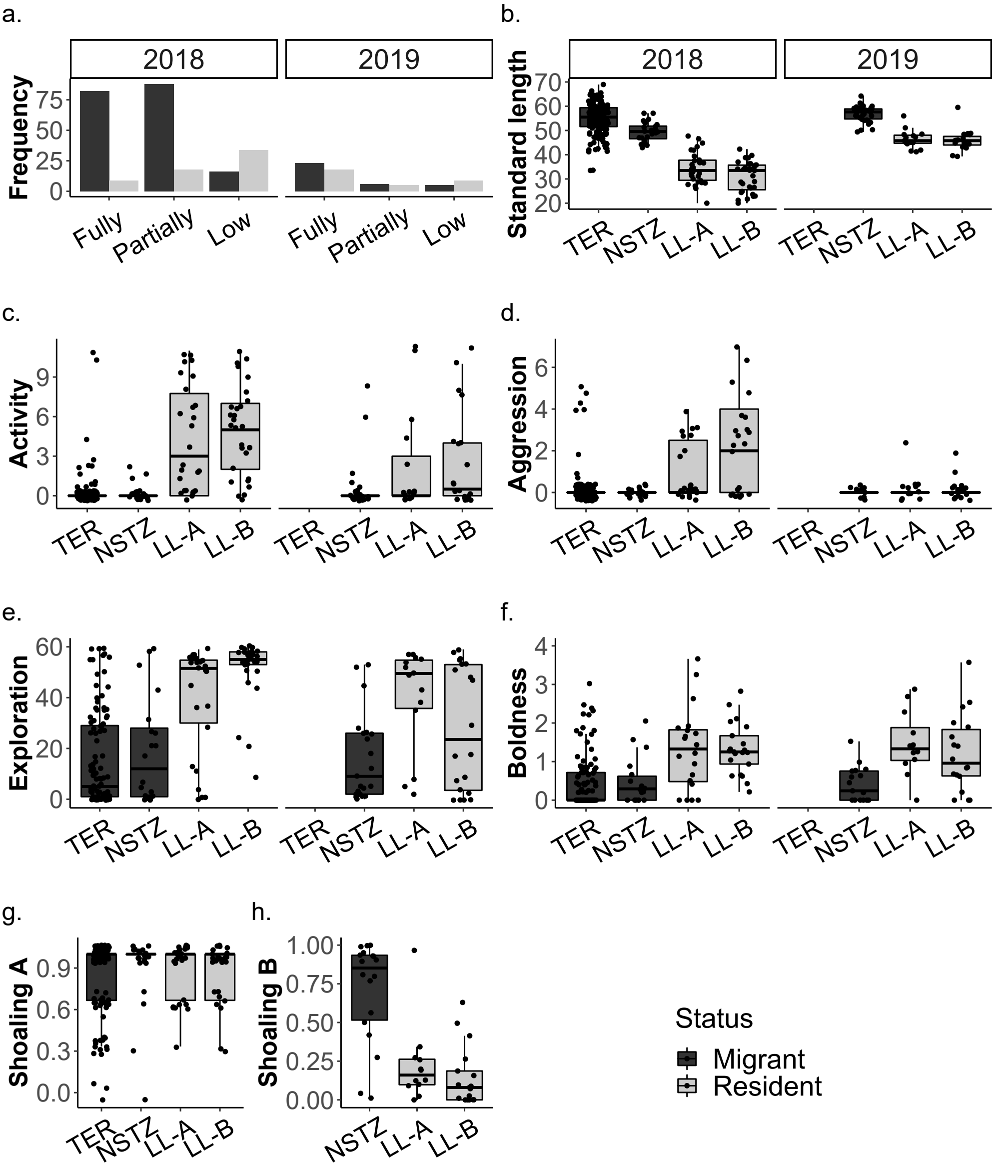


Figure 2.2 – ***Individual morphology and behaviours (median ± quartiles) of populations of residents and migrants over two years.*** *a. Lateral plate morph distribution (N_{2018} = 247, N2019 = 66), b. Standard length (N_{2018} = 249, N_{2019} = 72), c. Activity – number of square changes (N_{2018} = 203, N_{2019} = 56) d. Aggression – number of bites to intruder (N_{2018} = 187, N_{2019} = 44), e. Exploration – number of square changes (N_{2018} = 183, N_{2019} = 54), f. Boldness – number of inspection bouts/minute (N_{2018} = 164, N_{2019} = 48), g. Shoaling A – only 2018, fraction of trials spent with shoal (N_{2018} = 180), h. Shoaling B – only 2019, fraction of time spent near stimulus shoal (N_{2019} = 46)*

Behavioral differentiation

In both years, residents were significantly more active (87.5% of the migrants did not exhibit movements at all in their home-tanks), more exploratory and bolder compared to migrants (Table 2.2, Fig. 2.2 respectively). Compared to previous studies in sticklebacks (Huntingford 1976; Bell 2005; Dingemanse et al. 2007), we found only a marginal proportion of aggressive individuals outside of the breeding period. In 2018, residents were significantly more aggressive than migrants and in 2019, this pattern disappeared (significant status × year in Table 2.2; Fig. 2.2). The shoaling A assay performed in 2018 did not reveal differences between residents and migrants. However, the shoaling B assay performed in 2019 showed that residents shoaled much less than migrants (Table 2.2; Fig. 2.2). In addition, males and females did not differ significantly in terms of behaviours within migrants and residents (Supplementary Table. 2.A2).

Behavioral syndromes

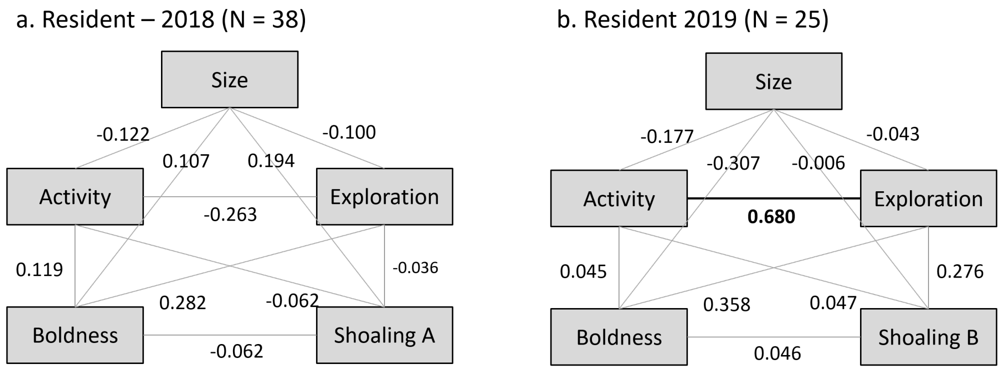
Behaviors were not correlated and there was little evidence for the existence of syndromes in both populations: only two of the 32 pair-wise correlations were significant after correcting for multiple testing and the correlation structure was not stable across years in either group (Fig. 2.3). In 2018, the only significant result was the positive correlation between exploration and predator inspection in migrants (Fig. 2.3, Supplementary Table. 2.A3; $\rho = 0.29$, $p_{corrected} = 0.009$). In 2019, the only significant result was the positive correlation between activity and exploration in residents (Supplementary Table. 2.A3; $\rho = 0.68$, $p_{corrected} = 0.002$). Most of the other correlations were far from significant.

Discussion

This study investigated if resident populations of sticklebacks, which are cut off from the sea due to human water management measures in the 1970s, exhibit consistent morphological and behavioural differences compared to their ancestral migrant counterparts. Our results reveal that ∼50 generations of isolation were sufficient to induce substantial morphological and behavioural differences.

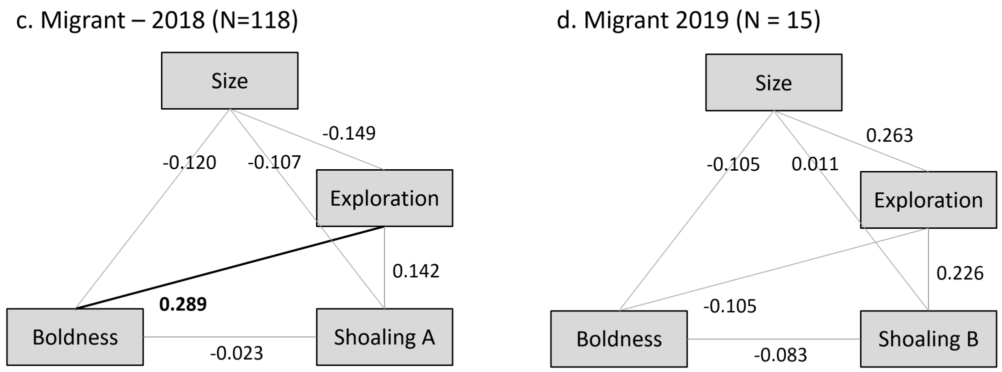
Phenotypic divergence between derived residents and ancestral migrants

We found clear phenotypic differentiation between migrants and residents in almost all traits studied in both years. In line with previous literature on morphological adaptations of sticklebacks to freshwater that occurred over the last glaciation event (∼12,000 years), we found that residents were about half the size of migrants and were characterized mostly by low-plated forms. Although the resident fish from 2019 comprised of more fully plated forms than the residents in 2018, they showed reduction in plate width (pers. obs.) in contrast to the robust armature spanning the width of the body, in fully- or partially-plated migrants. Lateral plate polymorphisms take many forms in sticklebacks, from variation in the number of plates (Reimchen et al. 2013; Eriksson et al. 2021) to variation in thickness and width of plates (Leinonen et al.



a. Resident – 2018 (N = 38)

b. Resident 2019 (N = 25)



c. Migrant – 2018 (N=118)

d. Migrant 2019 (N = 15)

Figure 2.3 – ***Syndrome structure of migrants and residents in two years.*** *Significant correlations after sequential Benjamini-Hochberg correction are represented with bold black lines. The numerical values represents pairwise Spearman correlation coefficients (rho).*

2012; Wiig et al. 2016), depending on the ecological conditions. In our case, we find reduction in lateral plate coverage via reduction in the width of plates, similar to that observed in populations with limited allelic variation for low-plated morph (Leinonen et al. 2012).

The morphological difference between populations are most easily explained by the necessity for flexibility to maneuver through vegetation in residents as compared to the demanding robustness and swimming abilities for migrants (Tudorache et al. 2007; Dalziel et al. 2012; Dalziel and Schulte 2012) and decreased resource availability in freshwater during growth (Snyder 1991; Wund et al. 2012). Furthermore, the reduction in number and size of lateral plates are also known to occur in response to different predator regimes present in the freshwater system (with fewer piscivorous predators and mainly dominated by invertebrate predators like dragonfly naiads) through selection on *EDA* gene underlying lateral plate polymorphism (Colosimo 2005; Marchinko and Schluter 2007; Leinonen et al. 2011). These observations therefore suggest that the reduced size and the reduced armament of our resident fish likely follow the same pattern of adaptive evolution seen during freshwater colonization of marine sticklebacks over the last glaciation event.

Table 2.2 – **Summary of linear mixed models on behaviour and morphology:** The table gives the summary of the LMMs on the selected variable for each behaviour and morphology. Estimates of fixed effects (β) are given with their 95% confidence intervals (CI) computed by bootstrapping method and variance components (σ^2) are given with their standard deviation (SD). Significant fixed effects are denoted in bold. Sample size (N) represents number of individuals. For shoaling assays, the analyses were done separately for each year as they were different procedures and analyzed using linear models.

	Size (N=317)	**Activity (N=258)**	**Aggression (N=233)**	**Exploration (N=239)**	**Boldness (N=214)**	**Shoaling-a (N=181)**	**Shoaling-b (N=47)**
Fixed effects	β **(95% CI)**	β **(95% CI)**	β **(95% CI)**	β**(95% CI)**	β **(95% CI)** β **(95% CI)**	β **(95% CI)**	
Intercept	**52.428**	0.362	0.181	**19.280**	**0.754**	**0.846**	**0.706**
	(48.551, 56.051)	(-0.398, 1.108)	(-1.258, 1.562)	**(7.634, 30.471)**	**(0.080, 1.411)**	**(0.803, 890)**	**(0.580, 0.832)**
Status (Resident)	**-19.808**	**4.337**	**3.090**	**29.014**	**0.716**	0.036	**-0.526**
	(-25.109, -14.505)	**(3.402, 5.351)**	**(1.038, 5.239)**	**(16.430, 41.321)**	**(0.332, 1.064)**	(-0.043, 0.116)	**(-0.687, -0.366)**
Year (2019)	4.386	0.614	2.546	2.466)	-0.166		
	(-2.409, 11.190)	(-0.534, 1.722)	(-0.334, 5.555)	(-16.140, 18.475)	(-0.795, 0.475)		
Status Resident × Year 2019	**9.110**	**-3.03**	**-5.367**	-17.183	0.367		
	(0.430, 17.688)	**(-4.799, -1.154)**	**(-9.013, -1.501)**	3 (-37.133, 2.270)	(-0.509, 1.231)		
Random effects	σ^2 **(SD)**	σ^2 **(SD)**	σ^2 **(SD)**	σ^2 **(SD)**	σ^2 **(SD)**		
Observer ID	-	0.171 (0.414)	-	44.720 (6.687)	0.350 (0.591)		
Population - Year	6.736 (2.595)	0.000 (0.000)	0.814(0.902)	25.000(5.000)	-		
Residuals	32.780 (5.725)	6.109 (2.472)	10.138(3.184)	346.810 (18.623)	1.185 (0.920)		

As for individual behavioural scores, we found that residents were more active, aggressive (in the year 2018), exploratory, bolder and showed lower shoaling tendencies than migrants (in the year 2019). The majority of our findings with wild-caught sticklebacks are in line with the only other study that compared similar behaviours in populations of residents and migrants in lab-bred F1 sticklebacks (Di-Poi et al. 2014). In this study, the authors found that residents were more active, more aggressive and shoal less than migrants. Functional explanations for the behavioural differences can be given, but they include quite some speculation. Compared to the sea, land-locked ditches in our study sites are characterized by small and shallow streams, enriched with vegetation, low mean annual productivity (Gross et al. 1988), lower density of piscivorous fish yet with the presence of invertebrate predators (Reimchen 1980; Marchinko 2009) and birds. Hence for residents selection may favor higher levels of aggression and exploration that facilitate the discovery, acquisition and monopolization of limited resources (Budaev 1997; Brown et al. 2005; Huizinga et al. 2009; Herczeg et al. 2013; Greenwood et al. 2016; Moran et al. 2017). Such 'risk-prone' behaviours may then be traded-off against shoaling, explaining why residents shoaled less compared to migrants (Ward et al. 2004). Differences in shoaling tendencies may also stem from the fact that migratory lifestyle involves group schooling during migration and presumably high shoaling tendencies in the sea due to 'openness' of habitats. In migrants, lowered activity level could further be an indication that freezing is an adaptive response to higher perceived predation when not protected by a shoal (Huntingford and Wright 1993). Furthermore, the robust armature and larger spines, characteristic of migrants, are known to impede them in escape behaviour, thus potentially favoring freezing behaviour (Andraso and Barron 1995). In addition, reduced aggressive interactions could be due to the highly shoaling lifestyle of migrants as these two behaviours were shown to be incompatible in sticklebacks (Lacasse and Aubin-Horth 2014). Despite the substantial differences in ecological conditions across the two study years, the differences in morphology and behaviour between migrants and residents were relatively consistent, suggesting that the observed population differences are related to the different life styles of migrants and residents, rather than due to stochastic annual changes.

In our system, we know with reasonable certainty that the resident populations have been isolated from the migratory ancestors about fifty generations ago due to barriers. However, some divergence may already have taken place before the isolation event. For example, local stickleback populations that are located further away from the sea may exhibit partial migration (McKinnon et al. 2004), that is, a polymorphism where only part of the population migrates to the sea, while the rest of the population overwinters on site. If our 'resident' populations originated from such partially migrating populations, the adaptations to a resident lifestyle may, to a certain extent, already have been in place. Additionally, the barriers to migration, especially the pumping station at LL-B (Supplementary Fig. 2.A1) pose as impenetrable barriers only to adult sticklebacks. Juveniles and fry may potentially cross over, especially to the open river with the direction of water flow and consequently making it possible to have reduced and biased gene flow from resident to ancestral migrant population. There exist many other possibilities for gene flow, e.g. transportation of eggs/fry via birds. In any case, it is even more surprising that that the stark behavioural differences in wild-caught fish from these resident and migrant populations exist, despite the potential of reduced gene flow to hamper local adaptation (Raeymaekers et al. 2014). Further population genetic studies are needed to uncover exact population structure and divergence in our system.

Rates of phenotypic change

Is the rate of phenotypic differentiation observed in our study comparable to other documented instances of rapid evolution? The literature on population differentiation in response to anthropogenic changes (Hendry et al. 2008), specifically in sticklebacks, acts as a useful yardstick (even though most of this literature only considers morphological traits). Quantifying rates of change in 'haldanes' (Haldane 1949) yields a measure that allows comparisons across populations and study systems. One way of calculating this is by quantifying absolute change in trait standard deviations per generation. In our study, we found that rate of change in size was -0.007 haldanes; in other words, the size of resident fish decreased by 0.007 standard deviations per generation. The rates of change in our behavioural measures were 0.01 haldanes for activity, 0.001 haldanes for exploration, 0.014 haldanes for boldness and -0.149 haldanes for shoaling behaviour. Overall, these values are quite comparable to the evolutionary rates reported for diverse traits in sticklebacks (Bell and Aguirre 2013) and other organisms (Hendry et al. 2008). In contrast, the rate of change in size was much smaller than the rate reported in another anadromous-freshwater system of three-spined sticklebacks (0.234 haldanes for females and 0.365 haldanes for males, Baker et al. 2011). It is possible that the values reported above are underestimates of the rates of change that occurred in the initial phase after isolation: we averaged the rates of change linearly over 50 generations, while a major part of differentiation typically occurs in the first few generations of isolation. Indeed more recent studies on sticklebacks isolated from marine to freshwater habitats have found evidence for evolution on contemporary timescales of decades to even seasons (Lescak et al. 2015; Hosoki et al. 2020; Garcia-Elfring et al. 2021)

Population differences in syndromes

A previous study with twelve freshwater stickleback populations reported a positive correlation between boldness and aggression toward a conspecific in five out of the six populations where predators were present (Dingemanse et al. 2007, 2009). There were also tight correlations among other behaviours including activity, exploration, aggressiveness and boldness in predator-sympatric populations (correlation coefficients range from 0.03 to 0.74). These tight behavioural correlations are thought to result from predation that enhances habitat heterogeneity by creating risky and non-risky areas and thus favors alternative behavioural strategies (e.g. Bell and Sih 2007; Dingemanse et al. 2007; Dhellemmes et al. 2020). Surprisingly, (but in line with an earlier study on freshwater and marine sticklebacks; Di-Poi et al. 2014), none of our stickleback populations, including migrants that should be exposed to higher predation pressure, exhibited stable syndromes across years and only few correlations between traits were detected. Boldness–Exploration was one of the stronger correlations in migrants ($\rho = 0.289$), but still was weaker compared to previous studies ($\rho = 0.667$, Dingemanse et al.

2007). Activity–Exploration syndrome in residents was observed in the second study year ($\rho = 0.680$), which was comparable to those reported from predator-sympatric populations ($\rho = 0.754$, Dingemanse et al. 2007). This lack of syndromes could be because the behaviours selected are not under correlated selection or that we lack the power to detect syndromes. Alternatively, in our system, predation risk and change in life-history may not systematically select for phenotypic trait integration (Sommer-Trembo et al. 2017).

Conclusions

We have shown that behaviour and morphology diverged in sticklebacks after human disturbance, blocking migration over about 50 generations. The observed phenotypic differences between migrants and residents clearly show that barriers to migration have thus major consequences for the phenotype and potentially life-histories and population dynamics of sticklebacks as correlated life-history characteristics (growth rate, size at maturity, number and size of eggs) are also known to change on adaptation to freshwater in sticklebacks. Nevertheless, at least some populations can cope to a drastic loss of migration opportunity as they seem to thrive in land locked conditions. Next step would be to test whether the observed divergence is adaptive and to identify how it came about. One way to delineate the relative roles of genetic inheritance, non-genetic inheritance, developmental plasticity and phenotypic plasticity is through common-garden experiments combined with cross-fostering experiments and through experiments where juveniles are exposed to different selective regimes in semi-natural mesocosm. This would give us insight into role of personality in adaptation to novel environmental conditions.

Acknowledgements

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Appendix

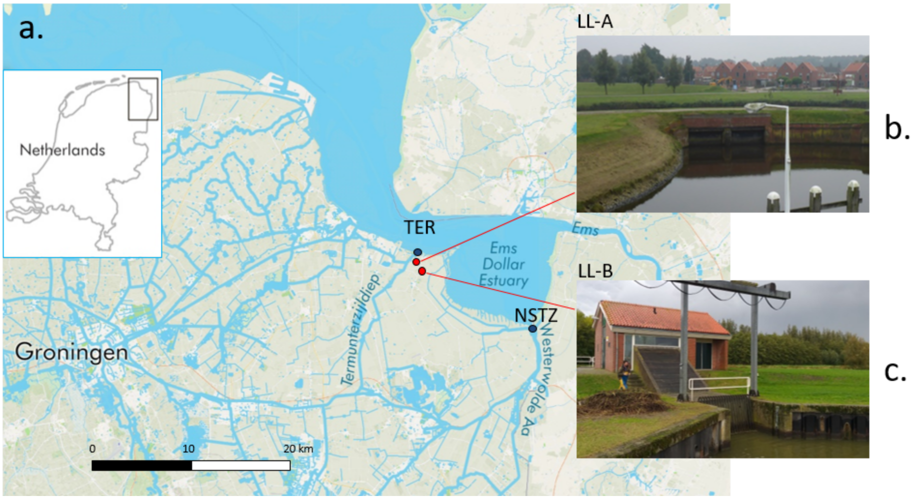

Figure 2.A1 – ***Map of the sampling sites and images of the barriers to migration in front of land-locked sites.*** *a). Blue dots indicate the locations of the two open sites TER and NSTZ where incoming migrants were caught and red dots indicate the two land-locked sites LL-A and LL-B. b) LL-A is blocked by a sluice, which has not been operational and hence closed for the last 50 years. c). LL-B is blocked by a pumping station, which allows water flow but blocks fish movement. The two land-locked locations are potentially connected but ∼3 km apart in two locally different habitats. LL-A is within wooded areas, with low human activity while LL-B is located in open farms with little shade and frequent human activity. Photo courtesy: P.P. Schollema, Waterschap Hunze en Aa's*

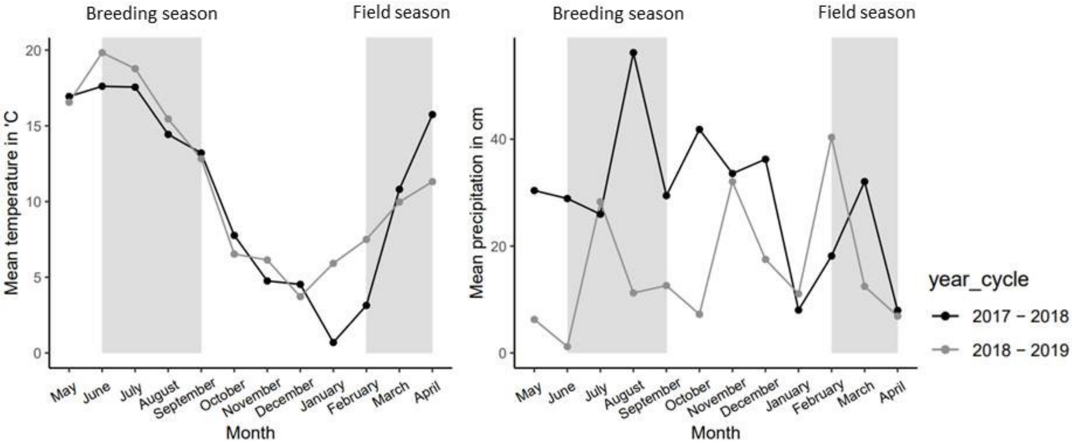

Figure 2.A2 – Mean monthly temperature and precipitation of two year-cycles of weather station Lauwersoog ($53°2′37.6″, 6°12′44.8″$), which closely represents the weather conditions of the coastal areas in the north of the Netherlands. Field season refers to the migratory period during which sticklebacks are caught. Breeding season refers to the breeding window, which coincides with spring and summer.

Table 2.A1 – Results of principal component analysis on morphology and individual behaviours containing more than one variable: The trait, its variables and their corresponding cos2 value (or) contribution to the principal components along with the percentage of variance explained by each principal component are given.

(a) **Morphology**	**Component (1)**
Weight	0.939
Total length	0.982
Standard length	0.969
Eigenvalue	2.9
% variance explained	96.68

(b) **Activity test**	**Component (1)**
Number of square changes	0.991
Number of unique squares	0.98
Total distance travelled	0.972
Eigenvalue	2.887
% variance explained	96.23

(c) **Exploration test**	**Component (1)**
Number of square changes	0.986
Number of unique squares	0.947
Total distance travelled	0.932
Eigenvalue	2.867
% variance explained	95.55

Table 2.A2 – Behavior does not significantly differ between the sexes. Means and corresponding Mann-Whitney U statistic for size and individual behaviours, are given separated for migrants and residents females ($N_{migrant} = 8$ females and 7 males, $N_{resident} = 14$ females and 8 males). Shoaling was not compared as we had limited sample size for the two different shoaling assays.

Trait (Status)	Mean (Male)	Mean (Female)	Mann-Whitney U	*p*
Standard length (Mig)	55.1	59.44	17.5	0.247
Standard length (Res)	42.49	44.66	57	0.729
Activity (Mig)	0.86	0.5	23	0.503
Activity (Res)	1.44	2.36	57	0.708
Aggression (Mig)	0	3.75	24.5	0.423
Aggression (Res)	4.89	0.21	78	0.206
Exploration (Mig)	14.71	23.88	24.5	0.728
Exploration (Res)	50.22	42.14	77	0.388
Boldness (Mig)	0.53	0.12	40.5	0.142
Boldness (Res)	1.41	1.57	65.5	0.9

Table 2.A3 – Correlations between behaviours and body size per status and year. The different behaviour-behavior and behaviour – morphology pairs are correlated using Spearman's correlation coefficient (ρ) and reported with p-values, along with Benjamini and Hochberg correction for multiple testing. The correlations are reported separately for residents and migrants in the years 2018 and 2019. Significant correlations after correction are denoted in bold.

Resident 2018 (N=38)				**Migrant 2018 (N=118)**			
Correlations	ρ	p	p_{corr}	**Correlations**	ρ	p	p_{corr}
Act – Exp	0.263	0.111	0.555	**Exp – Pred**	**0.289**	**0.0015**	**0.009**
Act – Pred	0.119	0.475	0.786	Exp – Shoal	0.142	0.124	0.248
Act – Shoal	-0.062	0.712	0.791	Exp - Size	-0.149	0.106	0.248
Act – Size	-0.122	0.464	0.786	Pred – Shoal	0.023	0.803	0.803
Exp – Pred	0.282	0.087	0.555	Pred – Size	-0.12	0.195	0.293
Exp – Shoal	-0.036	0.83	0.83	Shoal - Size	-0.107	0.247	0.296
Exp - Size	0.1	0.55	0.786				
Pred – Shoal	-0.062	0.711	0.791				
Pred – Size	0.107	0.522	0.786				
Shoal - Size	0.194	0.243	0.786				
Resident 2019 (N=25)				**Migrant 2019 (N=15)**			
Correlations	ρ	p	p_{corr}	**Correlations**	ρ	p	p_{corr}
Act –Exp	**0.68**	**0.0002**	**0.002**	Exp – Pred	-0.105	0.71	0.923
Act – Pred	0.045	0.831	0.93	Exp – Shoal	0.226	0.417	0.923
Act – Shoal	0.047	0.824	0.93	Exp - Size	0.263	0.343	0.923
Act – Size	-0.177	0.398	0.796	Pred – Shoal	-0.083	0.769	0.923
Exp – Pred	0.358	0.079	0.395	Pred – Size	-0.15	0.594	0.923
Exp – Shoal	0.276	0.182	0.455	Shoal - Size	0.011	0.97	0.97
Exp - Size	-0.043	0.837	0.93				
Pred – Shoal	0.046	0.827	0.93				
Pred – Size	-0.307	0.136	0.453				
Shoal - Size	-0.006	0.977	0.977				

3

Does genetic differentiation underlie behavioural divergence in response to migration barriers in sticklebacks? A common garden experiment

A. Ramesh
M. M. Domingues
E.J. Stamhuis
T.G.G. Groothuis
F.J. Weissing
& M. Nicolaus

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Abstract

Water management measures in the 1970s in the Netherlands have produced a large number of 'resident' populations of three-spined sticklebacks that are no longer able to migrate to the sea. This may be viewed as a replicated field experiment, allowing us to study how the resident populations are coping with human-induced barriers to migration. We have previously shown that residents are smaller, bolder, more exploratory, more active, more aggressive, exhibited lower shoaling and lower migratory tendencies compared to their ancestral 'migrant' counterparts. However, it is not clear if these differences in wild-caught residents and migrants reflect genetic differentiation, rather than different developmental conditions. To investigate this, we raised offspring of four crosses (migrant ♂ × migrant ♀, resident ♂ × resident ♀, migrant ♂ × resident ♀, resident ♂ × migrant ♀) under similar controlled conditions and tested for differences in morphology and behaviour as adults. We found that lab-raised resident sticklebacks exhibited lower shoaling and migratory tendencies as compared to lab-raised migrants, retaining the differences in their wild-caught parents. This indicates genetic differentiation of these traits. For all other traits, the lab-raised sticklebacks of the various crosses did not differ significantly, suggesting that the earlier-found contrast between wild-caught fish reflects differences in their environment. Our study shows that barriers to migration can lead to rapid differentiation in behavioural tendencies over contemporary timescales ($\sim$50 generations), and that part of these differences reflects genetic differentiation.

Introduction

Habitat fragmentation resulting from human activities is considered to be a major threat for many animal populations (Foley et al. 2005; Fischer et al. 2017). Habitat fragmentation is characterized by a reduction in habitat size, habitat loss, and loss of habitat connectivity (Fahrig 2003). This poses a threat to animal populations, especially for migratory species which rely on connectivity between functional habitats for reproduction and survival (Legrand et al. 2017). Migratory species would thus need to respond via adaptive changes in life history and behaviour to thrive in disconnected patches (Bohlin et al. 2001; Kraabøl et al. 2009; Junge et al. 2014). Therefore, understanding the underlying mechanisms of these responses is crucial as they directly affect the future adaptive potential and evolutionary trajectories of populations (Kawecki and Ebert 2004; Wang and Bradburd 2014) as well as conservation measures (Stockwell et al. 2003).

Individuals need to maintain a match between their phenotypes and the environment to enhance their local performance, thereby allowing populations to subsist or grow in an altered environment. Depending on the underlying mechanism involved, such adaptive responses may occur more or less rapidly and may influence population genetic structure (Hedrick et al. 1976; Hedrick 2006; Nicolaus and Edelaar 2018). For example, phenotypic adjustment may result from natural selection favoring some phenotypes over others, potentially leading to population genetic differentiation across multiple generations when phenotypic variation has a genetic basis (Kawecki and Ebert 2004). Non-exclusively, individuals may match their phenotype to local conditions through plasticity, be it reversible plasticity (or phenotypic flexibility sensu Piersma and Drent 2003, developmental plasticity, or transgenerational plasticity) through parental and epigenetic effects. Plasticity, defined as the ability of a genotype to exhibit different phenotypes in response to the environment (Via et al. 1995; Pigliucci 2005), can thus provide a rapid mechanism to respond to environmental changes (Ghalambor et al. 2007). Importantly, selection may favor genotypes with varying levels of plasticity (Scheiner 1993; Nussey et al. 2007), implying that mentioned mechanisms are intertwined (Edelaar et al. 2017) and that observed population divergence could reflect genetic differentiation and/or differences in the environments under which individuals grow up. In migratory species, migrants would have to exhibit phenotypic plasticity or bet-hedging strategies, as they are exposed to different environmental conditions (Botero et al. 2015). In the case where migrants are no longer able to migrate (forced 'residents'), we expect selection to act on either the traits themselves or on the degree of plasticity.

In this study, we focus on behaviour as it is the primary way through which animals interact with their environment and respond to changes (Wong and Candolin 2015). behaviour is often considered highly flexible and hence less prone to genetic divergence in response to environmental changes. However, plastic responses could evolve rapidly through genetic divergence compared to fixed traits (Van Gestel and Weissing 2018). In addition, 'animal personality' research points that behaviours are highly structured and form correlations over time (consistency) and over contexts (syndromes; Réale et al. 2007; Stamps and Groothuis 2010b; Wolf and Weissing 2012). Furthermore, individual differences within populations are often repeatable (Réale et al. 2007) and to some extent, heritable (Dochtermann et al. 2019). As a consequence, personality variation may retard or accelerate rates of microevolution and population divergences (Wagner and Altenberg 1996; Wolf and Weissing 2012; Dochtermann and Dingemanse 2013; Van Gestel and Weissing 2018). Here we aim to study whether genetic differentiation underlies the rapid behavioural differentiation following habitat fragmentation. We capitalize on an unintended field experiment in the north of the Netherlands, where the construction of pumping stations in the 1970s has led to the forced residency of replicate populations of anadromous three-spined sticklebacks (*Gasterosteus aculeatus*). A previous study in this system has revealed extensive phenotypic differentiation (morphology and behaviour) between the ancestral 'migrant' and its derived 'resident' populations (Ramesh et al. 2022b). Compared to migrants, wild-caught residents are smaller, more active and aggressive, more exploratory, bolder, and showed reduced shoaling and migratory tendencies (Ramesh et al. 2022b). These differences parallel the behavioural divergence reported between freshwater and marine populations of sticklebacks over ∼12,000 years (Di-Poi et al. 2014). However, it remains to be determined if similar behaviourally relevant genetic differentiation has evolved in our system over much shorter time scales (∼50 years). This knowledge is important because conservation efforts are underway to reconnect the waterways and therefore, we need to better understand the current state of fish populations in order to predict the eco-evolutionary consequences of barrier removal.

We conducted a common garden experiment to test whether genetic differentiation underlies the observed divergence in morphology and behaviour . We raised F1 juveniles from four types of crosses (migrant parents (MM), resident parents (RR), hybrids with a migrant mother (RM) and hybrids with a resident mother (MR); Fig 3.1a) under similar laboratory conditions and quantified variation in activity, exploration, shoaling, boldness, and migratory tendencies among these crosses. We expect that 1) if the behavioural differentiation is genetic, individuals of MM crosses will differ significantly from RR crosses (similar to their wild-caught parents, Fig. 3.1b) if the behavioural differences between wild-caught residents and migrants are induced by differences in their environments, there will be no differences between the 'common-garden' crosses (Fig. 3.1b) if parental effects are involved, we will see asymmetric changes in the reciprocal hybrid crosses (Fig. 3.1b). Specifically, if behavioural variation is strongly influenced by maternal effects, the hybrids resulting from the MR cross will have a similar score as the RR cross and the hybrids resulting from the RM cross will have a similar score as the MM cross (Fig. 3.1b). A similar trend can be expected in the case of paternal effects, but we eliminated that possibility to a large extent by raising juveniles without paternal care (Giesing et al. 2010; McGhee and Bell 2014; Heckwolf et al. 2018).

Methods

Study populations

The waterways in the Netherlands consist of rivers and canals that are open to the sea and of land-locked smaller ditches (<1 m deep) located in side polders. We caught incoming migrants at two sea locks ("TER" (53°18'7.24", 7°2'17.11") and "NSTZ" (53°13'54.49", 7°12'30.99")) whereas residents were caught in two land-locked polders ("LL-A" (53°17'56.14", 7°2'1.28") and "LL-B" (53°17'16.52", 7°2'26.46"); Ramesh et al. 2022b). Sticklebacks were caught over a period of four weeks between March and April in 2019. All individuals were transported to the laboratory within two hours of capture in aerated bags (5-6 fish / 3L bag). They were housed outdoors separated by their origin in groups of five fish in 50 liter aerated tanks filled with freshwater, exposed to the natural day-light cycles and temperatures. They were fed brine ad libitum with brine shrimps and blood worms (3F Frozen Fish Food bv.). Males were separated once they reached breeding colors, and females were checked daily for signs of gravidity.

Lab-bred F1 juveniles

Lab-bred F1 juveniles of resident, migrant, and hybrid sticklebacks arose from a partial factorial breeding design (Fig. 3.1a) using three resident males, three resident females, three migrant males, and three migrant females (six migrants from "NSTZ", five residents from "LL-A" and one resident female from "LL-B"). Each family consisted of all combinations of crosses between a male and female migrant and male and female resident, leading to F1 offspring of different crosses: pure migrant (MM) or resident (RR) and hybrids with migrant father and resident mother (MR) and vice versa (RM).

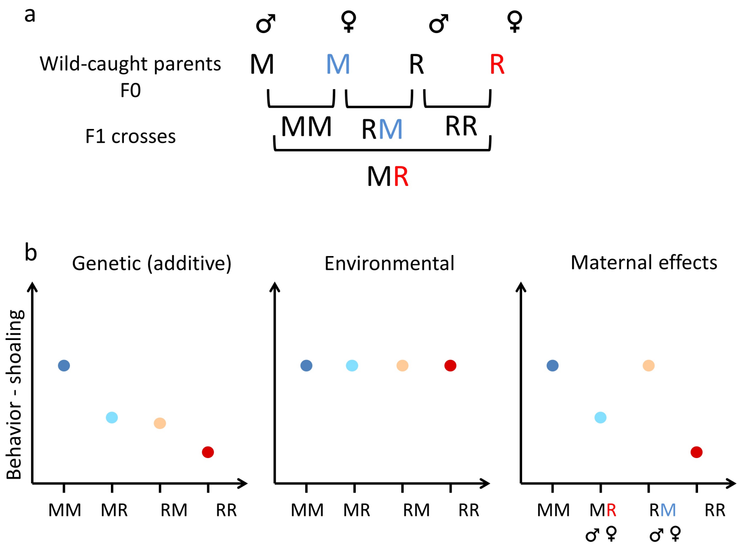

Figure 3.1 – ***Schematic of the breeding design.*** *a) We obtained four F1 crosses: migrant male × migrant female (MM), resident male × resident female (RR), migrant male × resident female (MR) and resident male × migrant female (RM); b) Expectations of mean behavioural scores (e.g., shoaling) if the underlying basis for behavioural differentiation in wild-caught parents is due to genetic differentiation, environmental experiences during development or through maternal effects. Letters of migrant and resident female in the maternal effects prediction plot are colored according to the origin for ease of interpretation of patterns in hybrids, when they are under the control of maternal effects. The expected mean value of hybrids, would correspond to the migrant or resident status of the female.*

From the offspring pool, a total of 40 fish were used per cross for the experiment, with each cross containing at least five fish from each family.

For obtaining F1 juveniles, we followed a split-clutch in-vitro fertilization protocol, where eggs of ripe females were stripped, then weighed and split into two halves for artificial insemination with sperm extracted from freshly euthanized migrant and resident fathers respectively (Barber and Arnott 2000). All offspring were raised without paternal care to prevent undesired long-lasting effects of father on offspring behaviour (McGhee and Bell 2014). The larvae hatched five to seven days after fertilization and started maintaining buoyancy and independent feeding one week after hatching. The fish larvae were fed a mixture of frozen cyclops, freshly hatched Artemia nauplii, and zebrafish diet (GEMMA Micro 75, Skretting, Tooele, Utah) daily. The densities never exceeded 40 fish larvae in 5 liter "home-tanks" (30 × 16 × 18 cm (L × W × H)). Once fish reached ~2 cm, they were isolated, assigning ten random individuals from the same family into separate home tanks. After this, the individuals were fed ad libitum with brine shrimps and blood worms (3F Frozen Fish Food bv.), and tanks were connected to the same water system at 16° C. The photoperiod was set at 16:8 (L:D), mimicking summer conditions during juvenile growth. When the fish reached a length of ∼4 cm, they received a unique identification (see below). We induced autumn conditions when the fish were ∼12-13 months old, characterized by 12:12 (L:D) photoperiod and temperatures being lowered to 13°C – 14°C. All fish were in non-breeding conditions and kept in autumn conditions during the period of experimentation. Experimentation started when fish were ∼15-16 months old.

Individual identification

When the juveniles reached 4 cm length (∼12 months), we used clipped spines or injection of an 8 mm Passive Integrated Transponder (PIT tag; Trovan, Ltd., Santa Barbara, California) for unique individual identification, We used PIT tag injection only for half of the tested fish (20 fish × 4 crosses = 80 fish), while the rest were tagged using a combination of dorsal and pelvic spine clipping (20 fish × 4 crosses = 80 fish). This was because PIT tag retention was low in these fish (∼15 % loss in the first week after tagging) and we did not re-tag the fish to prevent excess handling. PIT tags were injected in the abdominal cavity and under anesthesia following the standard protocol (following Cousin et al. 2012). During tagging/clipping, we also measured weight and standard length (the length from the tip of the snout to the base of the tail) as a proxy for size. Lateral plates were not very clearly visible in juvenile fish and hence were not measured. After individual tagging, we mixed juveniles from different families to be housed together in groups of ten in their home tanks while keeping them together with the same cross (MM, RR, MR or RM).

Large-scale movement tendencies in mesocosm (migratory tendencies)

For the subset of PIT tagged fish, movement assays were performed in semi-natural mesocosm before subjecting them to the lab-based tests. The mesocosm system consisted of five connected outdoor ponds of diameter 1.6 m connected by four pipes of length ∼1.5 m and diameter 11 cm, filled with water from a nearby freshwater ditch, with a linear flow in the system of connected ponds similar to those typically experienced in the canals and ditches (flow speed < 0.7 cm/s; Fig. 3.2a). This was done to create a cue for migration-like movement. All connecting tubes were fitted with circular PIT antennas around the entrance and exit of each pond to record fish movement between ponds. The sticklebacks were tested in pond experiments after at least one week of recovery from tagging. A group of ten fish of one cross (MM, RR, MR or RM) was introduced in the first pond and acclimatized for 5 hours in the first morning, after which the connection to the rest of the ponds was opened. We then recorded the movement of fish as the number of crossings between ponds for the next 16 h (∼4 p.m.-8 a.m.). We attempted to have 20 tagged fish/cross and tested them in groups of ten each, making it two groups/cross. However, due to tag loss, we ended up with <20 fish/cross. Instead of changing group size, which could have an effect on behaviour, we decided to spread the final number of tagged fish between two groups and supplement the remaining with untagged fish from the same cross to make up to ten. In total, two groups, each from a randomly chosen cross, were tested, making a total of eight groups

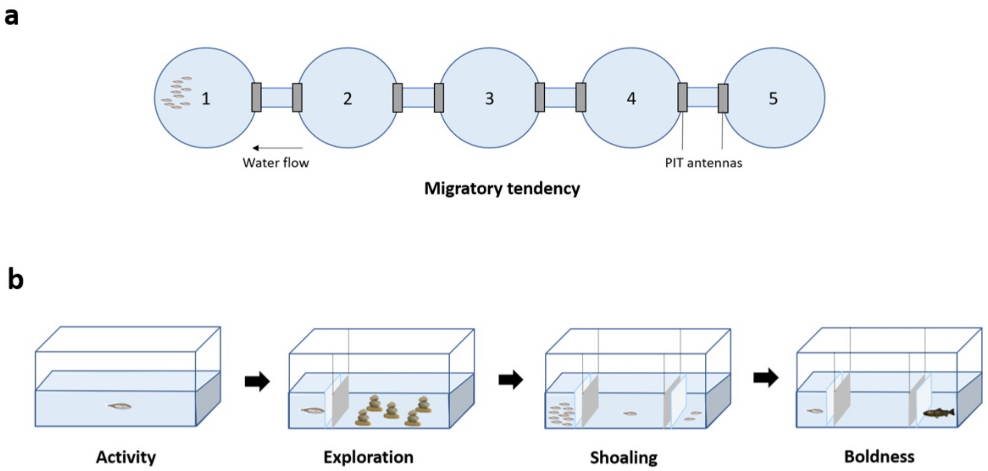

Figure 3.2 – ***Schematic of behavioural assays.*** *a) Migration tendencies were tested in a linear setup of five connected pond mesocosm with groups of 10 fish. There is water flow (rate ∼ 0.7 cm/s). PIT antennas are present at both ends of the corridors connecting the ponds. b) Lab assays were performed in the following order: Activity (Day 1), Exploration (Day 1), Shoaling (Day 2), Boldness (Day 3).*

with 56 fish ($N_{MM} = 12$, $N_{MR} = 17$, $N_{RM} = 15$, $N_{RR} = 12$). In groups with less than ten tagged individuals, untagged fish from the same cross were added to maintain constant group size.

Lab behaviour assays

Three days before testing, fish were selected randomly and acclimatized in visually separated and isolated tanks, same as their home tanks, at an ambient temperature of 19°C. We attempted to test 40 fish/cross, but some fish were lost due to mortality. Hence in total, 154 fish ($N_{MM} = 40$, $N_{MR} = 39$, $N_{RM} = 35$, $N_{RR} = 40$) were randomly selected for testing and split into four batches. One round of testing consisted of four batches of approximately ten fish of each cross and lasted one week where we assayed activity, exploration, shoaling, and boldness in that order (Fig. 3.2b). Overall, roughly 40 fish were tested each week. The interval between the first and the second round of testing of each individual was thus at least four weeks. Fish were returned to their home tanks between the testing rounds. The sample sizes for the second round was lower ($N = 151$) due to mortality between the two rounds ($N_{MM} = 39$, $N_{MR} = 38$, $N_{RM} = 34$, $N_{RR} = 40$). All lab-assays were filmed from the top using a Raspberry Pi camera (Raspberry Pi NoIR Camera Board V2 – 8MP, Raspberry Pi Foundation, UK) in tanks placed in illuminated wooden boxes to prevent external disturbance. behavioural assays were conducted in fixed order as below, and videos were analyzed using EthovisionXT (Noldus Information Technology bv.). In all tests, observers were blind with respect to the cross to which the test fish belonged and further bias was reduced by analyzing the videos using automated video tracking techniques.

Activity (day 1)

Activity of the fish was measured as the total distance the fish swam in a tank identical to its home tank during a total of 20 min (with 5 min for acclimatization).

Exploration (day 1)

Just after activity was recorded, the fish was isolated to one corner of the tank using a sheet partition, and the setup in the tank was changed. Five stone pillars extending above the water's surface were added in a specific position, forcing the fish to move around them. After 5 min, the sheet was removed remotely without opening the box, and the fish was recorded in this novel arena for 20 min. The total distance travelled by the fish in this novel environment was used as a proxy for exploratory tendency of fish as it highly correlates with space use (Ramesh et al. 2022b).

Shoaling (day 2)

For the shoaling assay, a larger tank (60 × 30 × 30 cm) was filled with water up to 10 cm height. The tank was divided into three compartments: the central testing arena where the focal fish was released and two end compartments containing the stimulus shoal (N = 10 unfamiliar conspecifics of mixed crosses), and the distractor fish (N = 2 unfamiliar conspecifics; adapted from Wark et al. 2011a). The position of the distractor and shoal fish compartments was switched to prevent biases and replaced with new distractor and shoal fish every seven tests. At the start of the test, the focal fish was allowed to acclimatize for 5 min in the central arena without viewing the end compartments which were covered with opaque barriers. Then the opaque barriers were lifted remotely from outside the box, and the response of the focal fish was recorded for the next 20 min. The water was refreshed after testing seven fish in the arena. In total, we had four groups of shoal fish and five pairs of distractor fish, which were randomly used to avoid biases. The proportion of time the focal fish spent within one-fish distance (6 cm) from the side containing the stimulus shoal was used as a proxy for shoaling.

Boldness (day 3)

In the boldness tests, we measured the responses of the focal fish toward visual cue of an European perch (*Perca fluviatilis*; model with soft body, Kozak and Boughman 2012) and olfactory predation cues (50 ml of water from freshly dead sticklebacks mixed with 50 ml of water containing live perch scent, Sanogo et al. 2011). The focal fish was moved from its home-tank into a bigger, novel tank (60 cm × 30 cm × 30 cm) with three compartments filled with 10 cm of water. The predator model was randomly presented in one of the end compartments, while the focal fish was acclimatized in the other end compartment (Kozak and Boughman 2012). After 5 min of acclimatization, the fish was released remotely into the arena with view of the predator model, and the assay lasted for 20 min. We changed the side of predator compartment systematically in order to avoid biases. Further, the water was refreshed and new predatory olfactory

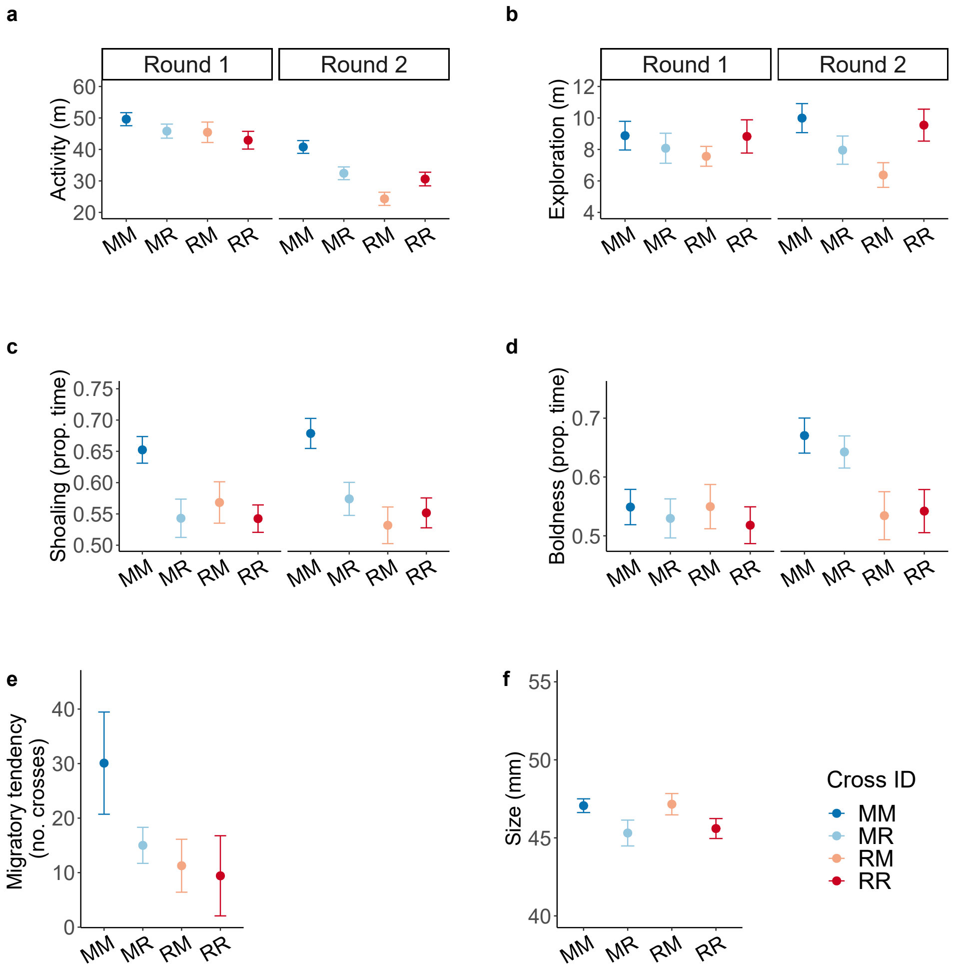

Figure 3.3 – ***Mean scores and standard errors for behaviours and size of F1 fish of different crosses.*** *a) "Activity" – total distance travelled in meters (m); b) "Exploration" – total distance travelled in a novel arena in m; c) "Shoaling" – Proportion of time spent near shoal compartment; d) "Boldness" – Proportion of time spent near predator. Means along with standard error are represented in the plots. For lab-based behaviours, the mean behavioural scores for the two repeats are represented separately. (Sample sizes round 1:* $N_{MM} = 40$, $N_{MR} = 39$, $N_{RM} = 35$, $N - RR = 40$; *round 2:* $N_{MM} = 39$, $N_{MR} = 38$, $N_{RM} = 34$, $N_{RR} = 40$)*; e) "Migratory tendency" – total number of pond crosses (*$N_{MM} = 12$, $N_{MR} = 17$, $N_{RM} = 15$, $N_{RR} = 12$*); f) "Size" – Standard length in mm (*$N_{MM} = 40$, $N_{MR} = 39$, $N_{RM} = 35$, $N_{RR} = 40$*).*

cues were added after testing seven fish in the arena. The proportion of time the focal fish spent within one-fish distance (6 cm) from the predator compartment was taken as a proxy for boldness.

Statistical analyses

Variation in size and behaviours (activity, exploration, shoaling, and boldness) was analyzed using Linear mixed models (LMM) in which repeat (first vs. second round) and cross identity (MM, MR, RM or RR) were included as fixed factors. We also included the interactive effects (cross × round) to test for cross-specific habituation effects. Individual identity (Fish ID), mother identity (Mother ID) and father identity (Father ID) were included as random effects. For shoaling behaviour, we added identity of the test shoal (Shoal ID) as an additional random effect. For migratory tendencies, only one round of tests was performed and we fitted a Poisson generalized linear mixed model with log-link function (GLMM), with number of pond crosses as the response variable and cross identity as fixed factor. As random effects, we included mother identity (Mother ID) and father identity (Father ID) and further, to prevent overestimation of predictive power caused due to overdispersion we added observation level random effects (OLRE; Harrison 2014). All LMMs/GLMMs were constructed in R v. 3.6.1 (R Core Team 2021) using the 'lmer' function of the 'lme4' package, package version 1.1-27.1 (Bates et al. 2015). The statistical significance of fixed effects was assessed based on the 95 % confidence interval (CI): an effect was considered significant when its 95% CI did not include zero. In addition, Tukey's HSD post-hoc test was performed using the functions 'emmeans' and 'pairs' to give pairwise comparisons using the package 'emmeans', package version 1.6.1 (Lenth 2021). LMMs were used to decompose the phenotypic variance of behaviours into between-individual (VFish ID), between-mother (V_{Mother} ID), between-father (V_{Father} ID) and within-individual (V_{Residual}) variances that we subsequently used to calculate repeatabilities, i.e the proportion of total phenotypic variation (Vp) attributable to differences between individuals (R_{Fish} ID), between mothers (R_{Mother} ID) and between father (R_{Father} ID):

$R_{\text{FishID}} = V_{\text{FishID}}$ / Vp
$R_{\text{MotherID}} = V_{\text{MotherID}}$ / Vp
$R_{\text{FatherID}} = V_{\text{FatherID}}$ / Vp
with $\text{Vp} = V_{\text{FishID}} + V_{\text{MotherID}} + V_{\text{FatherID}} + V_{\text{Residual}}$.

Raw (without fixed effects), adjusted repeatabilities (after accounting for fixed effects, cross × round), and their confidence intervals were calculated using 'rpt' function with 1000 bootstraps in 'rptR', package version 0.9.22 (Stoffel et al. 2017).

Results

Our prime goal was to test if RR and MM crosses that were raised under similar conditions, exhibited similar behavioural differences as observed in their wild-caught population of origin and if these differences were consistent over time. We found that RR crosses were consistently less active than MM crosses in the two rounds (Fig. 3.3a; Table 3.1; overall effect of crosses on activity: $\chi^2 = 17.35$, $df = 3$, $p < 0.01$; Table 3.A1). We further found that shoaling and migratory tendencies varied significantly and consistently between RR and MM crosses in the same direction, with RR crosses exhibiting lower shoaling and migratory tendencies than MM crosses (Fig. 3.3c, 3.2e; Table 3.1; overall effect of crosses on shoaling: $\chi^2 = 17.91$, $df = 3$, $p < 0.01$, on migratory tendency: $\chi^2 = 14.37, df = 3, p < 0.01$). MM but not RR crosses shoaled more than expected by chance (score of > 0.5; Table 3.1). RR and MM crosses did not differ consistently in levels of exploration and boldness (Fig. 3.3b, d; Table 3.1 & 3.A1). For boldness, RR cross differed from MM cross but only in round 2 (Fig. 3.3d, significant effects of round and round × cross RR, Table 3.1 & 3.A1), implying that the observed difference was not consistent over time (Fig. 3.3d). Crosses did not differ in body size (Fig. 3.3f, Table 3.1 & 3.A1). We did not find evidence for parental effects. For all traits investigated, we did not observe a clear directional asymmetry between the reciprocal hybrid crosses or trends in the distribution of individual behaviour (Fig 3.3 & 3.A2). Overall, only a small fraction of the variance in behaviours was attributable to differences between fathers and mothers (between 0 and 0.18, Table 3.2). In contrast, individual identity explained a significant part of the behavioural variation across the two rounds of measurement (adjusted $R_{ind} = 0.31$ to 0.38; raw $R_{ind} = 0.14$ to 0.43; Fig. 3.A1, Table 3.2), i.e. individual behaviour is consistent (to a certain extent), despite potential effects of habituation or sensitization to handling (Fig. 3.3, Table 3.1).

Discussion

We aimed to study whether genetic differentiation underlies the behavioural differentiation following habitat fragmentation in sticklebacks. Using a common garden experiment, we showed that the differences between residents and migrants in shoaling and migration tendency (and to some extent also activity) have a genetic basis. In contrast, there were no clear patterns regarding differences in other behaviours or size between crosses. The earlier observed differences in these traits between wild-caught residents and migrants might therefore reflect differences in the respective developmental environments of the two ecotypes of fish. We discuss below the likely causes of divergence in our system and compare the patterns to those observed in post-glacial divergence of marine and freshwater sticklebacks. Then we discuss the eco-evolutionary implications of our findings in link with conservation plans of our study area.

Our common garden experiment revealed that the divergence in at least two of the five behavioural traits studied have a genetic basis. This corroborates a previous study on sticklebacks showing that the expression of heritable variation, i.e. the fraction of phenotypic variance owing to additive effects of genes (Lynch et al. 1998), substantially varied depending on the personality trait considered and the evolutionary history of the populations (Dingemanse et al. 2009). An interesting future avenue will be to quantify population specific trait heritabilities and the relative contribution of genetic and non-genetic sources of variation in those behaviours. Furthermore, it remains to be tested if the genetic differences we uncovered reflect local adaptation as opposed to other processes such as genetic drift or founder effects. Shoaling and migration tendencies are very crucial for the ancestral migratory fish. Their migratory lifestyle involves group schooling tendencies and potentially higher shoaling tendencies due to increased predator pressure owing to ‘openness’ of habitats in the sea. In residents, shoaling tendencies may be less strongly selected for, leading to the pattern of random association with the shoal that we have recovered in our experiments (Fig. 3.3c). Alternatively, lower shoaling tendencies may be selected for due to increased competition, for instance in winter, when resources are scarce leading to a trade-off between intra-specific aggression and competition (Lacasse and Aubin-Horth 2014). Studies on marine-freshwater stickleback pairs have also revealed potential genetic underpinnings of shoaling via *EDA* gene (Wark et al. 2011a; Di-Poi et al. 2014; Archambeault et al. 2020) and migratory tendencies via genetic divergence in Thyroxine response mechanisms (Kitano et al. 2010). One next step will be to test whether the genetic differentiation of shoaling and migratory tendencies reflect local adaptation

Table 3.1 – *Effect of type of cross (migrant MM, resident RR, hybrid RM and MR) on behaviour and morphology of common garden raised three-spined sticklebacks. For lab-based behaviours, the additive and/or interactive effects of rounds are included. Summaries of linear mixed models on traits are presented with estimates of fixed effects (β), with their 95 % confidence intervals (CI) and variance due to random effects with corresponding standard deviation. Significant fixed effect compared to the reference factor are denoted in bold. The corresponding significant pair-wise comparisons (Tukey's HSD post-hoc tests) are given in Fig. 3.2. Sample size (N) represents number of observations.*

	Activity **N = 305**	**Exploration** **N = 304**	**Shoaling** **N = 302**	**Boldness** **N = 303**	**Migratory tendency** **N = 52**	**Size** **N = 154**
Fixed effects	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Intercept (Round First, Cross MM)	**49.56**	**9.11**	**0.63**	**0.55**	**2.86**	**47.01**
	(44.15, 56.64)	**(6.47, 11.76)**	**(0.57, 0.70)**	**(0.47, 0.63)**	**(2.07, 3.62)**	**(44.98, 49.04)**
Cross (MR)	-4.51	-1.79	**-0.10**	-0.03	-0.51	-0.79
	(-16.10, 7.03)	(-5.53, 1.92)	**(-0.18, 0.03)**	(-0.12, 0.07)	(-1.63, 0.54)	(-2.86, 1.21)
Cross (RM)	**-8.28**	-1.86	**-0.09**	-0.03	**-0.98**	0.54
	(-15.07, -0.29)	(-4.63, 1.14)	**(-0.17, -0.01)**	(-0.15, 0.08)	**(-1.92, -0.04)**	(-2.52, 3.28)
Cross (RR)	-5.54	-0.55	**-0.10**	-0.02	**-2.03**	-1.35
	(-17.05, 5.98)	(-4.25, 3.14)	**(-0.17, -0.02)**	(-0.13, 0.09)	**(-3.20, -0.86)**	(-4.18, 1.51)
Round (Second, Cross MM)	**-8.66**	0.91	0.03	**0.11**	-	-
	(-13.93, -3.42)	(-1.21, 3.02)	(-0.03, 0.10)	**(0.03, 0.19)**	-	-
Cross (MR) × Round (Second)	-4.91	-0.93	0.004	0	-	-
	(-12.04, 2.26)	(-3.80, 1.95)	(-0.09, 0.10)	(-0.11, 0.11)	-	-
Cross (RM) × Round (Second)	**-11.62**	-2.31	-0.78	-0.08	-	-
	(-19.13, -4.09)	(-5.33, 0.71)	(-0.18, 0.03)	(-0.20, 0.03)	-	-
Cross (RR) × Round (Second)	-5.35	-0.02	-0.02	**-0.12**	-	-
	(-12.48, 1.81)	(-2.88, 2.87)	(-0.12, 0.07)	**(-0.23, -0.01)**	-	-
Random effects	Estimate (std.dev)	Estimate (std.dev)	Estimate (std.dev)	Estimate (std.dev)	Estimate (std.dev)	Estimate (std.dev)
Fish ID	80.05 (8.97)	13.10 (3.62)	0.007 (0.09)	0.01 (0.12)	-	-
Father ID	0.00 (0.00)	0.00 (0.00)	0.001 (0.03)	0.00 (0.05)	0.00 (0.00)	2.21 (1.49)
Mother ID	41.69 (6.46)	3.39 (1.84)	0.00 (0.00)	0.00 (0.02)	0.08 (0.28)	0.59 (0.77)
Shoal ID	-	-	0.00 (0.03)	-	-	-
OLRE	-	-	-	-	1.20 (1.10)	-
Residual	111.7 (10.56)	18.04 (4.25)	0.02 (0.13)	0.03 (0.16)	-	11.21 (3.35)

Table 3.2 – *Repeatabilities of lab-based behaviours. Raw repeatabilities and adjusted repeatabilities after controlling for cross ID are given for individual ID, father ID and mother ID along with their 95% confidence intervals (CI).*

Behavior	Fish ID	Father ID	Mother ID
	R_{ind} (95% CI)	R_{father} (95 % CI)	R_{mother} (95% CI)
Activity - Raw	0.14 (0.00, 0.30)	0.08 (0, 0.25)	0.019 (0, 0.12)
Activity – Adjusted	0.34 (0.22, 0.53)	0	0.18 (0, 0.40)
Exploration – Raw	0.43 (0.29, 0.55)	0	0.03 (0, 0.13)
Exploration –Adjusted	0.38 (0.25, 0.54)	0	0.10 (0, 0.27)
Shoaling – Raw	0.31 (0.15, 0.46)	0.03 (0, 0.13)	0.03 (0, 0.12)
Shoaling – Adjusted	0.31 (0.15, 0.46)	0	0
Boldness – Raw	0.29 (0.08, 0.45)	0.06 (0, 0.18)	0.01 (0, 0.08)
Boldness - Adjusted	0.33 (0.19, 0.49)	0.05 (0, 0.19)	0.01 (0, 0.13)

using either a genomic approach to detect signature of adaptive divergence (using, for example, a whole genome and/or a candidate gene (*EDA* allele) approach) or a transplant experiment where we would raise crosses in different environmental conditions (marine vs freshwater) to infer fitness.

We expected similar differentiation in other traits, as they were found to be different between wild-caught migrants and residents over two study years (Ramesh et al. 2022b). For instance, studies have shown moderately heritable and additive genetic components in behaviours such as exploration and boldness in sticklebacks (Dingemanse et al. 2009). However, in our experiment, body size and behaviours such as exploration and boldness did not show differences between crosses. For body size, responses may be potentially plastically adjusted to the ecological conditions as seen in previous studies (e.g., predation pressure; Frommen et al. 2011, niche specialization, Day and McPhail 1996, Wund et al. 2008). Similar to body size, behaviours such as exploration and boldness may also be environmentally determined. Alternatively, these behaviours could also be state-dependent (state, being size or mass in this case), owing to differences in resource availability during growth of migrants and residents (Luttbeg and Sih 2010; Wolf and Weissing 2010). It also remains possible that the differences in behaviour in wild migrants are due to plastic responses of migrants in freshwater vs sea conditions, which has not been tested here.

In our current study, we found little evidence for maternal effects as maternal contribution to trait variation was small and not significant (19% for activity, 3% for exploration, and 3% for shoaling tendencies) and we did not find clear systematic differences between the reciprocal hybrid crosses (RM and MR). However, we raised the juveniles in the absence of paternal care. Hence, it remains possible that the behavioural differences observed between wild-caught migrants and residents (Ramesh et al. 2022b) are related to differences in paternal care. This is an interesting avenue warranting further investigation because there is evidence for parental programming through maternal effects and paternal care in sticklebacks (Giesing et al. 2010; McGhee et al. 2012, 2015; McGhee and Bell 2014; Stein and Bell 2014). Our studies revealing genetic differentiation between ancestral migrant and resident populations in behaviours related to migration and shoaling are timely and have important consequences for conservation efforts. Water authorities are currently implementing conservation measures which aim at restoring river connectivity via barrier removal or the construction of fishways. Reconnecting migratory and genetically differentiated land-locked populations can be viewed as a large scale eco-evolutionary experiment that raises exciting questions such as: will migratory and resident sticklebacks intermix and introgress in sympatry (Ravinet 2021)? Will hybrids be selected against? Will we have incomplete gene flow and partial migration occurring in these populations (Berner et al.; Ingram et al. 2015; Hanson et al. 2016; Lackey and Boughman 2017)? From our studies, residents and hybrids show lowered migratory and shoaling tendencies. This could potentially drive divergent selection, and lead to the genetic differentiation of sympatric populations with partial migration upon reconnection. Divergence may also be maintained or enhanced by size-assortative mating of migrants and residents as size difference at maturity has been detected in the wild (Ramesh et al. 2022b) or by phenotype-dependent microhabitat choice (Maciejewski et al. 2020; Dean et al. 2021). Irrespective of the mechanisms involved in the observed phenotypic differentiation between migrants and residents, whether the migrant-resident ecotype divergence will persist in the absence of migration barriers needs to be investigated. Overall, using a common garden experiment, we found evidence for genetic differentiation in shoaling, migratory tendencies and potentially activity. These results suggest that residents may have locally adapted to their novel environmental conditions in our system. Few imminent questions that follow this finding are whether our results can be generalized to other freshwater and migratory fish species that have undergone isolation and how conservation plans may be affected (Tuomainen and Candolin 2011; Franssen et al. 2013). Conservation methods indeed should not only aim at restoring the ecosystem to its original state because this may lead to unwanted consequences (Stockwell et al. 2003). For example, reversal of responses to restorations may not be possible if newly adapted populations or species lack genetic variation, leading to a rapid population decline after conservation measures are in place (Lahti et al. 2009; Mable 2019). Alternatively, newly adapted populations or species may, in fact, have selected for invasive phenotypes such as novel foraging tactics and increased aggression and boldness, leading to unwanted expansions causing unpredictable effects on other species and communities (Holway and Suarez 1999; Sol et al. 2002). Hence conservation efforts should be aimed at implementing methods taking an informed approach of the current state of the system and assessing the evolutionary changes undergone in the species assemblages they are aimed at.

Acknowledgements

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Appendix

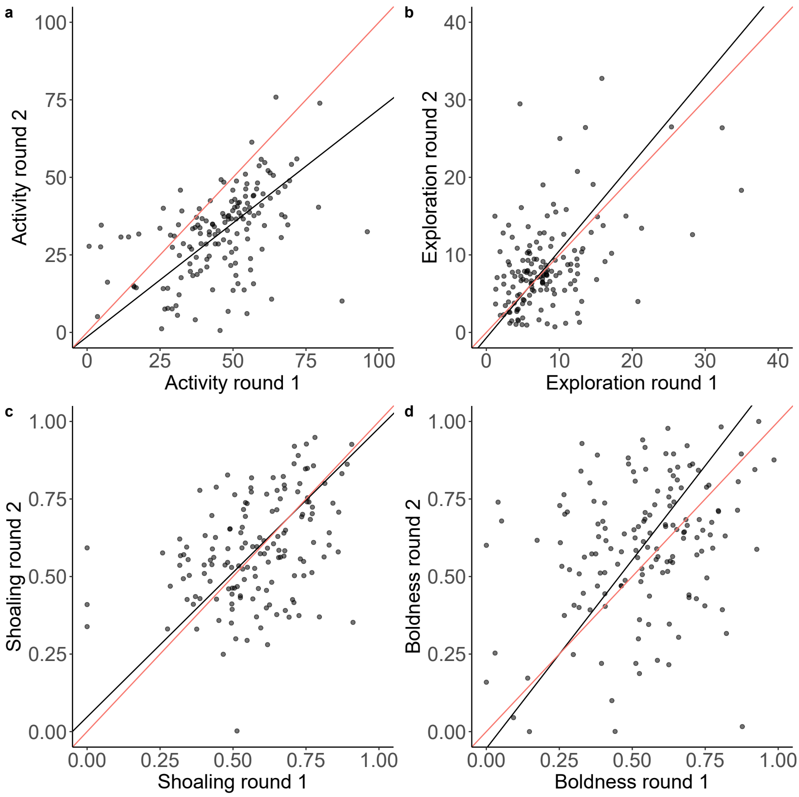

Figure 3.A1 – ***Repeatability of lab-based behaviours.*** *Major axis regression between behaviours measured in the first and the second round. The black line is the major-axis regression line, and the red line is the main diagonal (where* $y = x$*). Pearson's correlation coefficients are show at top left of each plot. (Sample size:* $N_{\text{Total}} = 151$*;* $N_{\text{MM}} = 39, N_{\text{MR}} = 38, N_{\text{RM}} = 34, N_{\text{RR}} = 40$)

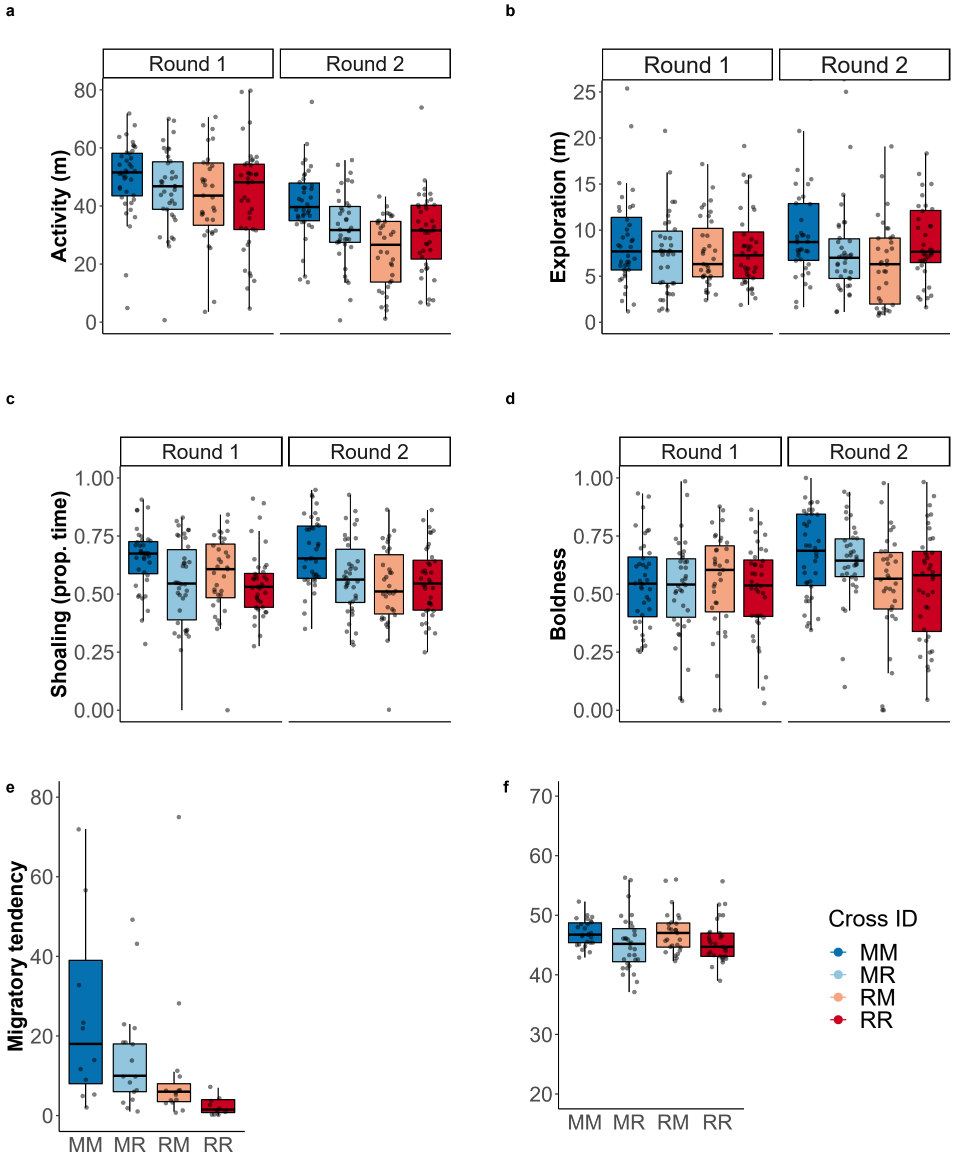

Figure 3.A2 – Median scores and quartiles for behaviours and size of F1 fish of different crosses along with variation within crosses. The whiskers represent the range of values, excluding the outliers. a) ‘Activity’ – total distance travelled in meters (m); b) ‘Exploration’ – total distance travelled in a novel arena in m; c) ‘Shoaling’ – Proportion of time spent near shoal compartment; d) ‘Boldness’ – Proportion of time spent near predator. For lab-based behaviours, the mean behavioural scores for the two repeats are represented separately. (Sample sizes round 1: $N_{MM} = 40$, $N_{MR} = 39$, $N_{RM} = 35$, $N_{RR} = 40$; round 2: $N_{MM} = 39$, $N_{MR} = 38$, $N_{RM} = 34$, $N_{RR} = 40$); e) ‘Migratory tendencies’ – total number of pond crosses ($N_{MM} = 12$, $N_{MR} = 17$, $N_{RM} = 15$, $N_{RR} = 12$); f) ‘Size’ - Standard length in mm ($N_{MM} = 40$, $N_{MR} = 39$, $N_{RM} = 35$, $N_{RR} = 40$)

Table 3.A1 – *Table of pairwise significance with adjusted p-values resulting from Tukey's HSD test using 'emmeans' package. Significant pair-wise comparisons are represented in bold.*

Contrast	**Estimate**	**S.E**	**p-value**	**Estimate**	**S.E**	**p-value**
Activity Round 1				*Activity Round 2*		
MM-MR	4.303	6	0.8867	8.965	6.02	0.4969
MM-RM	7.327	3.72	0.2526	**18.531**	**3.78**	**0.0015**
MM-RR	4.978	5.91	0.8329	10.039	5.93	0.4029
MR-RM	3.023	6.12	0.9577	9.566	6.13	0.4598
MR-RR	0.675	3.31	0.9968	1.074	3.31	0.9874
RM-RR	-2.348	6.12	0.9791	-8.491	6.13	0.5483
Exploration Round 1				*Exploration Round 2*		
MM-MR	1.541	2.08	0.8774	2.741	2.09	0.5851
MM-RM	1.774	1.57	0.6798	3.977	1.6	0.1134
MM-RR	0.256	2.07	0.9993	0.573	2.09	0.9921
MR-RM	0.233	2.17	0.9995	1.236	2.17	0.9385
MR-RR	-1.286	1.41	0.8003	-2.168	1.41	0.4585
RM-RR	-1.518	2.13	0.8894	-3.404	2.14	0.4386
Shoaling Round 1				*Shoaling Round 2*		
MM-MR	0.1015	0.0413	0.0979	0.09749	0.0416	0.1202
MM-RM	0.08918	0.0461	0.2383	**0.16751**	**0.0474**	**0.007**
MM-RR	0.09593	0.0409	0.1135	**0.11702**	**0.0408**	**0.0382**
MR-RM	-0.01232	0.0463	0.9932	0.07001	0.047	0.4604
MR-RR	-0.00557	0.0403	0.999	0.01952	0.0402	0.9613
RM-RR	0.00675	0.0494	0.9991	-0.05049	0.0496	0.7406
Boldness Round 1				*Boldness Round 2*		
MM-MR	0.02566	0.0538	0.9627	0.02347	0.0545	0.972
MM-RM	0.03091	0.0671	0.966	0.1148	0.068	0.3764
MM-RR	0.02042	0.0642	0.9882	0.13654	0.0648	0.2151
MR-RM	0.00525	0.0679	0.9998	0.09133	0.0681	0.5591
MR-RR	-0.00523	0.0627	0.9998	0.11307	0.0627	0.3368
RM-RR	-0.01048	0.0563	0.9976	0.02174	0.0566	0.9797

4

Mesocosm experiments reveal the loss of migratory tendencies in a recently isolated population of three-spined sticklebacks

A. Ramesh*
J. Gismann*
T.G.G. Groothuis
F.J. Weissing
& M. Nicolaus

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* Joint first authors

Abstract

In the 1970s, water management in the Netherlands resulted in numerous isolated populations of three-spined sticklebacks, which can no longer migrate from freshwater to the sea. We tested whether ∼50 years of isolation resulted in reduced migratory tendencies in these 'resident' sticklebacks. Lab-based individual testing showed behavioural divergence between residents and migrants, but also produced counter-intuitive results, especially with regards to movement tendencies. To detect differences in migration tendencies, we set up a semi-natural mesocosm, consisting of connected ponds, where movements of numerous individuals could continually be tracked at larger spatial scales. We found that wild-caught residents and migrants exhibited no differences in movement tendencies 'within ponds', but residents moved significantly less 'between ponds' than migrants. Between-pond movements were consistent and the observed differences were robust across contexts (changes in water flow and group size). Our study reveals that larger-scale movement tendencies can diverge over short time scales in response to human-induced isolation, and highlights the importance of observing behaviour in ecologically relevant setups that bridge the gap between lab and field studies.

Introduction

Habitat fragmentation is one of the major threats for biodiversity, particularly for migratory species that depend on multiple habitats to complete their life cycle (Legrand et al. 2017). In the north of the Netherlands, pumping stations have disrupted the connectivity between marine and riverine habitats, confining some fish populations to freshwater habitats without the possibility to migrate to the sea. Such forced isolation can cause rapid phenotypic responses and life-history changes (mammals and birds: Soriano-Redondo et al. 2020; fish: Quinn and Myers 2004; Closs et al. 2013; Dodson et al. 2013; Augspurger et al. 2017). Using individual lab-based assays, we have previously shown that this is indeed true for three-spined sticklebacks (*Gasterosteus aculeatus*): 'resident' populations, isolated for ∼50 years, were found to diverge in morphology and in behaviour from their 'migrant' ancestors (Ramesh et al. 2022b), with part of the divergence having a genetic basis (Ramesh et al. 2021). Regarding movement-related behaviours, population differences uncovered in the lab were surprising at first because residents, that were expected to exhibit lower movement tendencies than migrants, were instead more active and more exploratory (Ramesh et al. 2022b). We hypothesized at that time that this may be due to stress, induced by testing in social isolation, which might have affected wild-caught migrants disproportionately more than wild-caught residents, as migrants are thought to shoal extensively as an anti-predator strategy to higher predation risk in the open sea. Alternatively, small-scale experimental settings in the lab may not be suited to study larger-scale processes like migration. More generally, for wild-caught animals, lab conditions necessarily present a novel environment and fail to mimic natural complexity in biotic and abiotic factors, including the animals' social environment (Burns et al. 2009; Calisi and Bentley 2009; Niemelä and Dingemanse 2014; Pritchard et al. 2016). However, studying dispersal or migration behaviour in the field is often logistically challenging (especially in aquatic

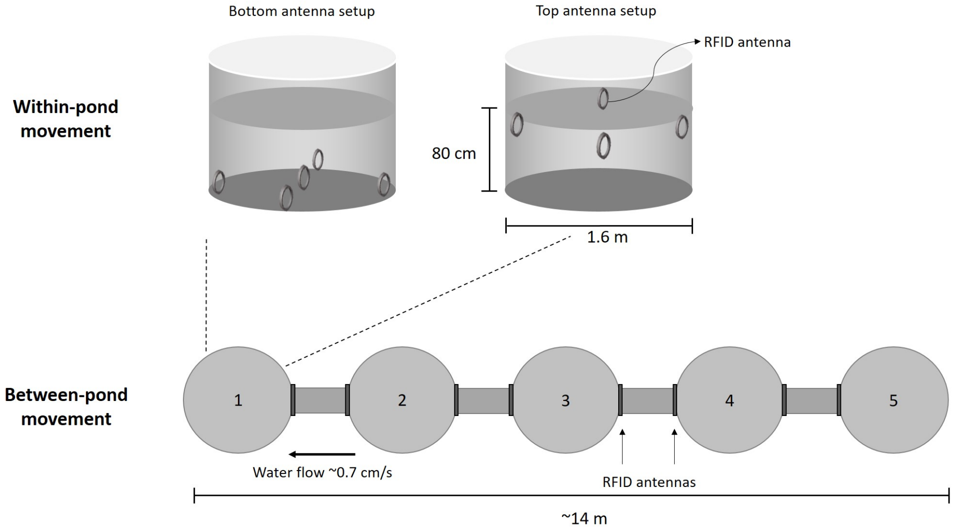

Figure 4.1 – ***Experimental setup.*** *The mesocosm consisted of two sets of five linearly connected ponds (1 to 5) equipped with circular RFID antennas that automatically detect crosses of PIT tagged individuals. Fish were released into pond 1. This pond was equipped with nine RFID antennas (five on the bottom and four on top of the water column), allowing us to quantify within-pond movements. The connections between adjacent ponds were equipped with two RFID antennas, allowing us to quantify the number and direction of movements between ponds.*

environments and for small fish) and frequently lacks data about the animals' social groups (Krause et al. 2013).

To bridge the gap between lab and field studies, we set up a semi-natural mesocosm consisting of connected ponds, in which groups of fish can be remotely tracked over extensive periods of time. We here report the first experiment that aimed to test for consistent differences in movement tendencies between wild-caught 'resident' and 'migrant' sticklebacks and to disentangle the effects of spatial scale (within and between ponds), social environment (group size), and ecological conditions (water flow) on movement patterns. The results of the second experiment, aimed at disentangling genetic and non-genetic effects, are reported in (Ramesh et al. 2021). Under these experimental conditions, we tested (a) if residents and migrants exhibit differences in their movement tendencies, (b) if the spatial scale of movement matters, and (c) how consistent these patterns are under varying conditions (group size and water flow).

Methods

Mesocosm system

The mesocosm consists of two independent systems of five ponds (each $\varnothing$ 1.6 m, with water depth of 80 cm), connected linearly with opaque corridors (each of length $\sim$1.5 m and $\sim$ 11 cm), spanning a linear distance of $\sim$14 m (Fig. 4.1). The system is supplied with freshwater from a natural ditch, with the possibility of creating water flow ($\sim$0.7 cm/s), mimicking the wild conditions, which also acts a cue for migration (Jonsson 1991). This system allowed to measure the movement of individual sticklebacks within and between ponds. The first pond (labelled 1 in Fig. 4.1), enriched with plastic plants, was used to quantify within-pond movements, while the whole system of five connected ponds was used to record between-pond movement tendencies (see details in Supp. info. 1).

We used a Radio-Frequency-Identification (RFID) system consisting of circular RFID antennas ($\varnothing$ 10 cm), data loggers and Passive Integrative Transponders (PIT tags; Trovan, Ltd., Santa Barbara, California) to record movements of tagged sticklebacks (details in Supp. info. 1). Nine circular antennas were placed in the first pond to record within-pond movements and two antennas were placed at both ends of each of the four connecting corridors to measure between-pond movement tendencies (Fig. 4.1). Each antenna records the unique PIT-tag ID of the fish along with a time stamp, stored on a USB drive in the central data logger. The sensitivity of the system was set to three reads per second per unique tag. In a pilot study, we validated the reads using video recordings and found that it corresponded well with the entry and exit times of fish.

Experiment 1

We created five groups of migrants and six groups of residents, each consisting of 10 randomly selected individuals (total: $N_{\text{mig}} = 49$ and $N_{\text{res}} = 60$). While we always tried to maintain the group size to 10 fish, tag-loss and other technical difficulties led to one group of migrants having nine fish and another with 11 fish. Groups were housed in separate small holding ponds for 24 hours before the start of the experiment. On the experimental day, one resident and one migrant group were released simultaneously (to avoid temperature or seasonal biases) into separate mesocosms. The individuals in each group were first monitored for within-pond movement by confining the fish to the starting pond for the first five hours (Fig. 4.1) and then for between-pond movement for $\sim$16.5 hours, after opening the connection to the other ponds (Fig. 4.1; Supp. info. 2).

Experiment 2

In a next step (after $\sim$one month), we combined all migrants and, separately, all residents (after excluding 12 fish which either had died or lost tags) into two large groups ($N_{\text{mig}} = 45$, $N_{\text{res}} = 52$) and quantified between-pond movements in these two groups in the same separate mesocosm setups over four days. In addition, we alternated flow and no-flow conditions on consecutive days (see Supp. info. 1).

Analyses

For each individual, we quantified within-pond movement as the number of times a fish crossed different bottom and surface antennas separately (Fig. 4.1). We deemed the number of separate visits to a particular antenna unreliable for measuring movement patterns because fish that stayed longer near an antenna were recorded as multiple disconnected set of reads, as if they visited the antenna multiple times. Between-pond movement was quantified as the number of crosses a fish made through the corridors connecting two ponds (Fig. 4.1). Fish that did not get detected by any antenna were given a score of zero crosses.

We then analysed if residents and migrants differed in the number of crosses for within- and between-pond movements (Experiment-1) and whether they were consistent across contexts (group size and flow; Experiment-2). Briefly, we considered the number of crosses within or between ponds as response variable separately in univariate generalized linear mixed models with Poisson errors. In all models, we included origin (resident vs. migrant) as a fixed factor and group-ID and an observation-level 'Obs' (Observation-level random effects to control for overdispersion (Harrison 2014), as random effects. For Experiment-2, treatment (flow vs. no flow) and its interaction effect with origin were added as fixed effects and individual-ID as a random effect to account for individual repeats. Additionally, we analysed whether the fraction of fish that did not exit the first pond differed between migrants and residents using Fisher's exact test. Repeatability and correlation of number of crosses across contexts were also calculated (Supp. info. 3). All analyses were carried out in R (R Core Team 2021). For complete description of the analyses see Supp. info. 3.

Results

In Experiment-1, residents and migrants showed a broad distribution of number of crosses at both bottom and top antennas (Fig. 4.2 a, b) and the differences between the groups were in both cases not statistically significant (Table 4.1; Median bottom-antenna crosses: Residents=23, Migrants=14; Median top-antenna crosses: Residents=3.5, Migrants=8). In contrast, residents exhibited much lower numbers of crosses between ponds than migrants (Fig. 4.2 c; significant effect of Origin in Table 4.1; Median pond crosses: Residents=0, Migrants=16). Furthermore, the proportion of 'non-leavers', i.e., individuals that did not exit the first pond, was significantly higher in residents than in migrants (55% in residents vs. 28.6% in migrants, odds ratio=3.02, p=0.007). In Experiment-2, as in Experiment-1, residents moved consistently less between ponds than migrants (Fig. 4.2d). Furthermore, fish moved more between ponds in the presence of flow and the trend was slightly stronger for residents than migrants (Fig. 4.2d; significant Origin × Treatment effect in Table 4.1). Individual movement tendency between ponds was moderately repeatable across ecological contexts but very weakly correlated over social contexts (Supp. info. 3). However, we clearly see from

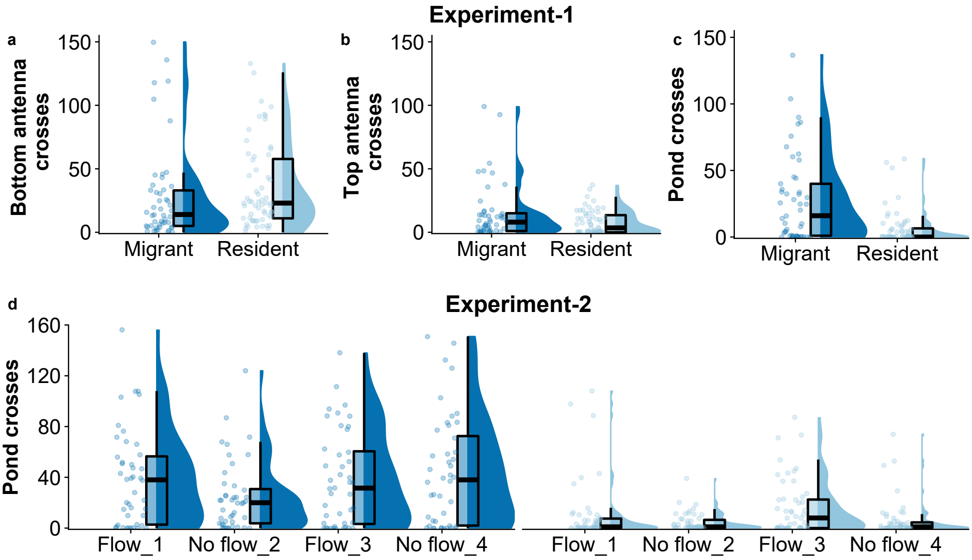

Figure 4.2 – ***Within-pond and between-pond movement of resident and migrant sticklebacks.*** *a,b) within-ponds crosses at the bottom and top antennas respectively (Experiment-1); c) between-pond crosses in Experiment-1; Sample size: Nmig=49, Nres=60; d) between-pond crosses in relation to the daily flow treatment in Experiment-2. Sample size: Nmig=45, Nres=52. In all graphs, individual crosses (dots), boxplots and density kernels are shown for migrant (dark blue) and resident (light blue) sticklebacks.*

Fig. 4.2 and Table 4.1 that the difference between residents and migrants was maintained across different contexts.

Discussion

We have previously shown that ∼50 years of isolation potentially led to rapid behavioural and morphological divergence of residents from migrants (Ramesh et al. 2022b), which mimics the divergence observed in another long-isolated population of sticklebacks (Di-Poi et al. 2014). Both studies assayed individual movement tendencies under artificial housing conditions in the lab and showed counter-intuitive patterns: residents showed either higher (Ramesh et al. 2022b) or inconsistent patterns (Di-Poi et al. 2014) in activity/exploration levels compared to migrants. Here, we show that the same populations as in (Ramesh et al. 2022b) exhibited movement tendencies as predicted previously, when they were tested in a semi-natural setting (relevant social/ecological context and spatial scale): Resident populations exhibited lower movement tendencies than their migrant counterparts. These differences, detected only at large spatial scale, remained consistent across ecological and social contexts. Together with the previous results on F1 lab-born juveniles (Ramesh et al. 2021), this study suggests

Table 4.1 – *Results of the statistical analysis of movement within and between ponds using generalised linear mixed models. Estimates of fixed effects (β) in log-scale are given with their 95% confidence intervals (CI) and variance components are given with their standard deviation. Fixed effects that significantly differ from zero are denoted in bold. Sample sizes experiment-1: N_{mig}=5 groups (49 individuals), N_{res}=6 groups (60 individuals); experiment-2: N_{mig}=1 group (45 individuals), N_{res}=1 group (52 individuals). 1:'migrant' is used as reference category; 2:'flow' is used as reference category*

		Experiment-1		**Experiment-2**
	Bottom crosses	Top crosses	Pond crosses	Pond crosses
Fixed effects	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Intercept	**2.61** **(2.13, 3.08)**	**1.98** **(0.30, 3.63)**	**1.90** **(0.63, 3.13)**	**2.53** **(1.87, 3.17)**
Origin^1	0.51 (-0.12, 1.15)	-0.68 (-3.03, 1.53)	**-2.26** **(-4.04, -0.58)**	**-1.77** **(-2.68, -0.87)**
Treatment^2	-	-	-	-0.14 (-0.44, 0.16)
Origin^1 × Treatment^2	-	-	-	**-0.72** **(-1.18, -0.27)**
Random effects	Var (sd)	Var (sd)	Var (sd)	Var (sd)
Group-ID	0.11 (0.33)	2.94 (1.72)	0.95 (0.98)	-
Obs	1.21 (1.10)	1.14 (1.07)	5.02 (2.24)	0.81 (0.90)
Individual-ID	-	-	-	4.11 (2.02)

that our mesocosm setup, by allowing water flow, testing in groups and larger spatial scale (14 m length), is much better suited to characterize individual movement patterns related to migratory behaviour than lab-based assays in social isolation in small tanks.

Our study reveals that the detection of population differences in stickleback behaviour was scale-dependent (only detectable between, but not within ponds). This is probably because in the wild, sticklebacks exhibit considerable foraging movements over days (median of 40 m upstream, (Bolnick et al. 2009) and hence their within-pond movements, representing foraging movements, may not differ between populations. However, wild migrants in our field system travel 10s of kilometres inland within a few days (pers. comm. from water authorities) and thus require sufficient space and navigation cues (e.g. flow velocity; Sommer-Trembo et al. 2017) to express their natural behaviour.

Tests in the lab, though invaluable for studies on animal behaviour owing to controlled settings, are not without drawbacks. Firstly, they cannot offer the more natural conditions mentioned above (e.g. spatial scale, appropriate social or ecological contexts), which may be particularly important for wild-caught animals. They may constrain the level of behavioural expression to some extent, such as the 'freezing' behaviour of wild-caught migrants in our previous studies (Ramesh et al. 2022b). Reassuringly, we observed that this was much less of an issue for lab-bred animals: lab-born F1 juveniles did not freeze in lab tests and their movement-related behaviours measured in the lab and in the mesocosm positively correlated (Fig. 4.A1). Secondly, lab-tests are performed in highly-controlled or novel setups. This can lead to homogenization of behavioural expression (e.g. decreased variance over time; Sommer-Trembo et al. 2017) or uncovering ‘cryptic’ behavioural variation (with novel behaviours and increased variance in behavioural expression (Schlichting 2008). We thus advocate using mesocosm or other semi-natural setups (e.g. Thorlacius et al. 2015; Sudo and Tsukamoto 2015; Hirsch et al. 2017; Thorlacius and Brodin 2018; Coates et al. 2019; Schirmer et al. 2019; Dhellemmes et al. 2020; Niemelä et al. 2021), to bridge lab and field studies. They circumvent the mentioned drawbacks and provide valuable insights undetectable in classical behavioural setups, especially for wild populations.

Our results further support the idea that forced isolation in freshwater is followed by phenotypic changes as reported for sticklebacks isolated after the last glacial retreat (e.g. reduction in lateral plates and reduced swimming abilities; Tudorache et al. 2007; Dalziel et al. 2012; Kitano et al. 2012). Many of these morphological and behavioural changes are underlined by genetic differentiation and are true adaptations to a resident lifestyle (Colosimo 2005; Chan et al. 2010). Additionally, we show that freshwater-induced phenotypic changes in sticklebacks can occur even on contemporary timescales (see also Lescak et al. 2015; Hosoki et al. 2020; Garcia-Elfring et al. 2021) and can have a genetic component (Ramesh et al. 2021). Residents in our study populations are thus likely on a trajectory to losing their migration tendencies and already (partially) adapted to complete residency. Current conservation management includes building fishways to reconnect land-locked and migratory populations. In this context, it is important to consider that residents may be less likely to use fishways due to lowered migration tendencies. This may require a revision in the evaluation criteria for the success of these conservation efforts. An exciting future avenue will be to study to what extent and how quickly individual migration tendencies will be affected when the two populations reconnect.

Acknowledgements

We thank Dennis Worst and Willem Diderich for help with fish care and advice on experimental design and other animal caretakers for looking after the sticklebacks. We thank Peter Paul Schollema from the Water Authorities Hunze en Aa’s and Jeroen Huisman from van Hall Larenstein, University of Applied Sciences for help with catching sticklebacks.

Appendix

Supplementary information 1: Description of the mesocosm and the tracking setup

Experiment-1

Within-pond movements:

On the morning of testing (∼10 a.m.), one test group of each origin was released into the first pond that was temporary disconnected from the other ponds by a cap blocking the entrance to the corridor. There was no water flow when recording within-pond movement tendencies. Five circular antennas were placed upright on the bottom of the pond (‘bottom antennas’), and four antennas were placed just below the water surface (‘surface antennas’; Fig. 4.1). To assess within-pond movements, we computed crosses that an individual made between bottom antennas or the surface antennas separately. Crosses that were made between a bottom and surface antenna were excluded as these hardly occurred. The experiment lasted for five hours.

Between-pond movements:

After five hours, we gently removed all antennas from the first pond. At this point we also turned on the flow in the system to create a cue for migration (Fig. 4.2c). Fish were given 30 minutes to recover from the disturbance caused by removing the antennas after which the connection from pond 1 to the other ponds was gently opened. We then recorded the movement of fish between the five connected ponds (‘crosses’) for the next 16.5 h (∼3.30 p.m. – 8 a.m.). At the end of the experiment, fish were returned to their original smaller housing ponds. Testing all 5 migrant and 6 resident groups took place over a week (temperature ranged between 12°C and 15°C). All fish were checked at the end of the experiment to see if they still carried the tags and if the tags functioned correctly.

Experiment-2

Between-pond movements Two weeks after we finished recording each individual for movement tendencies as above, we created one large group each of migrant and resident by combining all the fish ($N_{\mathrm{mig}} = 1$ groups, 45 individuals; $N_{\mathrm{res}} = 1$ group, 52 individuals) and monitored only the movement tendencies between-ponds simultaneously for the two groups and continuously for four consecutive days. During the study period, we furthermore alternated days with and without water flow (flow turned on / off at 10:00 a.m each day and hence kept in that condition for $\sim$24 hours). The flow treatment allowed testing whether the populations react differently to the presence of a migration cue.

Supplementary information 2: Study populations and housing of fish

We caught incoming migrants at a sea lock at the mouth of a river in Nieuwe Statenzijl (‘NSTZ’; 53°13′54.49″, 7○12′30.99″), and resident sticklebacks in an adjacent land-locked polder (‘LL-A’; 53°17′56.14″, 7°2′1.28″) in the province of Groningen, The Netherlands (1). Fish were caught at the onset of inland migration, over a period of four weeks in March and April 2020. Fish of $\geq$ 4 cm in total length (from the tip of the snout to the tip of the tail) were transported to the lab in aerated plastic bags within two hours of capture. After acclimatization, fish were housed in groups of 25, separated by their origin (migrant or resident), for a week prior to experimentation in small holding ponds ($\sim$100 L tanks filled with freshwater from a nearby ditch) under natural temperature and light conditions. Fish were fed a mixture of brine shrimps and blood worms (3F Frozen Fish Food b.), once a day, ad libitum. Fish were tagged with 8 mm Passive Integrated Transponders (PIT tag; Trovan, Ltd., Santa Barbara, California) for individual identification, under anaesthetization in buffered MS-222 solution (0.25 – 0.30 g/L ; pH = 7.5 - 8.0). PIT tags were injected in the abdominal cavity (following (2)). Before experiments, all fish were allowed at least five days of recovery in the housing pond with the same group. Mortality rate after PIT tagging was very low (<1% in the first week).

Supplementary information 3: Estimating consistency of between-pond movements

To quantify individual consistency in between-pond movements across ecological contexts (flow/no flow), we ran univariate generalised linear mixed model (GLMMs) with Poisson errors using the dataset from Experiment-2 and the lme4 package (3). For repeatability across social environments (small vs large group size), we combined the crosses data from Experiment-1 and day 1 and 3 of Experiment-2 with flow. We used the number of crosses between ponds as the response variable, with origin (resident vs. migrant), treatment (social context: small vs large group size or ecological context:

flow vs no-flow in two separate models) and their interaction (*origin* × *treatment*) as a fixed factors and individual-ID as a random effect. In addition, we added Obs as observation-level random effects to control for over-dispersion (OLRE, (4)). We used these 'full' and 'simplified' models (omitting all the fixed effects) to calculate 'adjusted' and 'raw' repeatabilities respectively. Repeatabilities are defined as the ratio of among-individual variance (V_{ind}) to total variance ($V_{total} = V_{ind} + V_{residual}$). We calculated repeatabilities in their original scale, along with their confidence intervals using the 'rpt' function with 1000 bootstraps using the 'rptR' package (5). We were not able to calculate repeatabilities for different social contexts due to lack of model convergence. Hence we resorted to using Spearman correlation as the data is not normally distributed. All analyses were carried out in R (R Core Team 2021).

Between-pond movement was moderately repeatable across ecological contexts (Adjusted R(95% CI) = 0.42 (0.34, 0.51) and Raw R(95% CI) = 0.38 (0.30, 048)). Across social context, individuals were not very consistent with low correlation coefficients (Spearman $\rho = 0.35$, $p < 0.001$). This could be because timescale and sample size were not balanced between Experiments 1 and 2. While repeated data were collected over consecutive days in Experiment 2, single data points were collected a month apart in Experiment-1. However we see that the residents were consistently moving less than migrants in all contexts.

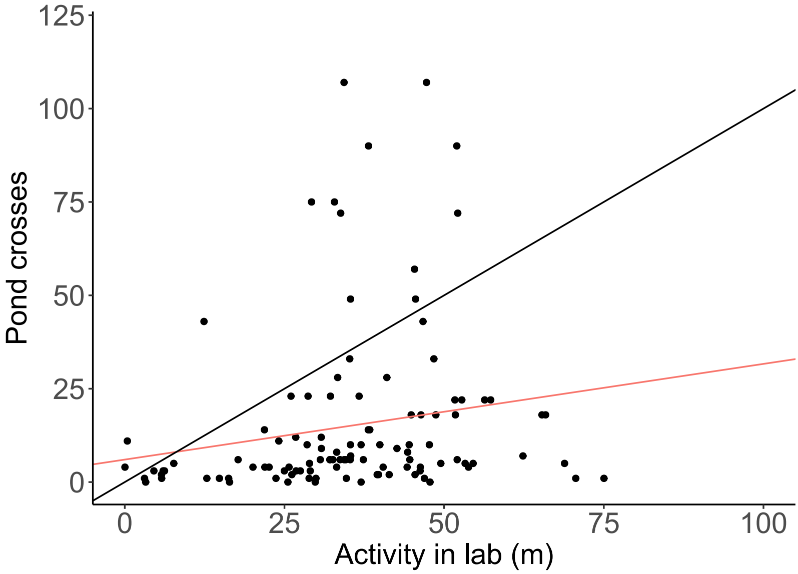

Figure 4.A1 – *Correlation of movement tendencies of lab-raised F1 migrants, residents and hybrids tested in the lab and in the mesocosm. In a separate experiment and set of animals, (F1 sticklebacks raised in the lab from (1)), we performed both, an activity assay in the lab, where individual fish were assessed for general movement tendencies for 20 minutes in their home tank (30 × 16 × 18 cm (L × W × H)) (according to methods in (6)) and movement tendencies across-ponds in the mesocosm as in Experiment-1. Black line represents the identity lines, $x = y$. The red line is the ordinary least squares regression line. Lab-based activity (total distance covered in meters in 20 mins) and number of pond crosses in the mesocosm were positively and significantly correlated (Spearman $\rho = 0.33$, $p < 0.01$).*

5

Individual personalities and not the social context predict movement tendencies in three-spined sticklebacks

A. Ramesh*
J. Gismann*
T.G.G Groothuis
F.J. Weissing
& M. Nicolaus

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* co-first authors

Abstract

It is now widely accepted that animals of the same age, sex and population often differ consistently in suites of behaviours (animal personalities). However, some behavioural plasticity should still be present to reap the benefits of a group living, as individuals often need to adjust their behaviour to some extent to match that of their interacting partners (social modulation of behaviour). Yet, assessing social effects on individual behaviour is challenging because knowledge of an individual's social environment is often difficult to gain, especially for animals which form dynamic social groups. We here capitalize on the availability of populations of migrant and resident sticklebacks (*Gasterosteus aculeatus*) that exhibit strong differences in movement tendencies. By creating mixed shoals of different proportions of migrants and residents in a series of linearly-connected ponds, we tested if individuals modulated their movement tendencies (latency to exit the first pond and crosses between ponds) to the social environment via social conformity and if this depended on individuals' origin (migrant or resident). In both populations, we found almost no effects of the social environments on individual movement tendencies although residents tended to leave the first pond faster in presence of migrants, while migrant were unaffected. Instead, individual personality was the main predictor of the observed variation. We thus conclude that in our stickleback populations, movement tendencies over larger scales are stable across social environments and we provide proximate and ultimate explanations for why this may be the case.

Introduction

Most of life is social and hence individuals' social environments can play an important role throughout their lifetime (Krause et al. 2002). Social interactions form the basis for most behaviours from fighting, feeding to fleeing and mating. The benefits of social interactions and group living especially have most commonly been ascribed to more efficient foraging (Snijders et al. 2021), improved anti-predator vigilance, dilution of risks (e.g., Foster and Treherne 1981; Lehtonen and Jaatinen 2016), predator confusion (e.g., Treherne and Foster 1982; Jeschke and Tollrian 2007), cooperative breeding (e.g., Groenewoud et al. 2016), collective learning and decision making in the face of unpredictable environments (e.g., Couzin et al.), and improved locomotion performance (e.g. in birds, Lissaman and Shollenberger 1970; in fish, Marras et al. 2015). In all these cases, social interactions are highly dynamic and have the potential to cause strong and instantaneous feedback to one's own behaviour with respect to others' (Couzin and Krause 2003).

While behavioural consistency, one of the hallmarks of animal personality, may arise from repeated social interactions (Wolf and Weissing 2010; Dingemanse and Araya-Ajoy 2015), particularly during critical periods of development (e.g. Fischer et al. 2017), social environments are rarely stable during an individual's lifetime. Thus, when young animals are adapted to a particular social niche, they may need later in life to either (1) maintain themselves in similar social environments that match their personality (social niche choice or specialization, Bergmüller and Taborsky 2010; Montiglio et al. 2013), or (2) adjust their personality to match that of their interacting partners (social modulation of behaviour Webster and Ward 2011; Van Den Bos et al. 2013).

Social modulation of behaviour often takes the form of conformity, where individuals adopt the behaviour of their social group. For example, in three-spined sticklebacks (*Gasterosteus aculeatus*), individuals chose to forage on suboptimal patches to stay within a larger shoal even when they know it to be of lower quality (Webster and Ward 2011). In this species, shy individuals also spent more time out of cover in the presence of a bold than a shy partner (Jolles et al. 2014). Similarly in guppies (*Poecilia reticulata*), individuals preferred staying with a shoal rather than following a single individual toward a food source (Day et al. 2001). These examples clearly illustrate that social interactions can affect an individual's behavioural expression (Dingemanse and Araya-Ajoy 2015).

One challenge for animal personality studies is that detailed knowledge of an individual's social environment is often difficult to gain. For example, one would ideally not only need the data on behavioural responses of the focal individual but also those of all the interacting individuals. This is especially hard for animals which form dynamic social groups with individuals moving between groups. Another difficulty lies in reciprocal interactions: e.g. whether the behaviour of a focal individual is caused by its interacting partners or whether the interacting individuals changed to match the behaviour of the focal individual (Dingemanse and Araya-Ajoy 2015). These have typically led to studies testing conformity by comparing an individual's behavioural tendency in isolation vs in a social group. While this offers valuable insights, for a highly social species, behaviour in isolation may reflect responses to a 'novel' situation or stress. These challenges can be overcome by collecting longitudinal behavioural data of individuals across time and social groups and manipulating the compositions of social groups.

We here make use of a unique system of behaviourally divergent populations of three-spined sticklebacks ('migrants' and 'residents', Ramesh et al. 2022b) to investigate whether social context can modulate individual movement tendencies. Mixed groups of residents and migrants were studied in a mesocosm (system of connected semi-natural ponds where fish movements can be tracked remotely (Ramesh et al. 2022a), to quantify their movement tendencies over longer periods of time. This study aims to test whether and to what extent individuals modify their movement tendencies according to the composition of the social group (i.e. varying proportions of residents and migrants) and whether residents or migrants differ in their propensity to modulate their behaviour. We propose four main (non-exclusive) hypotheses through which social effects may affect individual movement tendencies (adapted from Webster and Ward 2011; Fig. 5.1.): A) No effect: migrant and resident sticklebacks do not modulate their movement tendencies in response to social group composition. B) Majority effect: individuals in a group tend to conform to the behaviour of the majority, such that e.g., residents placed in a majority migrant group would display behaviour more similar to that of migrants (or vice versa). C) Averaging effect: all individuals in a group adjust behaviour to some extent, such that we observe an averaging effect. D) Differential responsiveness between

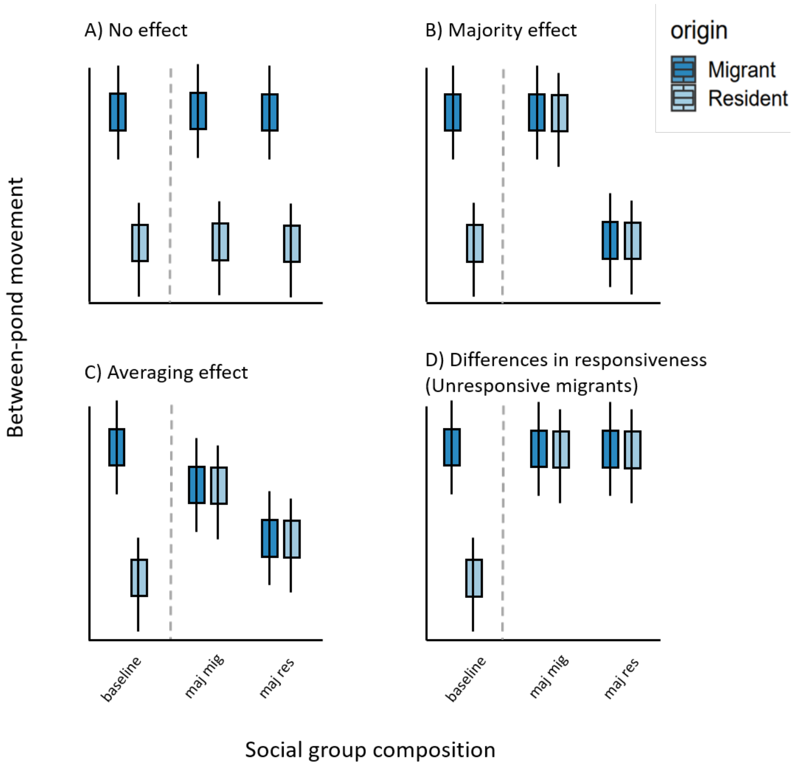

Figure 5.1 – *Four potential non-exclusive scenarios for social effects based on social conformity, adapted from Webster and Ward (2011). We compare these expectations with the patterns obtained from different measures of movement tendencies – i.e. the number of pond crosses and the latency to exit the first pond.*

groups: either migrants or residents respond by changing their movement tendencies to match that of the others (Guayasamin et al. 2017). For example, if migrants exhibit a 'fast' pace-of-life (associated with higher dispersal, higher activity and low HPA axis reactivity) and residents a 'slow' pace-of-life (associated with lower dispersal, lower activity and higher HPA axis reactivity; Réale et al. 2010), then migrants are expected to be less responsive to changes in their environment while residents are expected to conform to migrants regardless whether they are in majority or minority.

Methods

Study populations

We used the same fish as in Ramesh et al. (2022a). These fish were caught between March and April 2020 from two populations in the north-east of the Netherlands. Migratory sticklebacks were caught during inland migration at the mouth of a river ("NSTZ": $53°13'54.49'', 7°12'30.99''$), whereas resident sticklebacks were caught in a permanently closed-off sidearm of another river close by ("LL-A": $53°17'56.14'', 7°2'1.28''$). For the purpose of individual identification and monitoring using the RFID system all fish were anaesthetised using MS-222 (0.3 g/l) and injected with a PIT tag (ID-100A/1.4 Mini Transponder (8 mm); Trovan, Ltd., Santa Barbara, California) into the abdominal cavity. Prior to testing, all fish were given at least 10 days to recover in 100 L housing ponds outside. Fish were fed brine shrimp and red bloodworms (3F Frozen Fish Food bv, The Netherlands) *ad libitum* daily.

Experimental groups

In a previous experiment, the same fish were tested in connected ponds ('mesocosm') in 'pure' groups consisting of 10 fish of either migrant or resident origin (Ramesh et al. 2022a). We found that between-pond movements were repeatable and that residents consistently moved less than migrants (individual behaviour in these pure groups is used as 'baseline' in our later models). Building-up on this first experiment, we here tested for social conformity effects by manipulating the animals' social environment. We thus created mixed treatment groups consisting of a majority of residents ('majres': 7 residents + 3 migrants) or a majority of migrants ('majmig': 3 residents + 7 migrants). First, fish were randomly assigned to one of the two treatments regardless of their own origin (e.g., 'majres' or 'majmig'). Next, the same fish were re-tested in the opposite treatment (e.g., first test 'majres', second test in 'majmig'). As a result, all individuals were tested in all social contexts, i.e. a minimum of three tests per fish ('pure' group; see Ramesh et al. 2022a, 'majres' and 'majmig'), some fish had to be tested more often but we only kept the first three tests in the analyses. In total, we tested 49 migrants and 60 residents in 17 'majres' and 18 'majmig' groups. For each of the experimental rounds, we monitored within-pond and between-pond movements (see below). Between tests, fish were kept according to their origin in groups of 30 in smaller holding ponds (100 L filled with natural freshwater) outdoors and were given a minimum of 2 days between behavioural tests. Experimental groups were created 24h prior to testing and kept in the same holding ponds during that acclimatisation period.

The mesocosm

The mesocosm consists of a row of five connected ponds (each 1.6 m diameter; 1200 L; connected with tubes of 11 cm diameter). Ponds were fitted with circular RFID (Radio-frequency Identification) antennas (11 cm diameter) at different positions to remotely monitor movements within- and between-ponds (see below). The system was supplied with freshwater from a nearby water body, similar to natural stickleback habitats in the Netherlands (see details in Ramesh et al. 2022a).

Monitoring movements

Within-pond movements

On the morning of testing (10 a.m.) a group of 10 fish was released into the first pond in the row of 5 connected ponds. Connecting tubes were closed off during the first 5 hours of the experiment so that fish were confined to pond 1. To record movement within ponds, five circular RFID antennas were placed upright on the bottom of the pond, and 4 antennas were placed just below the water surface in a square pattern. In the previous study on pure groups (‘baseline’; Ramesh et al. 2022a), we did not find any differences between migrants and residents in terms of movements within a pond and hence did not expect social effects based on population composition to act here. In this study we will mainly focus on between-ponds movements. However see Fig. 5.A2 for comparison of migrants and residents in within-pond movements.

Between-ponds movements

Five hours after the start of the experiment we gently removed all RFID antennas used to monitor movement within the first pond. At this point a pump connected to the nearby water body was started, creating a flow (Fig. 5.A1). Fish were given 30 minutes to recover from the disturbance caused by removing the antennas after which the connection from pond 1 to the other ponds was gently opened. All connecting tubes were fitted with circular RFID antennas on both ends, and we recorded the fish’s movement between ponds for the next 16.5h (∼3.30 p.m. - 8 a.m.). Specifically, we monitored each fish’s latency to exit the first pond (i.e. time in hours to enter the second pond) and the number of crosses from one pond to another – regardless of direction (i.e. back-and-forth movements count the same as consecutive movement in one direction). After the experiment, fish were returned to holding groups according to their origin.

Statistical analysis

Our aim was to test whether the social group composition affects individual movement tendencies characterised by the individuals’ number of crosses between ponds and their latency to exit the first pond. Hence, we analysed variation in these two behaviours separately in univariate general(ised) linear mixed models (GLMMs) where individual baseline (i.e. number of pond crosses or latency to exit the first pond assayed in ‘pure’ groups, Ramesh et al. 2022a), treatment (‘majmig’ or ‘majres’ to test the effect of social group), origin (resident or migrant - to test if residents and migrants differ in their responses) and finally, treatment × origin (to test for differential responses of migrants and residents to the treatment) were fitted as fixed effects and Individual ID as random effect. GLMMs were fitted with Poisson errors for the number of crosses between ponds and Gaussian errors for the latency to leave the first pond. For the number of crosses, an observation-level random effect (‘Obs’) was added to control for overdispersion (OLRE, Harrison 2014). We used the *confint* function to obtain 95% confidence interval (CI) around the estimates. The statistical significance of fixed effects was assessed based on these 95% CI. We consider an effect to be significant in the frequentist's sense when its associated 95% CI does not overlap with 0. All GLMMs were constructed in R v. 4.1.0 (R Core Team 2021) using the *lmer* and *glmer* functions of the 'lme4' package (Bates et al. 2015).

Results

Pond crosses

Results show that fish behave similarly to their baseline with residents making consistently fewer pond crosses than migrants, regardless of the social group composition (Fig. 5.2a; significant effect of baseline but no clear effect of Treatment, Origin or Treatment × Origin, Table 5.1). There was a slight tendency for both migrants and residents to increase their pond crosses between tests, as seen by increased variance in their number of crosses in Fig. 5.2a, supplementary Table 5.A1 and Fig. 5.A3.

Latency to exit the first pond

Our results show that residents in mixed groups tended to exit the first pond quicker than their baseline (Fig. 5.2b; asymmetric CI of Origin and Treatment × Origin in Table 5.1). Notably, the latency to leave the first pond of residents was much lower in mixed groups compared to their baseline in pure groups and residents tended to behave as migrants did (median baseline = 16 h vs. median = 0.65 h and 0.73 h in majority migrant and majority resident groups, respectively) whereas the difference was much less pronounced for migrants (median baseline = 1.59 h vs. median = 0.62 h and 0.78 h in majority migrant and majority resident groups, respectively), supporting the prediction in Fig. 5.1d.

Discussion

Overall, our results show that individuals maintain their personality (here movement tendencies) irrespective of the changes in their social environment, i.e., the effect of 'baseline' on subsequent movement tendencies is strong. Nonetheless, residents tended to initiate movements faster, up to the level of migrants in mixed groups while migrants did not change their behaviour (supporting the prediction in Fig. 5.1d). In addition, we found that over repeated tests fish moved more between ponds, regardless of the social group. This could be explained by habituation, which is often seen when animals are tested repeatedly in the same environment that is no longer novel (e.g., Dingemanse et al. 2002, Supplementary table 5.A1, Fig. 5.A3).

A previous study in sticklebacks showed that personality variation within groups was not affected by the social environment (i.e. familiar vs. unfamiliar social groups did not affect an individuals' behavioural tendency, Laskowski and Bell 2014). Similarly,

Table 5.1 – ***Results of general(ised) linear models of movement tendencies (pond crosses and latency to enter pond 2).*** *Estimates of fixed effects (β) are given with their 95% confidence intervals (CI) and variance components are given with their standard deviation. Significant fixed effects are denoted in bold, when the confidence intervals do not overlap with zero. Sample sizes: N migrant = 49 individuals, N resident = 60 individuals. 1:'major migrant' is used as reference category; 2:'migrant' is used as reference category*

	Pond crosses	**Latency**
Fixed effects	β (95% C.I.)	β (95% C.I.)
Intercept	**0.89 (0.10, 1.63)**	**3.90 (1.88, 5.91)**
Baseline	**0.04 (0.02, 0.05)**	**0.24 (0.09, 0.38)**
Treatment^1	0.32 (-0.30, 0.94)	0.04 (-2.12, 2.20)
Origin^2	0.35 (-0.56. 1.27)	-2.19 (-4.84, 0.46)
Treatment^1 × Origin^2	-0.60 (-1.47, 0.25)	1.40 (-1.57, 4.39)
Random effects	Variance (std. dev)	Variance (std. dev)
Obs	1.84 (1.36)	-
Individual ID	2.20 (1.48)	15.27 (3.91)
Residual	-	28.83 (5.31)

we did not find evidence for social interactions leading to a smaller differences between migrants and residents, i.e., no effects of conformity. We propose several explanations for the lack of social effects on personality. From a proximate view, it is known that the early social environment may be particularly important for juvenile sticklebacks, where they tend to form groups and have strong social interactions (Ostlund-Nilsson et al. 2006). Social effects may thus mostly affect personality variation during sensitive periods of development that lead to stable personalities in adults (Groothuis and Trillmich 2011; Langenhof et al. 2016). Beyond such sensitive periods, individuals may be much less responsive to changes in their social environment (Fischer et al. 2017). Furthermore, the specific behaviours tested here may also not be sensitive to changes in the social environment, at least for migrants during the migratory period, as migrants depend on their inland migrations for reproduction. Hence in our experiment, we may have tested fish outside such sensitive periods. Furthermore, the differences in movement tendencies between migrants and residents may be too large to conform by behavioural plasticity alone, especially when considering between-pond movements.

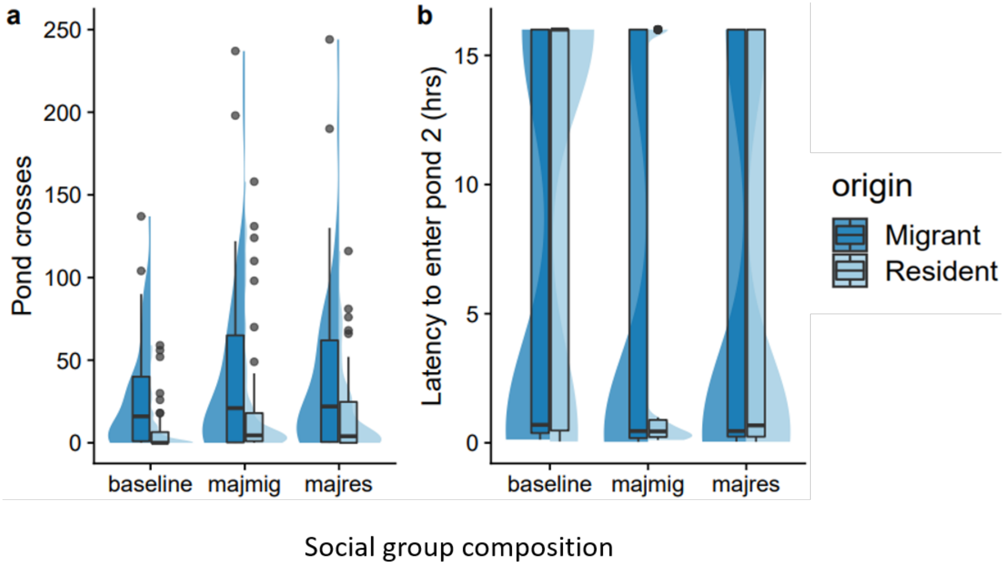

Figure 5.2 – *a) Number of pond crosses in each of the social groups. b) Latency of migrants and residents to enter second pond. h) by social group. Sample sizes:* $N_{migrant} = 49$ *individuals;* $N_{resident} = 60$ *individuals.*

Residents in our population are much smaller than migrants (Ramesh et al. 2021) and hence may have lower swimming capacities than migrants (Tudorache et al. 2007; Dalziel et al. 2012). However, we note that there is a tendency for residents to exit the first pond earlier in the presence of migrants, showing some level of conformity. This could be because the latency to exit the first pond does not require the same morphological and physiological adaptations necessary for swimming long distances between ponds.

From an evolutionary point of view behavioural plasticity in response to the social environment may not be favoured in a species with a highly dynamic, fission-fusion social system, such as in sticklebacks. Alternatively, variation in individual behaviours within a group (i.e. heterogeneity) could in some cases indeed outweigh the benefits of conformity. In guppies, individuals in small shoals consisting of a mix of bold and shy individuals were found to forage more efficiently in the lab than in shoals consisting of only shy or bold individuals (Dyer et al. 2009). Similarly, in collective decision making, heterogeneity in behaviours may lead to the emergence of leaders and followers and ultimately more efficient decision making than in homogeneous groups (Couzin et al.). In lions (*Panthera leo*) and other group hunting predators, heterogeneity among individuals comprising the group enables them to assume different roles, important for a successful hunt (Stander 1992). In all these cases, individual heterogeneity allows for some division of tasks or behavioural specialization within the group, increasing the overall group efficiency, and thus acting as a key mechanism maintaining individual differences (Montiglio et al. 2013).

In conclusion, in our study, we found that individual movement tendencies in adult sticklebacks override social effects to a large extent. Potential future studies may be done using similar-sized sticklebacks to control for potential assortative shoaling by size, or by doing experiments during ontogeny to identify sensitive periods in which social effects influence personality. Furthermore, an interesting avenue would be to use the mesocosm system to allow free movements of fish of different and test whether there is assortative shoaling and non-random associations among groups (social network analyses) and if that affects group performance.

Acknowledgements

We thank Dennis Worst and Willem Diderich for help with fish care and advice on experimental design and other animal caretakers for looking after the sticklebacks. We thank Peter Paul Schollema from the Water Authorities Hunze en Aa's and Jeroen Huisman from van Hall Larenstein, University of Applied Sciences for help with catching sticklebacks.

Appendix

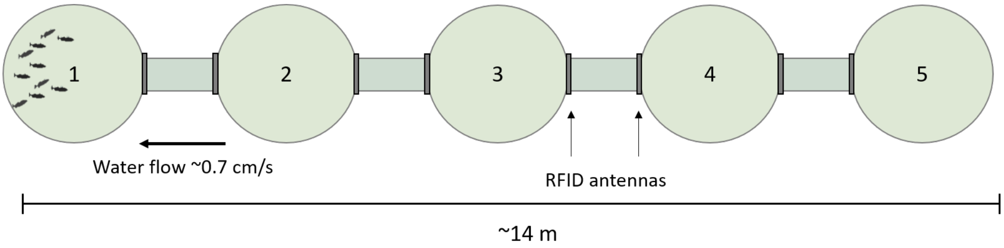

Figure 5.A1 – The mesocosm setup of linear ponds used to assess 'between-pond' movement of sticklebacks. A mixed group consisting of 10 sticklebacks is introduced in the first pond as shown. The corridors are fitted with RFID antennas on both ends to assess the movement and direction of movement of individual fish. In total, 35 groups were tested, with $N_{migrant} = 49$ individuals; $N_{resident} = 60$ individuals.

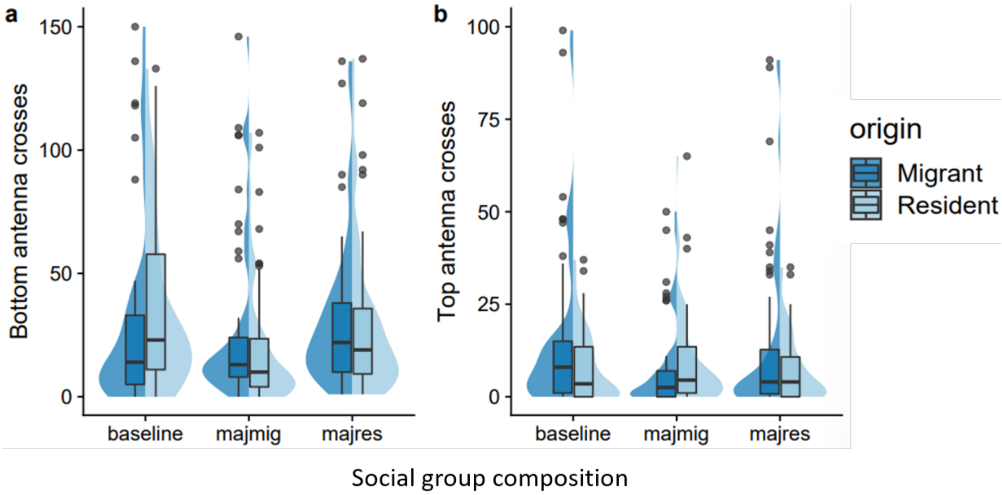

Figure 5.A2 – Within-pond movements across different social group compositions. a) Number of bottom antenna crosses in each of the social groups. b) Number of top antenna crosses in each of the social groups. The plots show that migrants and residents within-pond movements are not affected by the social environment. Sample sizes: $N_{migrant} = 49$ individuals; $N_{resident} = 60$ individuals.

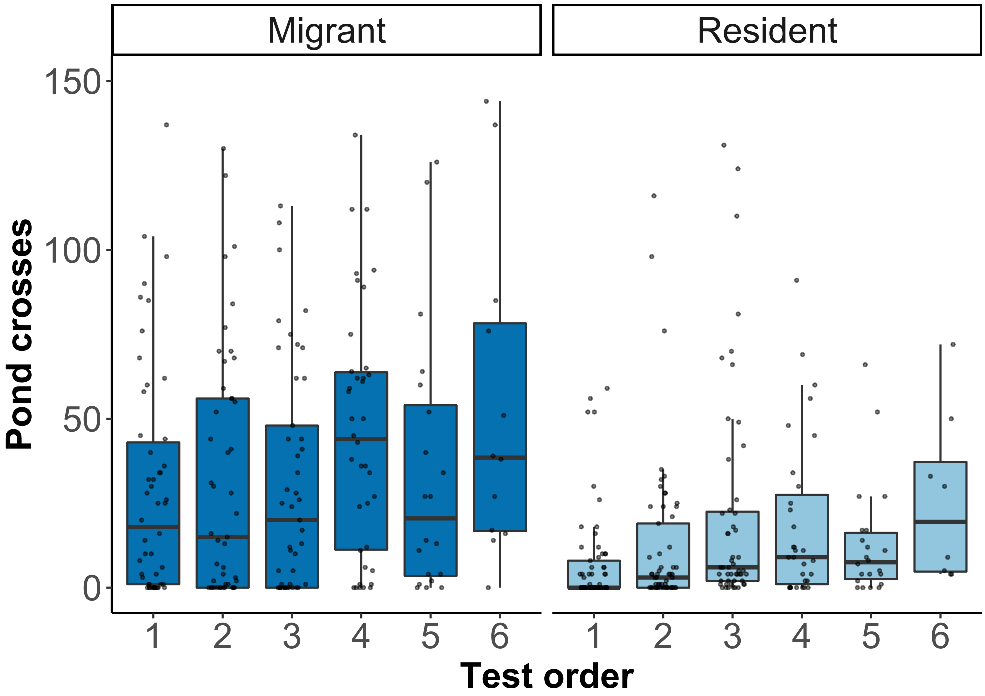

Figure 5.A3 – Plot of movement tendencies over all the test rounds. Individuals were tested multiple times as non-focal, to form the social group and hence some individuals were tested in their majority group for as much as seven times. There is a small tendency to increase pond crosses with test order (see Table 5.A1), suggesting some habituation effect.

Table 5.A1 – ***Summary of general(ised) linear models of between-pond (pond crosses and latency to exit pond 1).*** *For pond crosses, we used a GLMM with Poisson errors and for latency to exit pond 1, we used a LMM with Gaussian error structure. Estimates of fixed effects (β) are given with their 95% confidence intervals (CI) and variance components are given with their standard deviation. Significant fixed effects are denoted in bold, when the confidence intervals do not overlap with zero. Sample sizes: N migrant = 49 individuals, N resident = 60 individuals. The effect of 'test order' indicates the order of repeats. 1:'migrant' is used as reference category.*

	Pond crosses	**Latency**
Fixed effects	β (95% C.I.)	β (95% C.I.)
Intercept	**1.97 (1.21, 2.72)**	**6.09 (3.92, 8.25)**
Origin[1]	**-1.59 (-2.61, -0.59)**	**3.01 (0.08, 5.95)**
Test order	0.11 (-0.06, 0.29)	0.53 (-1.11, 0.05)
Origin[1] × Test order	0.16 (-0.09, 0.41)	**-0.87 (-1.69, -0.06)**
Random effects	Variance (std. dev)	Variance (std. dev)
Obs	1.42 (1.19)	-
Individual ID	2.67 (1.63)	15.27 (3.91)
Group ID	0.45 (0.67)	2.94 (1.71)
Residual	-	25.59 (5.06)

Intermezzo: Details matter when modelling the effects of animal personality on the spatial distribution of foragers

C. Netz
A. Ramesh
J. Gismann
P.R. Gupte
& F.J. Weissing

Published in Proceedings of the Royal Society B: 2022, 289(1970), 20210903. A comment on DiNuzzo and Griffen (2020)

By means of a simulation study, DiNuzzo and Griffen (2020) investigate whether individual variation in a personality trait can explain ‘undermatching’, an often-observed deviation from the ideal free distribution (IFD). Here, we raise five points of concern about this study, regarding (i) the interpretation of the results in terms of personality variation; (ii) deficiencies in the technical implementation of the model, leading to wrong conclusions; (iii) the effects of population size on deviations from the IFD; (iv) the measure used for quantifying deviations from the IFD and (v) the analysis of the mud crab data. Finally, we provide an overview of the evolutionary ramifications of the relation between animal personality and the IFD.

Personality variation and the IFD

The individuals in DiNuzzo & Griffen’s model tend to maximize their intake rate. At each point in time, they are perfectly informed about the distribution of resources (which remains constant) and the distribution of foragers (which can change due to movement). Individuals differ in ‘activity’, that is the rate at which they recognize that their current intake rate is sub-optimal; once they observe a discrepancy, they move instantaneously to the habitat patch yielding a maximal intake rate. In this model, each individual has to move at most once: if all individuals have moved (or stayed at their initial position, as this already yielded a maximal intake rate), the IFD is reached. It is therefore obvious that less active individuals that, by definition, take on average more time steps for making a movement decision, retard the approach of the population to the IFD. Hence, it is also obvious that the ‘time to reach IFD’ increases with an increase of the proportion of inactive individuals. In other words, it is not personality variation per se that retards the approach to the IFD but rather the presence of inefficient movers.

Problems with the technical implementation of the model

Above we argued that it is obvious that the ‘time to reach IFD’ increases with the proportion of inactive individuals. In view of this, it is surprising that DiNuzzo & Griffen report a hump-shaped relationship in one of their simulation scenarios (their Fig. 4e) and even a monotonic decline in the time to reach IFD with increasing proportions of inactive individuals in case of a type II functional response (their electronic supplementary material, Fig. S1, reproduced here in Figure 1a). We think both results are artefacts. The pattern in their electronic supplementary material Fig. S1 is caused by a comparison between intake rates calculated with two different formulas. As a consequence, individuals can ‘believe’ that they are already in a habitat maximizing their intake rate, while really they are not.

In addition, an incorrect formula of a ratio-dependent functional response type II is used (following Abrams and Ginzburg 2000). A detailed explanation of these mistakes can be found in our electronic supplementary material (Netz et al. 2021). If these mistakes are corrected, the time to reach IFD shows the expected increasing trend with the proportion of inactive individuals (Figure 1b), rather than the decreasing trend reported by DiNuzzo & Griffen. Hence, a saturating type II functional response leads to a similar relationship between the proportion of active consumers and time-to-IFD as an unlimited linear (type I) functional response. Special explanations for discrepancies between type I and type II models (the 'domino effect' explanation in electronic supplementary material, 1.4 of DiNuzzo and Griffen 2020) are not needed and are actually misleading.

We can further show by a simple mathematical argument that the correspondence between the two model variants considered by DiNuzzo & Griffen should be even stronger: the special version of the type II functional response used by DiNuzzo & Griffen (following Abrams and Ginzburg 2000) should lead to exactly the same time-to-IFD and the same consumer distribution over patches as their type I functional response (see part 3 of our electronic supplementary material Netz et al. 2021). We were therefore surprised that our figure 6.1b does not exactly match with Fig. 3 in DiNuzzo and Griffen (2020): it generally takes 100 time steps longer to reach the IFD. Re-running the scenario underlying Fig. 3 in DiNuzzo and Griffen 2020 with DiNuzzo & Griffen's published NetLogo code, we did obtain an exact replicate of our Figure 1b. We conclude that DiNuzzo & Griffen must have used a different version of their simulation program to produce their Fig. 3.

In addition, the simulation program in DiNuzzo and Griffen (2020) produces a substantial bias in reported time to reach the IFD. Each simulation run stops once movement has ceased for 50 time steps, assuming that this is a clear indication that the IFD has been reached. The problem is that movement can cease for 50 time steps even in situations where the population is still far from an IFD (Figure 2a). This easily happens in populations with a large proportion of highly inactive individuals. the lack of movement may just reflect the reluctance of these individuals to move (rather than having reached a habitat with maximal intake rate, where movement is no longer necessary). Figure 2 shows two replications of Fig. 4e in DiNuzzo and Griffen (2020), one with the published NetLogo code (Figure 2b) and a second with an improved version (see our electronic supplementary material; Netz et al. 2022) where DiNuzzo & Griffen's stopping criterion is replaced by a check whether the IFD has indeed been reached (Figure 2c). It is obvious that the stopping criterion has a large effect on the simulation outcome. Notice that neither outcome shows the puzzling 'hump' of Fig. 4e in DiNuzzo and Griffen (2020). As we produced figure 2b with DiNuzzo & Griffen's published NetLogo code, we have to conclude again that a different version of their simulation program was used to derive their Fig. 4e.

A more detailed account of the technical issues reported above (and some additional issues) and corrected versions of the NetLogo program can be found in our electronic supplementary material (Netz et al. 2021).

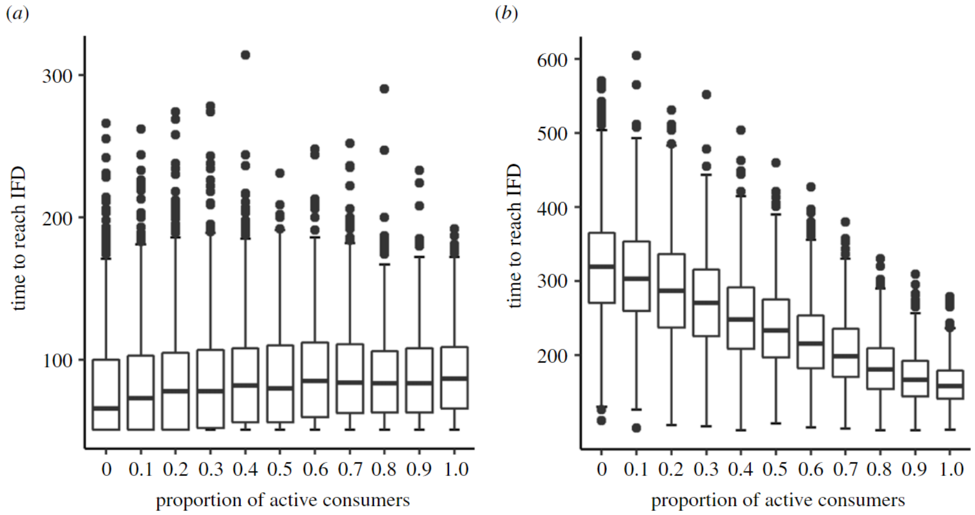

Figure 1 – ***Replication of DiNuzzo & Griffen's electronic supplementary material Fig. S1*** *(a) using their original NetLogo code and (b) using a corrected version of their code. Both panels show the time to reach the ideal free distribution (IFD) for various proportions of 'active' (80% activity) and 'inactive' (20% activity) consumers with a type II functional response in 1000 replicate simulations. According to DiNuzzo & Griffen's NetLogo code, the time-to-IFD increases with the proportion of active consumers. A corrected version of the code (see our electronic supplementary material, Netz et al. (2022) for details) yields the expected pattern of decreasing waiting times with increasing proportions of active consumers.*

Effects of population size

DiNuzzo & Griffen investigated the effect of population size on the time to reach the IFD. However, the timescale of their model implementation is quite different from a 'natural' timescale. In their simulation program, individuals make decisions sequentially, and only one individual can make a decision in each time step. As in a larger population more individuals have to take decisions, this automatically increases the time to reach a certain target distribution. Moreover, the time to reach the IFD is inflated by the fact that active individuals are restricted in their movement because they have to 'wait' for inactive individuals. For these reasons, it is more natural to use a continuous timescale, where individuals take movement decisions independently of each other, at a rate that is proportional to their activity level. This can be done in a straightforward manner, by translating the discrete-time model of DiNuzzo & Griffen into an otherwise equivalent event-based model (making use of the Gillespie algorithm, Gillespie 1976; a description and implementation of such a model can be found in Netz et al. (2021). Figure 3 shows how in the event-based version of the model the time to reach the IFD depends on the population size, N and the proportion of active individuals. For each population size, the time to reach the IFD is, as expected,positively

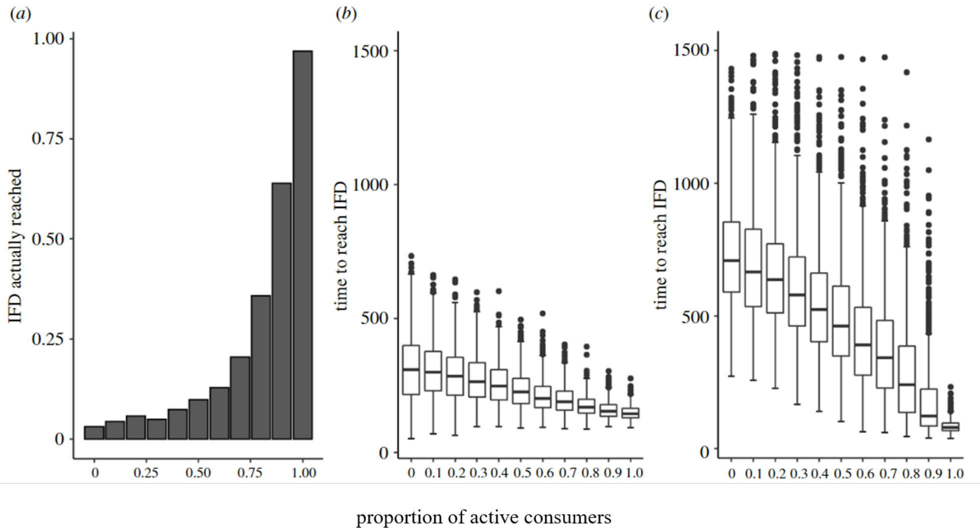

Figure 2 – ***Systematic bias in outcomes due to premature termination of simulations.*** *The NetLogo code underlying the simulations in [1] assumes that the IFD is reached after 50 time steps of inactivity. (a) The proportion of simulations that have actually reached the IFD after 50 time steps of inactivity in the scenario underlying Fig. 4e in DiNuzzo and Griffen (2020). (b) Replication of Fig. 4e, using DiNuzzo & Griffen's NetLogo code. (c) The same set of simulations for an improved version of the NetLogo code, where a simulation now stops when the IFD is actually reached. In all simulations,'active'consumers have an activity level of 90% while'inactive'consumers have an activity level of 10%.*

related to the proportion of inactive individuals. However, the event-based version of the model does not support DiNuzzo & Griffen's conclusion that the time to reach the IFD increases with population size. This only occurs for very low population densities (N=8 and N=40 in Figure 3),and even in these cases, the effect is small. At higher population sizes, the time to reach the IFD decreases with population size: as shown in Figure 3, the IFD is reached much faster in a population with N=1000 individuals than in any of the smaller populations. This can be explained as follows. In the case of the low population sizes considered by DiNuzzo & Griffen, the initial density of individuals is very low (typically only one individual per patch). In such a case, an individual can only improve its intake rate by moving to a more profitable patch. In case of a large population size (and a higher initial density per patch), there is an additional option: if an individual on a patch decides to leave in order to improve its intake rate elsewhere, all remaining individuals on that patch profit as their intake rate increases due to alleviated within-patch competition (see Wolf et al. 2008). This effect is not addressed by the study of DiNuzzo & Griffen, although the authors state, 'in most natural systems, there are many more consumers than patches'.

Quantifying the approach to the IFD

DiNuzzo & Griffen conducted their study in order to investigate whether personality differences can explain‘undermatching’, the commonly observed phenomenon that high-resource patches tend to be relatively under-exploited, while low-resource patches are relatively over-exploited. Yet, they devote only one figure (their Fig. 2) to this phenomenon. In general, they quantify deviations from the IFD by measuring the time to reach the IFD. This measure has at least three disadvantages. First, ‘time-to-IFD’ is determined by the last individual that moves to a patch with an optimal intake rate. In other words, a single individual with very low activity can have a very large effect on the time-to-IFD. Second, ‘time-to-IFD’ depends on the initial conditions; it takes longer to reach the IFD if the initial spatial distribution of individuals differs a lot from the IFD. Third, ‘time-to-IFD’ is only a sensible measure when the IFD is actually reached. This, however, will only be the case in highly standardized simulation models with a fixed resource distribution. As stated by DiNuzzo & Griffen: ‘In most systems, the IFD is a moving target owing to temporal environmental variation and directional change (i.e. habitat degradation)’. In 1.5 of their electronic supplementary material, DiNuzzo & Griffen show some simulation results for a scenario with temporally varying patch quality. Surprisingly, ‘time-to-IFD’ is also used for this scenario (their electronic supplementary material Fig. S2), where it is difficult for us to understand how the IFD can ever be reached in the case of rapid environmental change. How can movement cease for 50 time steps (the criterion for reaching the IFD) if the distribution of patch qualities changes completely every 10 or 20 time steps? Under such changing conditions, we would advocate using a more robust, population-level measure for deviations from the IFD, such as the variance in intake rates across patches.

Analysis of the mud crab system

We are puzzled by the fact that DiNuzzo & Griffen revert to a simple calculation of activity ratios in their analysis of the refuge use data on the mud crab, *Panopeus herbstii* (Toscano et al. 2014), instead of taking advantage of their individual-based model. The model becomes necessary because such a simple calculation does not suffice, as it ignores the distribution of personality in the population. Hence, their Fig. 5 illustrates the influence of personality on the IFD only in the sense that no single crab is ‘ideal’ in immediately leaving its refuge and moving to the patch with highest profitability, but not the implications of the distribution of activity levels in the population. Additionally, the data come from a special (predation cue) treatment, not from standard conditions, and the crabs differ substantially in size (actually body size is used as a proxy for activity level) and accordingly also in their resource needs and competitive abilities.

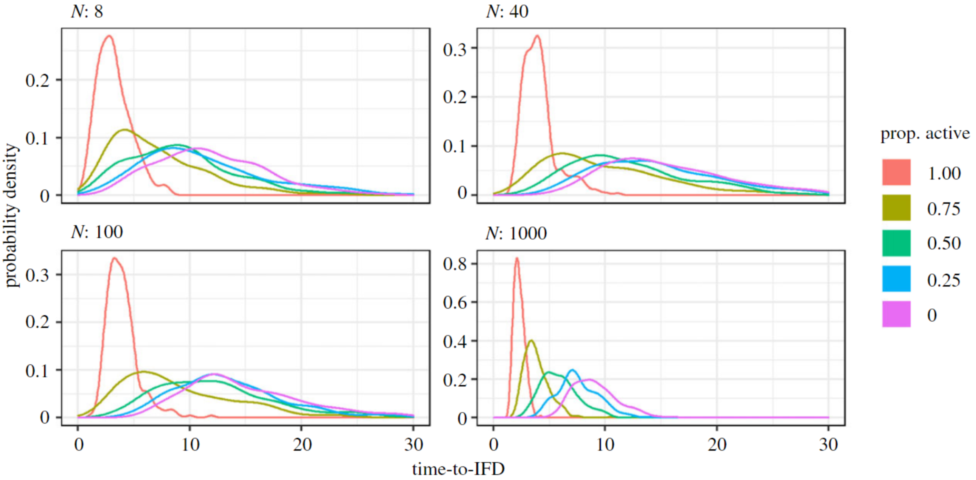

Figure 3 – ***Probability distributions of the time until the ideal-free distribution is reached, based on 1000 replicate simulations per setting.*** *In a system with 49 habitat patches, the panels show how the time to reach IFD depends on the proportion of 'active' (movement rate 0.8) and 'inactive' (movement rate 0.2) individuals for four population sizes, N.*

Outlook

We have the impression that DiNuzzo & Griffen view 'personalities' mainly as (mal-adaptive) deviations from optimal or efficient behaviour. By contrast, many studies show that personality variation is often shaped by adaptive evolution (Dall et al. 2004; Wolf et al. 2007; Dingemanse and Wolf 2010; Wolf and Weissing 2010; Luttbeg and Sih 2010; Bergmüller and Taborsky 2010; Wolf and Weissing 2012). For example, Wolf et al. (2008) demonstrate that 'inactivity' (called 'unresponsiveness') may be viewed as an efficient strategy in achieving a high foraging success and approaching an IFD. An adaptive perspective on personality variation leads to novel eco-evolutionary questions regarding the interplay of individual behavioural variation and the spatial distribution of foragers. The IFD is a prototype example of a model linking ecology (the spatial distribution of foragers) to evolution (optimal or evolutionarily stable movement decisions). Future research is needed to reconcile the IFD with the eco-evolutionary causes and consequences of personality for at least two reasons: first, the IFD model presupposes that the resource intake rate is a proxy for fitness (Tregenza 1995). But how, then, can different personality types persist at stable proportions, when inactive individuals consistently achieve a lower intake rate than their more active conspecifics? Second, a personality perspective may change what spatial distribution is optimal. In animals, differences inactivity are usually associated with (adaptive) differences in energy metabolism (Careau et al. 2008). When foraging individuals differ in energetic expenditure, they should not take maximizing the intake rate as their sole guiding principle (Campos-Candela et al. 2019). In other words, individuals differing in activity should use different decision rules, and the optimal behaviour of a polymorphic population may, even at equilibrium, deviate considerably from the IFD of a monomorphic population.

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6

Ideal free distribution of unequal competitors: spatial assortment and evolutionary diversification of competitive ability

C. Netz
A. Ramesh
& F.J. Weissing

Abstract

Ideal free distribution theory attempts to predict the distribution of well-informed ('ideal') and unconstrained ('free') foragers in space based on adaptive individual decisions. When individuals differ in competitive ability, a whole array of equilibrium distributions is possible, and it is unclear which of these distributions are most likely. In the first part of our study, we show that strong competitors have an intrinsically stronger preference for highly productive habitat patches than poor competitors. This leads to an equilibrium distribution where the average competitive ability on a patch is strongly correlated with the productivity of the patch. In the second part of our study, we consider what happens if differences in competitive ability are heritable and, hence, subject to natural selection. Under constant environmental conditions, selection eliminates such differences: a single strategy prevails that optimally balances the costs and benefits associated with competitive ability. If the productivity of patches changes during the lifetime of individuals, the spatial assortment of competitors of equal competitive ability gives poor competitors a systematic advantage in times of environmental change, while good competitors benefit from equilibrium conditions. Using evolutionary individual-based simulations, we demonstrate that environmental change may then lead to the diversification of competitive ability.

Introduction

Animals constantly have to make decisions on movement within or between habitats, especially in variable environments. The distribution of individuals depends on these decisions, which take into account the properties of the habitat and the distribution of conspecifics. The simplest forager distribution model (Fretwell 1969) assumes a population of identical foragers, which are 'ideal' in that they have complete knowledge of the distributions of resources and conspecifics and are 'free' in that they are unrestricted in their movement. Foragers are then expected to distribute such that any further movement between patches does not increase the intake of any individual, yielding the so-called ideal free distribution (IFD). If foragers do not interfere with each other and share resources equally, the distribution of foragers corresponds to the distribution of resources, termed 'input matching' (Parker 1978). Although the IFD serves as a useful null model, in reality, individuals are neither 'ideal' nor 'free', and there is increasing evidence that consistent individual differences influence habitat choice and spatial distributions (Ehlinger 1990; Holtmann et al. 2017a; Bonnot et al. 2018; Schirmer et al. 2019, 2020). This development is both a challenge and an opportunity for the theoretical framework of the ideal free distribution.

Several models have studied the distribution of foragers by relaxing key assumptions of the IFD, for example considering individuals that behave idiosyncratically and in non-optimal ways (Jackson et al. 2004; Matsumura et al. 2010) or incorporating individual differences that affect optimal decision making (Holt and Barfield 2008; Edelaar et al. 2008), specific examples including body size (Price 1983; Railsback and Harvey 2002), gizzard size (Van Gils et al. 2005) or competitive ability (Sutherland 1985; Houston and McNamara 1988; Sutherland and Parker 1992; Van de Pol et al. 2007; Smallegange and van der Meer 2009). In particular, individual variation in competitive ability has been the focus of several modelling studies. Such variation is incorporated into IFD models in two different ways. In interference competition models, competitive ability affects the impact of interference on individual intake rates (Sutherland and Parker 1992; Smallegange and van der Meer 2009). In this case, IFD theory predicts the segregation of unequal competitors over resource patches, where the most competitive types accumulate on patches with the highest resource levels, while weaker competitors occur at the lower resource levels. In exploitation competition models, the competitive ability of an individual determines the individual's share in the local resources, for example via the capacity to defend territories (Huxley 1934). In this case, IFD theory predicts that, at equilibrium, the competition intensity on each patch (= the sum of the competitive abilities of the occupants of the patch) is proportional to the resource abundance on that patch (Sutherland 1985; Sutherland and Parker 1992). Such an equilibrium distribution can be realized in many different ways, and in principle, it is possible that weak and strong competitors co-occur on all patches or that weak competitors accumulate on patches with the highest productivity. Sutherland (1985) hypothesised that the most likely distribution of foragers converges on the IFD with equal competitors, which corresponds to the situation where, at equilibrium, the distribution of competitive types is roughly the same for all occupied patches. In contrast, Houston and McNamara (1988) argued that strong competitors should be slightly overrepresented on resource-rich patches, simply as a consequence of the number of ways in which the equilibrium distribution can be realized. Further work showed that the sequence and mechanism, by which foragers distribute across both patches, can have a significant impact on the equilibrium distributions that are reached (Houston and McNamara 1988; Spencer et al. 1995).

Virtually all theoretical work on the distribution of unequal competitors has only considered the choice between two patches. The first goal of this study is to extend the theory to a more fine-grained environment with multiple patches. In addition, we consider a whole spectrum of competitive abilities. We show that stronger and weaker competitors differ in their patch preferences and that stronger competitors have, in comparison to weaker competitors, a systematic bias in favour of resource-rich patches. One would therefore expect competitor assortment, where strong competitors accumulate on resource-rich patches, while weak competitors typically occur on resource-poor patches. By means of individual-based simulations, we will show that such assortment does indeed take place under exploitation competition and that the effect is much stronger than the 'statistical mechanics' approach of Houston and McNamara (1988) suggests.

Most studies on the distribution of unequal competitors assume that differences in competitive ability are fixed and externally given. In many situations, it is likely that such differences are at least partly heritable (Baldauf et al. 2014). This implies that competitive ability is an evolvable trait. Therefore, we can ask not only how individual variation in competitive ability influences habitat choice and spatial distributions but also how (variation in) competitive ability is shaped by natural selection. Addressing this question is the second goal of this study.

One might expect that natural selection has the tendency to eliminate all variation in competitive ability, thus leading to a single strategy that optimally balances the costs and benefits associated with a given level of competitive ability. With a simple argument and some evolutionary simulations, we will show that this is indeed the case if the environment is stable, that is, if the resource level per patch remains constant. Making use of the assortment result derived in the first part of our study, we then argue that the situation may be different in case of a changing environment. With a simulation study, we will demonstrate that, under changing conditions, selection can lead to the diversification of competitive ability.

Our twofold purpose is therefore to first investigate the equilibrium distributions emerging from individual-based patch choice decisions, and secondly to study the evolutionary dynamics that this scenario implicates. We present a) an analytical description of how habitat preferences depend on individuals' competitive abilities, and b) a simulation model of how spatial assortment can lead to the diversification of competitive ability. We thus show that spatial distributions are not only determined by the interactions between unequal competitors but that the process of repeated redistribution can by itself propel the evolution of several competitive morphs.

Models and Results

We consider a population distributed across a number of patches, each of which provides a constant influx of resources that is shared among the foragers present on the patch. This situation is commonly known as a 'continuous input' model (Tregenza 1995). Individuals differ in their competitive ability, that is, their ability to defend resource shares against competitors. The intake rate of an individual on a habitat patch with resource influx R depends on the relation of the individual's competitive ability to the 'competition intensity' C on this patch, which is defined as the sum of the competitive abilities of all individuals present. In line with earlier work (Houston and McNamara 1988; Sutherland and Parker 1992; Tregenza 1995), we assume that the individual can consume a fraction c_i/C of the local resources, yielding the intake rate:

$$F(c_i, R, C) = \frac{c_i}{C}.R = c_i.\frac{R}{C} \tag{1}$$

The ratio R/C may be viewed as the 'resource availability' on a given patch (per unit of competitive ability). As long as patches differ in their resource availability, at least some individuals have an incentive to move to a patch with higher resource availability. This will continue until an 'ideal free distribution (IFD)' is reached where all occupied patches have the same resource availability R/C (Sutherland 1985; Tregenza 1995; Houston and Lang 1998).

Spatial assortment: good competitors prefer resource-rich patches

At the ideal free distribution, the ratio R/C is equalized across all patches. Hence, the ideal free distribution depends on the distribution of competition intensity over patches and not directly on the distribution of individuals. In fact, many different distributions of foragers may lead to the same competition intensity on a given patch. For example, the same value $C = 10$ occurs when a patch is occupied by 10 individuals with competitive ability 1.0 or by 100 individuals with competitive ability 0.1. This implies that the IFD criterion (equality of the ratio R/C) can be satisfied by many different distributions of competitors over the patches. The question is whether some of these distributions are more likely than others. Sutherland (1985) predicted that the most likely distribution should correspond to the ideal free distribution with equal competitors since such a distribution corresponds to a random mixture of competitors over patches. Houston and Lang (1998) noticed that among the many possible ways by which the IFD criterion can be satisfied those options where stronger competitors tend to occur on resource-rich patches are somewhat overrepresented. In analogy with statistical mechanics, they argue that it is, therefore, likely that at least some assortment of competitors over patches will occur. Although this argument is elegant, it is not immediately obvious whether principles of statistical mechanics can be applied to agents that do not move at random but by choosing the most suitable patch. Spencer et al. (1995) and Houston and Lang (1998) expanded on these results and showed that the sequence in which individuals move may have considerable influence on the resulting equilibrium distributions. Further, Houston & Lang showed that the movements of strong competitors may cause the subsequent movement of inferior competitors, providing a plausible mechanism by which spatial assortment may occur across patches. We here show that, more generally, the patch preferences of weaker competitors differ systematically from those of stronger competitors.

Consider an individual that compares two patches as to their suitability: patch 1 with resource influx R_1 and current competitive intensity C_1 and patch 2 with resource influx R_2 and current competitive intensity C_2. Assume further that patch 1 is the resource-richer patch, $R_1 > R_2$. An ideal and free individual with competitive ability c_i should prefer the resource-richer patch 1 if this patch, after the arrival of the individual, yields a higher intake rate:

$$c_i . \frac{R_1}{C_1 + c_i} > c_i . \frac{R_2}{C_2 + c_i} \tag{2}$$

Notice that the denominators in (2) take account of the fact that the competition intensity of each patch would increase by, should our individual move to that patch. Inequality (2) is equivalent to:

$$c_i > \frac{C_1 C_2}{R_1 - R_2} . \left(\frac{R_2}{C_2} - \frac{R_1}{C_1} \right) \tag{3}$$

As long as the resource-rich patch 1 has a higher resource availability ($R_1/C_1 > R_2/C_2$), the right-hand side of (3) is negative, implying that all individuals prefer this patch, regardless of their competitive ability. This changes when the resource-rich patch 1 gets crowded to such an extent that the resource-poor patch 2 has a higher resource availability ($R_2/C_2 > R_1/C_1$). In this case, (3) is a threshold criterion: only those individuals with a sufficiently large competitive ability (larger than the right-hand side of (3)) will prefer the resource-rich patch 1, while individuals with lower competitive ability will prefer the resource-poor patch 2.

The above argument shows that individuals with a large competitive ability have a higher likelihood to prefer resource-rich patches than individuals with a smaller competitive ability. We therefore expect the assortment of competitive abilities along a resource gradient. To investigate the strength of this effect, we ran some individual-based simulations. We consider 100 patches with resource levels running from 0.01 to 1.0 at increments of 0.01. A population of 10,000 individuals containing the five different competitive types (0.1, 0.2, 0.4, 0.8, 1.6) in equal proportions is initially distributed randomly over the patches. Individual foragers are chosen in random order to compare intake rates among patches and move to the patch offering the highest intake rate. The individuals redistribute until no single individual can improve their intake rate any further, at which point a stable distribution is reached. As shown in Fig. 6.1, the ensuing distributions are characterized by spatial assortment, where individuals of high competitive ability consistently occur more frequently on high resource patches, while individuals of low competitive ability occur on low resource patches. The degree of spatial assortment is surprisingly strong considering the relatively small influence of competitive ability on the comparison of potential intake rates between different patches ($C \gg c_i$; eqn (2)). As the IFD is approached, the difference between the R/C ratio of different patches becomes successively smaller, such that many patches offer relatively similar intake rates. In this case, the influence of individual competitive ability becomes temporarily decisive, producing the observed spatial correlations. As the differences between the R/C ratios decrease yet further, the threshold approaches zero and becomes irrelevant again.

Evolution of competitive ability

Differences in individual competitive ability may arise at all levels from genetics to development and environmental effects during adulthood. From an evolutionary perspective, the presence of different types of competitors in a population poses the question of how multiple competitive types can coexist in a population. In the following we will consider how competitive abilities evolve in a patchy environment, first for a population that is permanently at the ideal free distribution (within generations) and second for a population where the IFD is repeatedly perturbed by changes in the environment.

In an evolutionary model, we have to specify how differences in intake rates translate into differences in survival and reproduction (Darwinian fitness). In optimal foraging models, either average food intake rate or lifetime resource consumption is typically taken as a proxy for fitness. When considering the evolution of competitive ability, this would not make much sense: according to eqn (1), the intake rate on each patch is proportional to an individual's competitive ability. Hence, the highest possible com-

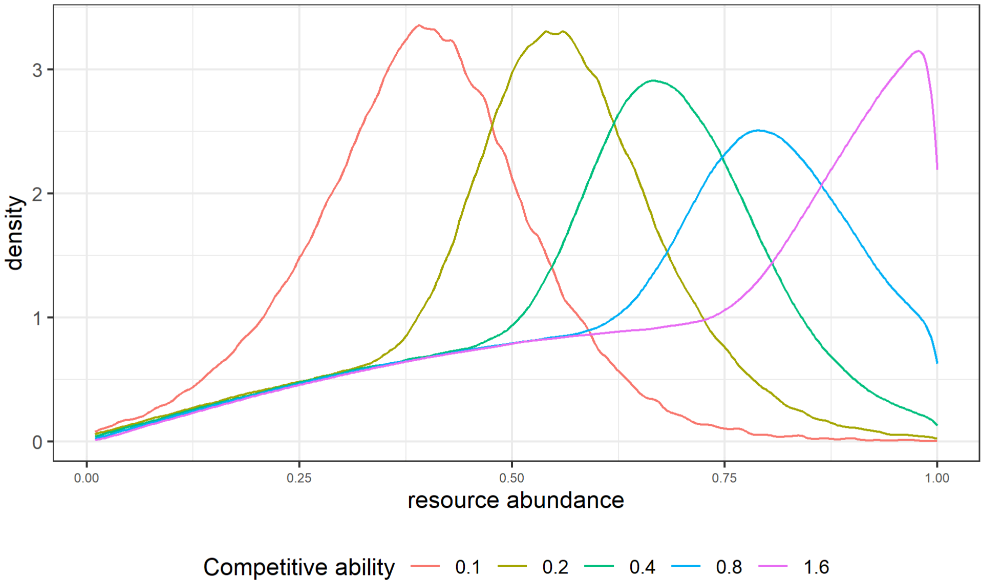

Figure 6.1 – ***Ideal free distribution of unequal competitors over habitat patches differing in resource abundance.*** *10,000 individuals were initially distributed randomly over 100 patches with resource abundance values running from 0.01 to 1.00 at intervals of 0.01. One of five competitive ability values was randomly assigned to each individual. Then individuals moved sequentially (in random order) to the best-suited patch, until an ideal free distribution was reached. The graph shows the distribution of each competitive type at the IFD by combining the results of 100 replicate simulations.*

petitive ability would evolve if it could be realized without costs. Here, we assume that a higher competitive ability is metabolically costly, and that the per-time-unit costs for a competitive ability c_i amount to kc_i resource units, where k is a constant of proportionality. Our fitness proxy is therefore based on the net intake rate:

$$W(c_i) = c_i.\frac{R}{C} - c_i.k = c_i.\Big(\frac{R}{C} - k\Big) \tag{4}$$

which, accumulated over the lifetime of an individual, is our measure of lifetime reproductive success. At the IFD, the resource availabilities R/C are equal across all patches and given by $R/C = \sum R_j / \sum c_i = \sum R_j/(N\bar{c})$ where N is the number of individuals and $\bar{c}$ is their average competitive ability. If we insert this expression into (4), we can conclude that the net intake rate W increases with c_i if $\sum R_j/(N\bar{c}) > k$ and decreases with c_i if $\sum R_j/(N\bar{c}) < k$. This implies that competitive ability will converge to a level c^* at which $\sum R_j/(N\bar{c}) = k$. As the corresponding population is monomorphic, the value c^* is equal to the average competitive ability ($c^* = \bar{c}$). This yields:

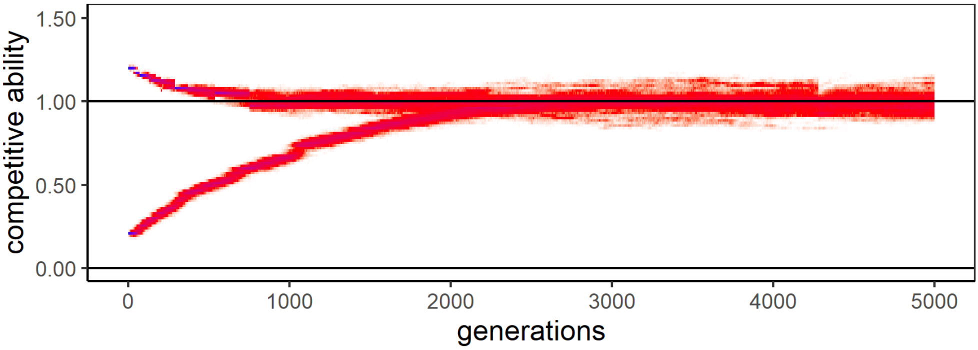

Figure 6.2 – ***Evolution of competitive ability under IFD conditions.*** *Two simulations, starting at different initial conditions, for the evolution of competitive ability in a system where 10,000 individuals distribute over 100 patches with resource abundances varying between 0 and 1. The cost parameter k had the value 0.005. Both simulations converge to the value* $c^* = 1.0$ *the value of competitive ability predicted by eqn (5). The relative frequencies of trait values within each generation are encoded by a colour gradient from 0.0 (= white) to 0.3 (= red) and 1.0 (= blue).*

$$c^* = \bar{c} = \sum \frac{R_j}{N.k} \tag{5}$$

To check this expectation, we ran individual-based evolutionary simulations. Each individual is endowed with a heritable competitive ability. Within generations, individuals move to a patch yielding the maximal intake rate (given their competitive ability); movement will stop once the ideal free distribution is reached. Between generations, individuals produce offspring that inherit the competitive ability of their parent (subject to rare mutations). As the number of offspring is proportional to the net intake, accumulated over lifetime, those competitive abilities will increase in frequency that realize the highest net foraging success. A more detailed description of the model is provided in the appendix. Figure 6.2 shows that, irrespective of the initial conditions, the simulations evolve to the value of c^* predicted by eqn (5) and therefore confirm our analytical expectations.

Changing environments: evolution of competitive diversity

If environmental conditions remain constant within a generation, a population of foragers will rapidly converge to the IFD. Accordingly, the population will converge to a monomorphic state where all individuals have the same competitive ability c^* . Some limited variation around c^* remains due to the ongoing influx of mutations (selection close to the evolutionary equilibrium is weak and not very efficient in eliminating mutations that are close to c^*), but larger-scale variation in competitive ability is eliminated. Resource environments are rarely static, however, and the ideal free distribution is therefore often a fleeting target. If the environment changes repeatedly

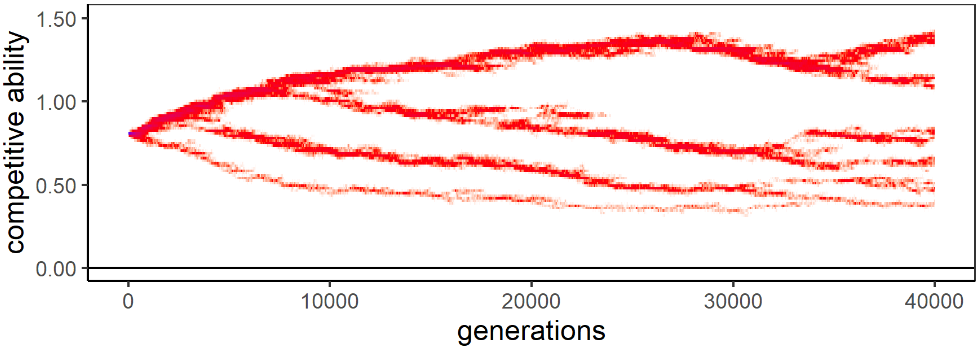

Figure 6.3 – ***Evolutionary diversification of competitive abilities under changing environmental conditions.*** *The graph shows one representative simulation for the same parameter settings as in Fig. 6.2. Now, however, the resource influx per habitat patch does not remain constant throughout a generation but randomly changes on average once every four time units. In the course of evolution, the population 'branches' into distinct competitive types.*

within a generation and if it takes time to re-establish the IFD after each change, it is no longer obvious that only a single competitive ability will persist.

To see this, consider a population with variation in competitive abilities. As we have seen above, strong competitors will, under IFD conditions, accumulate on resource-rich patches, while weak competitors will mainly occur on resource-poor patches. If the environment (i.e., the resource influx per patch) changes at random, previously resource-rich patches will, on average, deteriorate while previously resource-poor patches will, on average, improve. This implies that changing conditions will, on average, be detrimental for strong competitors (that have accumulated on the previously resource-rich patches) and beneficial for weak competitors (that mainly occur on the previously resource-poor patches). It is conceivable that this principle will facilitate the coexistence of different competitive types, where in times of stasis (under IFD conditions), strong competitors have a higher net intake rate, while in times of change, weak competitors have a higher net intake rate.

To test this idea, we ran our evolutionary simulations under a stochastic regime of change, where the patch-specific resource levels changed at a rate of 0.25 (i.e., on average every 4 time units). In this variant of the model (see the appendix for details), foragers scan their environment at a rate of 0.5, thus noticing on average every 2 time units whether changes have occurred that may induce them to move to a patch with a higher net intake rate. Figure 6.3 shows that, under these changing conditions, evolution does indeed not lead to a monomorphic state. Instead, the population diversifies into a large number of coexisting competitive types.

Figure 6.4 demonstrates that, as predicted, the coexisting competitive types receive a differential net intake at equilibrium and after a change. Under stable conditions (when the population is close to the IFD), the net intake rates increases with competi-

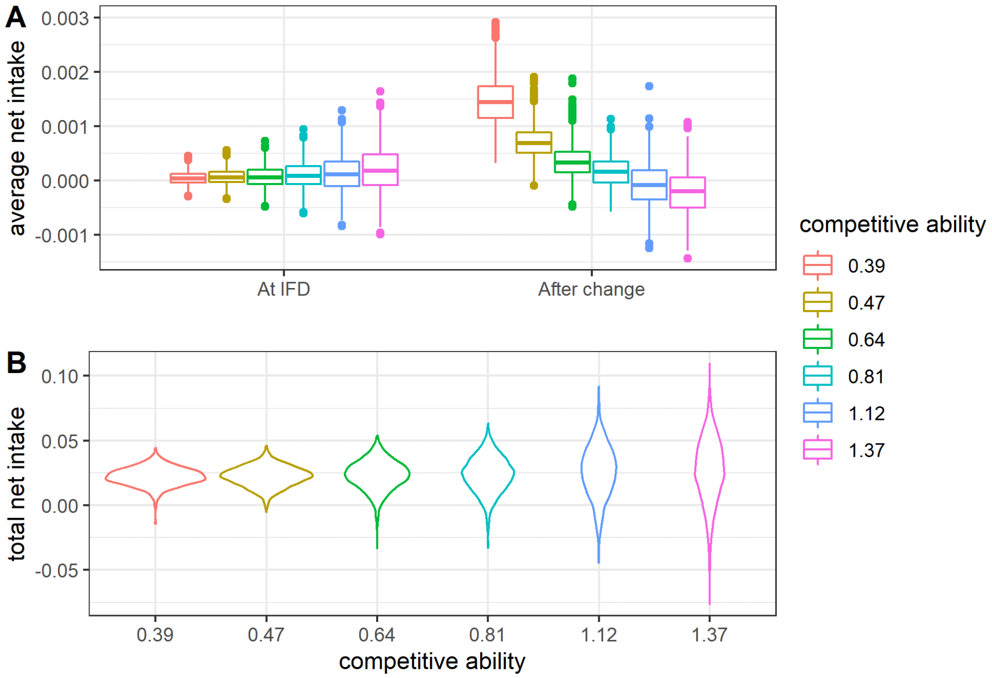

Figure 6.4 – ***Net intake rates under changing environmental conditions.*** *For the simulation in Fig. 6.3, we binned the six competitive types in generation 40,000 and (A) averaged their momentary net intake rates under IFD conditions (left part of the graph) and immediately after a change of the environment (right part of the graph). Net intake rate increases with competitive ability under stable conditions (at IFD), while it decreases with competitive ability under changing conditions. (B) The total net intake over individual lifetime is roughly the same for all six morphs.*

tive ability, while under changing conditions the weakest competitors have the highest net intake rate. The spatial assortment of less competitive individuals on poor patches and more competitive individuals on rich patches produces a transient benefit of spatiotemporal variation for the former.

Discussion

Competition is a central motive in ecology and evolution and may determine forager distributions as well as the course of natural selection. We here considered the patch choice decisions of individuals, the equilibrium distributions emerging from these decisions, and the evolutionary dynamics of competitive abilities under stable and changing environmental conditions. We arrived at two key insights. First, the ranking of habitat patches as to their suitability (= net intake rate) is systematically affected by the competitive ability of the decision-making individual. Quite generally, strong competitors have a higher tendency to prefer resource-rich patches than weak competitors. Although this bias is relatively small, it can result in strong spatial assortment, where stronger competitors accumulate on resource-rich patches, while weaker competitors mainly occur on resource-poor patches. Second, this spatial assortment has important implications for the evolution of competitive ability. Under constant environmental conditions, variation in (heritable) competitive abilities cannot persist, and the population will converge to a monomorphic state with one type of competitor. If, however, environmental conditions change within generations, spatial assortment leads to a situation where strong competitors have an advantage under stable conditions (at IFD), while weak competitors have an advantage in periods of environmental change. As a consequence, foragers differing in competitive ability can have the same fitness (= net intake rate, summed or averaged over lifetime), allowing coexistence. We have shown that such polymorphism does indeed evolve: through repeated 'evolutionary branching' (Geritz et al. 1998; Baldauf et al. 2014), a large number of competitive types emerges and stably coexists.

In contrast to interference models, continuous input models, such as the one considered here, do not predict the segregation of unequal competitors, as the IFD condition (equality of resource abundance R/C across patches) can be satisfied in a multitude of ways. Sutherland (1985) and Parker and Sutherland (1986) speculated that unequal competitors will typically occur in roughly equal proportions at all patches, which would lead to the same IFD as predicted in the absence of differences in competitive ability. This is not the case in our model implementation, where at the IFD strong competitors are over-represented on the resource-rich patches. For the special case of two patches, other studies (e.g., Houston and McNamara 1988; Spencer et al. 1995; Houston and Lang 1998) arrived at a similar conclusion, but based on different arguments. In Appendix B, we investigate in some detail how our findings relate to the results of these earlier studies. We confirm the findings of Spencer et al. (1995) and Houston and Lang (1998) that the degree of competitor assortment strongly depends on the way how individuals make their patch choice decisions, and we add one insight to those discussed in these papers. Both Spencer et al. (1995) and Houston and Lang (1998) consider foragers moving into the patches from the outside (a mechanism we call 'external initialisation'): two initially empty patches fill up due to the sequential arrival of individuals, each newly arriving individual choosing the patch offering the highest intake rate. In contrast, our study considers an 'internal initialisation' scenario, where the individuals are initially distributed randomly over the patches and subsequently sequentially relocate themselves if another patch offers a higher intake rate. In case of two patches, we show (Fig. 6.A1) that external initialisation leads to strong assortment, while internal initialisation does not lead to assortment at all. In other words, the distribution of ideal and free competitors over patches strongly depends on whether the competitors make their choices when entering the system from the outside (external initialisation) or from within (internal initialisation).

The no-assortment result of Fig. 6.A1 points at an interesting discrepancy between the two-patch scenario typically considered in the literature and the multi-patch scenario considered in our study. Why does one of our key findings, assortment of competitors at a multi-patch IFD, break down for the special case of two patches? In Appendix B, we provide an explanation. We show that our threshold criterion (3) is generally (i.e., also for the case of two patches) applicable to the external initialisation scenario, and that it therefore explains the assortment results of Spencer et al. (1995) and Houston and Lang (1998). However, the criterion ceases to hold in the special case of two patches and internal initialisation, where it needs to be replaced by an alternative criterion (see Appendix B), which no longer predicts assortment. Interestingly, assortment is re-established if the two patches are split into sub-patches that have the same properties as their 'mother patch' (Fig. 6.A2). This implies that the distribution of competitors over space may depend strongly on the 'graininess' of the environment. If, for example, the habitat choice situation is framed in a coarse-grained manner, such as a decision between deciduous and coniferous forest, our model would not predict assortment. In contrast, the same model would predict the accumulation of strong competitors in productive habitats if the otherwise identical situation is framed in a more fine-grained way, such as a decision between a multitude of deciduous and coniferous forest plots.

The existing models on the distribution of unequal competitors assume that differences in competitive ability are externally given. Such an analysis is incomplete if competitive differences have a heritable component. If this is the case, ideal free distribution theory, which is rooted in evolutionary optimality thinking (Netz et al. 2022), should pose the question whether unequal competitors can stably coexist in the course of evolution and, if so, how the distribution of competitive types is shaped by natural selection. We have shown that the evolutionary coexistence of unequal competitors is unlikely if the population is at an ideal free distribution all the time. This conclusion may change, however, if deviations from IFD conditions occur regularly. Such deviations are, for example, to be expected if sensory and/or locomotory constraints are taken into account (i.e., if the individuals are less 'ideal' and 'free' than IFD theory assumes). Here, we considered an alternative scenario, where IFD conditions are frequently perturbed due to environmental change. By means of a simple model, we demonstrated that distinct competitive types can emerge and stably coexist in the course of evolution. Consistent individual differences may therefore be as much a consequence as they are a cause of spatial distribution of individuals within the population (see also Wolf and Weissing 2010). As the evolved differences in phenotype (= competitive ability) lead to consistent differences in behavioural dispositions (= patch preferences; see (3)), we can conclude that spatiotemporal variation of the environment paves the way to the evolution of 'personality' differences.

It has been argued repeatedly (e.g., Dingemanse and Wolf 2010; Wolf and Weissing 2010; Dall et al. 2012) that spatiotemporal variation of the environment, coupled with constraints on matching the environment, may be a key driver of personality differences, but to our knowledge this has not been demonstrated in a formal model before. Empirical evidence for this is hard to collect in wild populations, but emergent spatial patterns have been studied in a number of taxa. In great tits (*Parus major*), spatiotemporal variation in resources (here, nest boxes) within and between populations and study plots have been implicated in the coexistence of different exploratory tendencies (Nicolaus et al. 2016; Mouchet et al. 2021). Similarly, dispersal syndromes have been reported to be present in heterogeneous environments with fluctuations in habitat quality, risks and competition leading to spatial structuring of a population (Duckworth 2006; Cote et al. 2010), much like in our simulations. Taborsky et al. (2014) found that habitat competition between cichlids of different body sizes leads to assortment and ultimately assortative mating, which is another potent factor by which spatial distributions can affect the course of evolution in sexually reproducing species. There is also empirical evidence for habitat choice based on personality, leading to a biased spatial distribution of behavioural types and behaviour-environment correlations (Edelaar et al. 2008; Pearish et al. 2013; Holtmann et al. 2017b). However, in these cases, the mechanisms underlying such spatial structuring of personality types are often in the dark.

Our two key results, the emergence of spatial assortment in a continuous input model of the IFD with unequal competitors, and the occurrence of polymorphism in an evolutionary model incorporating the very same, are both derived from an extension of a simple analytical model with certain mechanistic assumptions. We suggest that this is a constructive approach to study the robustness of these analytical models, and to uncover phenomena that would be otherwise overlooked. This model also acts as a useful starting point to relax further assumptions of IFD and extend to other dimensions of biologically relevant traits such as responsiveness to environmental change or limits to perception.

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Appendix

A. Description of the evolutionary simulation model

Ecological setting

We consider 100 patches, with resource densities drawn from a uniform distribution between 0 and 1 at initialization and during every change of the environment.

Individual movements and environmental change occur in an event-based approach, where each event occurs at a constant rate. Individual foragers scan their environment at a rate of 0.5, compare the potential intake across all patches and move to the patch providing the highest intake rate. Environmental change occurs at a rate of 0.25, and therefore on average every four time units. For computational convenience, foragers consume resources at discrete intervals of one time unit.

Reproduction and Inheritance

We consider discrete, non-overlapping generations of 100 time units, at the end of which reproduction occurs. For simplicity, reproduction is asexual. Individuals are haploid and have a single gene locus encoding for competitive ability that is inherited from parent to offspring. For each individual, the cumulative lifetime net intake W_{cum} is calculated. To prevent negative fitness values, a baseline value W_0 is added to W_{cum}, which can be interpreted as food intake that is unaffected by competitive interactions. The number of offspring produced per parent is determined by a weighted lottery that ensures that the expected number of offspring of an individual is proportional to $W_{cum} + W_0$ and that population size remains constant at 10,000 individuals. Offspring inherit the competitive ability from their parent, subject to rare mutations of small effect size. Mutations occur at a rate of 0.01 per reproduction event. When a mutation occurs, a random number, drawn from a normal distribution with mean zero and standard deviation $\sigma = 0.01$, is added to the parental value. At the beginning of the new generation, offspring are randomly distributed over the patches.

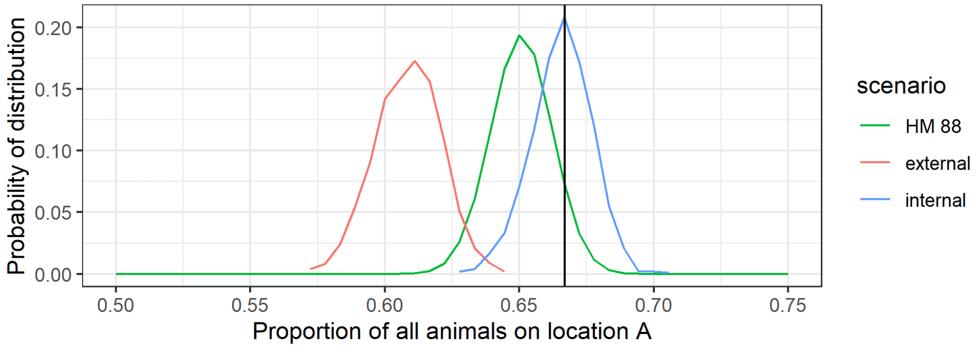

Figure 6.A1 – ***Implications of three habitat choice scenarios for the assortment of competitors.*** *Following Houston McNamara (1988), we consider a population of 180 individuals that distribute over two patches differing in quality. Resource abundance on patch A is twice the resource abundance on patch B. If all individuals were equal, 2/3 would occur on patch A at the IFD (vertical black line). Assume now that individuals differ in competitive ability: there are 90 good competitors that are twice as strong* $c_1 = 2c_2$ *as the 90 bad competitors. The green curve shows the probability distribution of the proportion of individuals on the resource-rich patch A, as derived from the 'statistical mechanics' analysis of Houston McNamara (1988). The major part of this distribution is to the left of the value 2/3, indicating that, on average, strong competitors accumulate on the resource-rich patch. The* ***red curve*** *shows the probability distribution resulting from the 'external initialisation' scenario, where two initially empty patches fill up due to the sequential arrival of individuals, each newly arriving individual choosing the patch offering the highest intake rate. This choice scenario leads to an even stronger assortment of competitors to patches. The* ***blue curve*** *shows the probability distribution resulting from the 'internal initialisation' scenario, where the individuals are initially distributed randomly over the patches and subsequently sequentially relocate themselves if the other patch offers a higher intake rate. No assortment does occur in this scenario. The distributions shown are based on 1,000 replicate simulations per scenario.*

B. Comparison with two-patch models

For the special case of two habitat patches, Houston and McNamara (1988) showed that the distribution of competitors over patches at the IFD is biased in such a way that strong competitors are more likely to occur on the resource-rich patch. This result reflects the fact that among the many possible distributions satisfying the IFD condition, those with an accumulation of strong competitors on the resource-rich patch are over-represented. To see this, consider two patches A and B, of which A is twice as resource-rich as B ($R_A = 2R_B$). If all competitors are equal, 2/3 of all individuals would therefore occur in patch A in the ideal free distribution. Consider now two types of competitors, of which type 1 is twice as strong as type 2 ($c_1 = 2c_2$); both types are equally frequent ($N_1 = N_2 = N/2$). In Fig. 6.A2, the green curve shows the frequency distribution of the number of individuals in patch A for all realisations of the IFD condition. In the majority of cases, the number of individuals on patch A is smaller than $2N/3$, implying that the strong competitors are over-represented on this resource-rich patch. The green distribution in Fig. 6.A1 represents the complete set of IFD realisations, and the validity of Houston and McNamara's 'statistical mechanics' argument relies on the assumption that the IFD that is actually realised is an unbiased sample of all IFD realisations.

A subsequent investigation by Houston and Lang (1998) showed that the distribution of actual IFD realisations strongly depends on the way the equilibrium distribution of competitors over patches is achieved. If, for example, the good competitors make their habitat choice decisions before the bad competitors, the number of individuals on the resource-rich patch will be $2/3N$ at the IFD, as in the case of equal competitors. If, in contrast, competitors make their decisions sequentially, in a random order, good competitors accumulate even more strongly on the resource-rich patch A than predicted by Houston and McNamara (1988). In either case, the solution set calculated by Houston and McNamara (1988) is not representative for the realized distribution of competitors over patches.

An important detail of Houston and Lang (1998) treatment is that their individuals sequentially enter the two patches from the outside, whereas in our model we assume that the foragers are already distributed across the patches and subsequently redistribute until an IFD is reached. Figure 6.A1 shows that the initialisation has a clear effect on the outcome. While 'outside initialisation' (red) leads to a pronounced assortment (i.e. the accumulation of strong competitors on the resource-rich patch A), this is not the case for the scenario where the individuals were first distributed randomly over the two patches (blue). In both cases, the realized distributions of competitors over patches are considerably different from the one predicted by Houston and McNamara (1988).

In view of our threshold criterion (inequality (3) in the main text), it is understandable that 'outside initialisation' leads to pronounced assortment: strong competitors have a higher tendency to choose the research-rich patch than weak competitors. But why does this argument break down in the case of 'random initialisation'? We see two reasons for this. First, strong and weak competitors only differ in their patch preferences if the difference in resource availabilities (= the difference in R/C-values) is such that the right-hand side of (3) is larger than the lowest competitive ability c_{min} and smaller than the highest competitive ability c_{max}. If the patches fill up sequentially ('outside initialisation'), the resource availabilities R_A/C_A and R_B/C_B will, due to the choices of the newly arriving individuals, remain similar to each other, implying that the threshold criterion (3) will often lead to different outcomes for weak and strong competitors. If, in contrast, the patches are initialised at random, the resource availabilities will initially differ a lot, implying that the threshold criterion (3) leads to the same outcome for different competitors. This, however, cannot be the whole story, as we showed in the main text that random initialisation does lead to pronounced competitor assortment in a multi-patch scenario.

Our second reason highlights a difference between the two-patch scenario (which is the standard scenario considered in the literature) and a multi-patch scenario (as the one considered in our study). Threshold criterion (3) is based on inequality (2), which implicitly assumes that the decision-making individual compares two patches that it does not occupy. This is the case if individuals enter the system from the outside, and it is typically the case if many patches are compared with each other (as an individual can only occupy one of the patches, most patch comparisons involve patches not occupied by the individual). The situation is different in the two-patch scenario: if an individual makes a choice 'from within', it must already occupy one of the two patches under comparison. Let us call the occupied patch P_{occ} and the other patch P_{other}. The individual should switch to the other patch if that other patch yields a higher intake rate:

$$c_i.\frac{R_{other}}{C_{other}+c_i} > c_i.\frac{R_{own}}{C_{own}} \tag{S1}$$

or, equivalently, if:

$$c_i < \frac{C_{own}C_{other}}{R_{own}}.(\frac{R_{other}}{C_{other}} - \frac{R_{own}}{C_{own}}) \tag{S2}$$

If the own patch has a higher resource availability $R_{own}/C_{own} > R_{other}/C_{other}$, the right-hand side of (S2) is negative, implying that individuals should never switch to the other patch, irrespective of their competitive ability. However, strong and weak competitors may differ in their patch preferences if the resource availability is higher on the other patch. Now, (S2) is a threshold criterion which is most likely satisfied for weaker competitors. This is in line with the findings of Houston Lang (1998), who noticed that weak (but not strong) competitors may revise their earlier patch choice decisions once a strong competitor has moved into their patch. Notice that the 2-patch criterion (S2) does no longer contain the difference in resource richness $R_{own} - R_{other}$ in the denominator of the right-hand side. This means that the bias between strong and weak competitors is not based on differences in resource richness per se, but on differences in resource availability. Accordingly, one should not expect the assortment of strong competitors to resource-rich patches, in line with Fig. 6.A1 (blue line).

This is where the difference between a two-patch scenario and a multi-patch scenario becomes decisive. In a multi-patch scenario, relevant patch comparisons occur predominantly between patches not currently occupied, and therefore threshold (3) applies rather than (S2). Likewise, the increased number of patches makes diverging patch choice decisions between individuals of different competitive ability more likely. Extending the Fig. 6.A1 to multiple patches, we observe that some spatial assortment indeed occurs when individuals (Fig. 6.A2, blue curves in the left panels), even if these patches are down-scaled versions of patches A and B in the two-patch scenario. Previous theoretical treatments have predominantly focused on the two-patch scenario, and this qualitative difference between two and multiple patches is therefore of some significance. We also observe a substantial increase of spatial assortment between two-and ten-patch scenarios if foragers are initialized outside of the patches (Fig. 6.A2, red curves in the left panels).

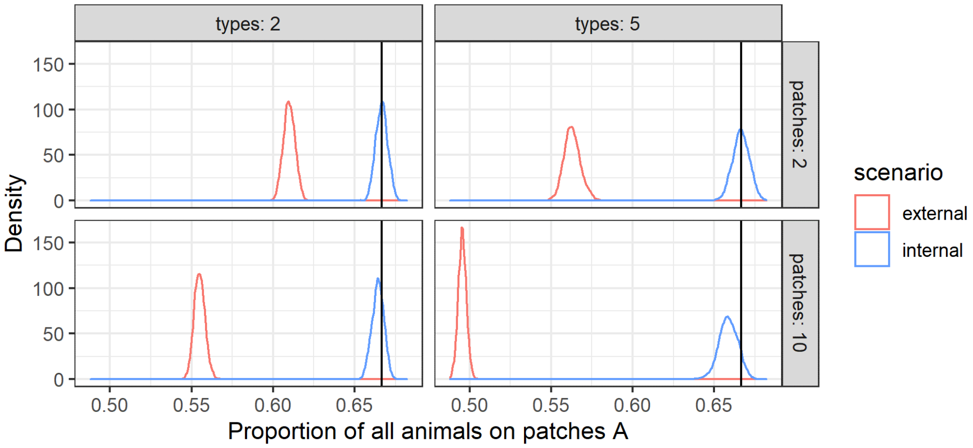

Figure 6.A2 – ***Effect of the number of patches and the number of competitive types on spatial assortment in two habitat choice scenarios.*** *As in Fig. 6.A1, the panels show the distribution of competitors over patches, based on 1,000 simulations for the external initialisation scenario (red) and the internal initialisation scenario (blue). The population now consists of 2,000 individuals, which can either be of two types (as in Fig. 6.A1) or of five types, with competitive abilities* $c_i = c_1/i$. *There are either two patches A and B (as in Fig. 6.A1) or ten patches, where five are resource rich, while the other five are resource poor. As before, the resource influx in the resource-rich patches is twice as large as in the resource-poor patches.*

By the same token, we can extend our simulations to consider the effect of more than two competitive types. Intuitively, the threshold criterion should become more relevant for a broader range of competitive types. Considering five instead of two competitive types, where competitive ability is given by $c_i = c_1/i$, we observe strengthened spatial assortment for external initialization (Fig. 6.A2, red curves in right panels). At random initialization (Fig. 6.A2, blue curves in right panels) an increased number of types does not automatically lead to spatial assortment: On two patches, competitive types are distributed randomly independent of the number of types considered. Only when 10 patches are considered, does an increased number of types lead to some reinforcement of spatial assortment. Again, this is explained by the difference between equations (3) and (S2). For the simulations shown in Fig. 6.A2, we used a population size of 2,000, but this parameter only affects the spread of the probability distributions and not their locations.

7

General discussion

Movement is integral to to the life of virtually all animals, and we see a baffling diversity in the types, scales and patterns of movement across taxa, across populations of the same species and even within populations. However, the diversity of patterns, especially within populations begs an explanation. Why do some animals migrate while others do not? Why are some animals sit-and-wait predators while others chase and pursue their prey? It is generally thought that the answer lies in the state of the animal (muscular strength related to food resources, hunger levels, having different energetic demands, being male/female). But some of these differences in movement tendencies occur even in the absence of such obvious state-differences. How can we then explain why the differences exist and how can we predict them?

The potential answer to these questions relates to the currently booming field of animal personality, which brought to attention the fact that individuals can have inherent general tendencies different from one another. Some individuals show more risk-prone behaviours than others (Wolf et al. 2007), some are more optimistic and others more pessimistic (Bateson 2016), some are able to detect and respond actively to environmental change while others passively accept the change and cope with it (Koolhaas et al. 2010). Movement is often a response to environmental changes and integrates multiple aspects of an animal (sensory capabilities, risk-reward assessment, motivation, social competence and responsiveness etc.) and therefore may be expected to be intimately related to the personality of an animal.

In this thesis, I set out to answer whether there are behaviours associated with movement tendencies forming a 'syndrome' in an integrative eco-evo-devo framework. To this end, I used empirical studies (three-spined stickleback system) and theoretical modelling studies. In this final chapter, I close my thesis first with some reflections on the trajectory that led to the thesis, especially with regard to the empirical study system as this was the pioneering work on the stickleback system regarding (partial) migration in the Netherlands. Second, I proceed to summarise our main findings using the system. Third, I summarise the main modelling approach that I used within and outside this thesis before reflecting on some of the future directions.

What we started out to do

Partial migration in sticklebacks

An excellent example for understanding the causes and consequences for syndromes is the migration syndrome, which found in many species (Dingle 2006). In many animal populations, individuals differ considerably in their migration tendency. The extreme case is 'partial migration,' where only a fraction of the population migrates while the others stay back as residents. Migrants often represent a non-random subset of the population that differ consistently in suites of phenotypic features (morphology, physiology, behaviour). Thus, partial migration is ideal to study the emergence of individual variation within populations from both a mechanistic and an evolutionary perspective.

To reach that aim we decided to use the three-spined stickleback as a study system. The system of waterways in the Netherlands is highly fragmented, owing to water management measures, by the implementation of barriers to fish migration. Sticklebacks in open waters are often partially migrating populations, with a subset of the population migrating to the North Sea where the migrants overwinter, while the others ('residents') stay at their freshwater site throughout the year. In addition, man-made barriers to migration have imposed a resident lifestyle to several populations. Thus, the 'natural experiments' in this field system led to stickleback populations forming different ecotypes namely, (1) migrants in open waters (facultative/ obligate?) (2) residents in open waters (facultative residents) at different distances to the sea and (3) land-locked residents in freshwater (obligate residents). The well-dated human intervention measures give rise to natural replicates, and the excellent field system of RFID antennas (see **Chapter 1**, Fig. 1.1) that are being set up by the water authorities of Groningen (Waterschap Hunze en Aa's) makes this system particularly unique. Furthermore, early growth conditions have been shown to influence migratory behaviour as well as personality (i.e. consistent individual differences that are consistent across contexts). For example, individuals born late in the season are smaller and have a higher migration tendency than individuals born early (Kitamura et al. 2006), and there is considerable evidence that personalities and behavioural syndromes can be shaped by environmental conditions during development (Bell and Sih 2007; Langenhof et al. 2016). Moreover, the extensive paternal care of sticklebacks can allow to disentangle maternal and paternal effects (Giesing et al. 2010; Stein and Bell 2014; Hellmann et al. 2021).

With such a field system, we set out to answer the following questions aimed at an integrative eco-evo-devo overview of migration syndrome:

1. Under which conditions of spatiotemporal variation of the environment should we predict partial migration? (eco)

2. Which factors determine differences in migration tendency between populations? (eco–evo)
3. Which individuals in a partially migrating population migrate and which individuals stay? (evo)
4. When is the migration decision taken (early in development vs later in life) and by whom (the migrant itself vs its parents, via parental effects)? (devo)
5. What are the eco-evolutionary implications of partial migration? (eco–evo)

However, the original plans of comparing different populations were soon found to be extremely difficult to execute for a variety of reasons. Most importantly, it proved to be challenging to find evidence for partial migration. Despite huge catching effort in 2018 and 2019, including electrofishing, outside the migratory period (September – October) when we expected to find residents, we caught only 5 sticklebacks from different open freshwater rivers that were similar to resident phenotypes. The years 2018 and 2019, where we wanted to firmly establish our new field system, turned out to be extreme and not representative in their weather conditions: the beginning of the year 2018 (following the winter of 2017), was marked with extreme cold and frost lasting up to March, leading to very late arrival of migrants and also very low catching success. The following summer was one of the worst heatwaves experienced in Western Europe (Yiou et al. 2020), leading to many side arms of the river getting completely dried up. This led to drastic effects in land-locked populations, in terms of reproduction and survival such that, in 2019, some of our sample populations faced extirpation. These effects also posed a logistical challenge to our catching efforts during migratory period.

A compromise: migrant – resident system

It is possible that partial migration does exist in this system but the densities of facultative residents were too low to allow formal conclusions. However, the stickleback system still is quite unique, and I could collect valuable information on migration syndromes by comparing migrants and the artificially land-locked resident populations of sticklebacks in the Dutch waterways. However, one needs to keep in mind that these comparisons are not made within a single population with partial migration, but between populations that may differ in other aspects than the decision to migration or stay residents. Hence, we instead phrased the questions differently as follows:

1. Are populations of migrant and resident sticklebacks different in movement tendencies?
2. If so, are there suites of morphological and behavioural traits (migration syndromes) associated with the two life cycles?
3. What determines the migration tendency and migration syndromes in three-spined sticklebacks?

Apart from these challenges, we also faced hurdles that are expected from any pioneering research. While lab-based assays gave us valuable insights, we were aware that a semi-natural system, with larger scales and a more realistic setting would be better for eliciting behaviours comparable to a wild settings. We thus had to secure multiple grants and set up a state-of-the-art mesocosm system, shown in Fig. 1.4, which led to considerable delays in the starting of the project. However, the mesocosm system is now well-equipped with a radio-frequency identification system and has offered insights regarding the stickleback system that were not obvious using only lab studies, as detailed below. Answering these questions formed a major part (empirical part) of my thesis.

What can we learn from the sticklebacks?

Migrants and residents: in a nutshell

In this thesis, we tried to take an integrated view to investigate migration and associated personality. Although, as previously mentioned, the integrative eco-evo-devo overview with the partial migration system would have been ideal but we still uncovered interesting differences between migrant and resident populations of sticklebacks, as a first step towards understanding this system.

We found that migrants and residents of different replicate pairs of populations differ consistently in phenotypic characteristics, indicating that these differences are related to divergent lifestyles (**Chapter 2**). With a common-garden experiment, we showed that some behavioural traits related to migration (movement and shoaling tendencies) have a heritable component. We found that differences in predator-inspection and exploratory tendencies were not recovered in F1 of various origins. In addition, the magnitude of differences in movement tendencies and shoaling in F1 offspring were much less compared to the differences in wild-caught parents. These insights indicate that these behavioural traits are crucially influenced by the developmental environment of sticklebacks (**Chapter 3**). Furthermore, using the mesocosm system, we were able to establish cross-context consistency in movement tendencies of migrant and resident sticklebacks over ecological contexts (flow, social group composition and group size). An interesting pattern was also that the scales of testing movement tendencies impacted the pattern of differences we found and hence can influence potential explanations we attribute to differences (**Chapters 4 & 5**). Thus we obtained insights on the use of semi-natural and methods incorporating appropriate scales for improved testing. See Fig. 7.1 for an overview of the results.

Is there a migration syndrome in sticklebacks?

In our studies, we tried to understand whether there exists a migration syndrome by comparing migrant and resident populations. On the whole, we may say conclusively that migratory sticklebacks are more active in larger-scale movements across ponds, shoal more and these formed a syndrome across migrant and resident populations. The other behaviours such as predator inspection, exploration, boldness, smaller scale

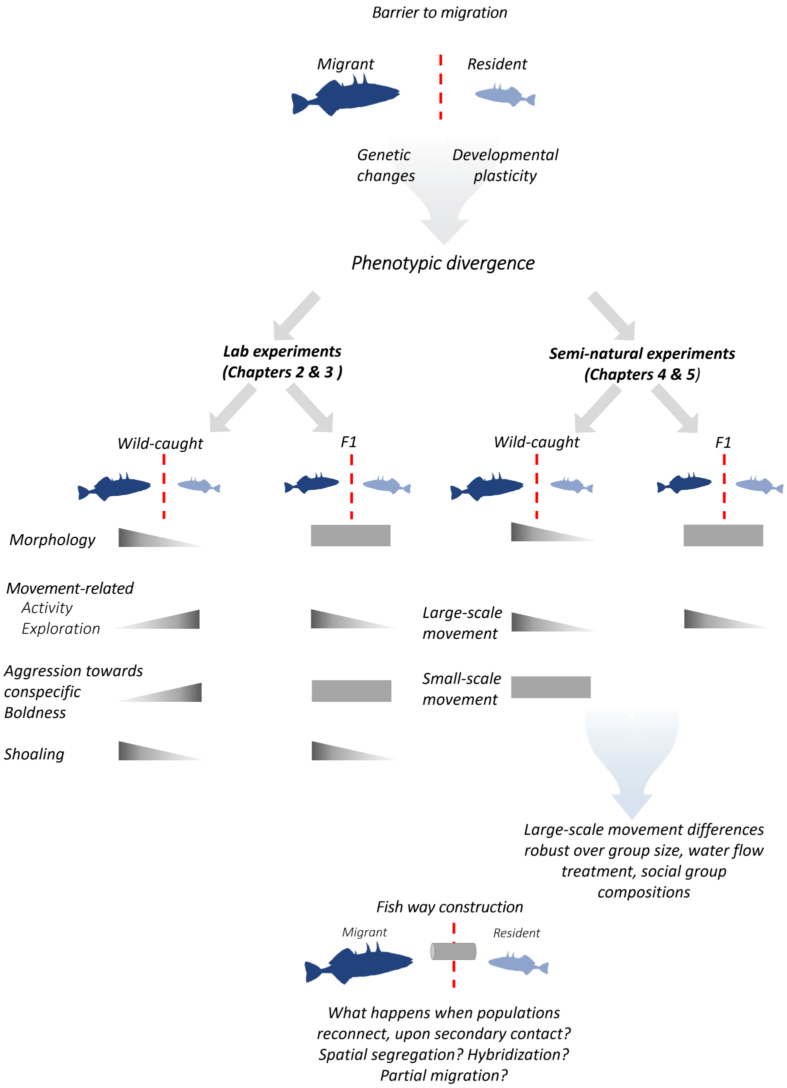

Figure 7.1 – ***Studies on sticklebacks in this thesis, in a nutshell.*** *In the flow chart, we present the current situation of stickleback migrant and resident in the wild along with the main results oh behaviours in both wild-caught and lab-bred migrant and residents in lab and mesocosm. When we find differences between migrants and residents, they are represented by the triangles showing the direction of differences. Rectangles indicate lack of differences between the two origins.*

activity did not show a conclusive pattern across lab and mesocosm and hence provide ambiguous pattern on syndromes.

Despite the drastic differences in several behaviours between migrant and resident populations and ‘syndrome’ across populations, there was little evidence of stable syndrome within populations, that is, in one year, we found evidence for exploration and predator inspection in migrants and in another, activity and exploration in residents. We find this interesting as the previous studies have extensively reported that sticklebacks often show evidence for personality, especially in the form of boldness-aggressiveness syndrome(or viewed broadly as risk-taking personality).

Predation pressure is thought to have an organisational effect on behaviours in sticklebacks and has been implicated in the existence of boldness-aggressiveness syndrome (Bell and Stamps 2004; Dingemanse et al. 2007). It is often hypothesised that predation pressure differs between sea (with high threat of predators, especially ones that pierce the skin of prey) and freshwater conditions (lower threat, mostly invertebrate predators; Reimchen 1994). This explanation has also been attributed to differences in morphological and behavioural variation in marine vs freshwater sticklebacks (Bell et al. 2004). Upon a closer inspection in our system, we find that the system of freshwater that harbour stickleback populations have not only avian threat but also piscivorous fish (European Perch, *Perca fluviatilis* and European eels, *Anguilla anguilla*) and the densities of predators varied between the years as well. Furthermore, in the freshwaters, the water levels, salinities and other abiotic factors fluctuated drastically within years and seasonally due to water management and the requirement of farmers (Pers. obs.). Hence we think the behavioural tendencies in individual residents could be quite flexible in response to an unpredictable and changing environment. However, the non-existence of syndromes in migrants is still puzzling. It is possible that by limiting ourselves to testing within the migratory period and in lab-based assays, we provided very peculiar conditions, especially for migrants. In the future, we may better use the semi-natural mesocosm to gather more data on both migrants and residents and test this in detail.

A notable pattern that we uncovered in **Chapter 2** is that we were able to disentangle the effect of morphology from other behaviours, i.e., we could rule out the confounding effects of morphology on behaviour. In the F1 sticklebacks, all fish of different origins were of similar size (lateral plates were usually small and undifferentiated even after one year of development, pers. obs.), potentially because they were all raised entirely in freshwater in larger densities. But we still uncovered systematic differences, with migrants having higher activity, shoaling tendencies and larger-scale movement tendencies with hybrids in-between and residents having lowest activities, shoaling tendencies, and larger-scale movement tendencies. It is thus interesting that these traits, could potentially form a syndrome between movement tendencies and shoaling across the different origins, in the absence of clear morphological differences, when raised in similar environmental conditions. In the wild, these differences can be exaggerated by interaction with different developmental conditions as well.

What happens when populations are reconnected?

The current conservation efforts in the Netherlands aim to reconnect the river systems to the sea by bypassing barriers via fishways (Fig. 7.2). From my viewpoint, we can address (1) fundamental questions of what happens when the two ecotypes, migrants and residents, come into secondary contact and (2) application-oriented research on this system can inform water management authorities about the efficiency of the conservation efforts.

Reconnecting migratory and genetically differentiated land-locked populations can be viewed as a large-scale eco-evolutionary experiment that raises exciting questions such as: will migratory and resident sticklebacks interbreed and introgress in sympatry (Ravinet 2021)? Will hybrids be selected against (as in the lake-stream sticklebacks, Berner et al.)? Or will we have incomplete gene flow and partial migration in these populations (weak assortative mating, Ingram et al. 2015; weak reproductive barriers and no selection against hybrids, Hanson et al. 2016)? From our studies, residents and hybrids show lowered migratory and shoaling tendencies. This divergence may be maintained or enhanced by size-assortative mating of migrants and residents as size difference at maturity has been detected in the wild (Ramesh et al. 2021) or by phenotype-dependent microhabitat choice (Maciejewski et al. 2020; Dean et al. 2021). Irrespective of the mechanisms involved in the observed phenotypic differentiation between migrants and residents, whether the migrant-resident ecotype divergence will persist in the absence of migration barriers needs to be investigated.

It is now recognized that the conservation of behavioural diversity (or 'ethodiversity', Cordero-Rivera 2017) is an important issue. The development of effective conservation methods taking ethodiversity into account is still in its infancy and currently most conservation methods do not consider how lack of ethodiversity may potentially bias their evaluation criteria. Focusing the conservation efforts on the population level while ignoring individual differences can have unintended and potentially negative side effects on the population structure as a whole. Recently, there has been renewed focus on the selectivity of conservation measures such as fishways on certain behavioural types, which can lead to negative effects on a population in the long term (e.g., bolder and more active individuals perform better at fishway passage attempts; Lothian and Lucas 2021). Furthermore, there is mounting evidence that personality-dependent habitat choice may exist leading to personality - environment correlation (Holtmann et al. 2017b). Hence, habitat alterations and restorations without complete understanding of individual differences (and differences between migrants and residents in our case) in habitat use may lead to favouring of only limited behavioural types potentially leading to loss of diversity, which goes against the conservation efforts. Awareness that between-individual differences in movement tendencies or migration can exist is important in these cases. In light of this, it may be crucial to study the behaviour types of fish that pass through the fish way.

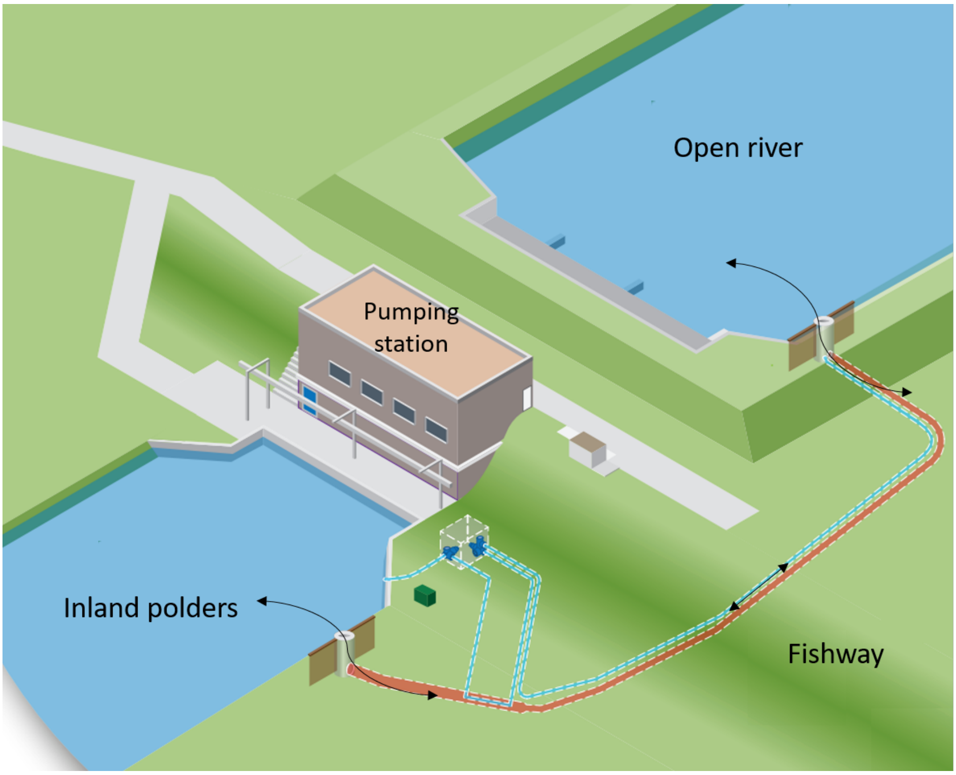

Figure 7.2 – ***Fishway at a pumping station.*** *This is a pictorial representation of a fishway that was constructed recently in Hongerige Wolf, Groningen. The pumping station at Hongerige wolf poses as a migration barrier to incoming migrating sticklebacks. The fishway bypasses the pumping station and connects the main river channel to the downstream polders. Picture credits: Hunze en Aa's*

Perspectives on theoretical modeling

In this section, I would like to reflect on the theoretical modelling studies I have used as part of my thesis and during my PhD. These have proven to be indispensable tools in the understanding of concepts and have the potential to be very useful when used complementary to empirical studies. I will discuss the models included in the thesis and also briefly touch upon additional models that I developed on the evolution of partial migration the emergence of a migration syndrome.

At present, there seem to be two main ways in which behavioural ecologists use modelling approaches to aid their studies. In the first, models are used as a tool for a deeper conceptual understanding of an idea or verbal argument. In the second, models are tailored to a specific empirical system, with the aim of deriving testable, quantitative predictions. In my PhD trajectory, I have gained some experience with both approaches, which I will detail below.

Conceptual models

My thesis includes one modelling chapter (**Chapter 6**) and a critique of a theoretical modelling study (**Intermezzo**). Both parts centre on conceptual models that aim to further our understanding of how individual differences affect the movement decisions of foragers. Both models make the assumption that the individuals are 'ideal' (fully informed about the environment and the distribution of their conspecifics) and 'free' (not constrained in any way in their movement). These assumptions are obviously a caricature of reality, but they allow a complete analysis of a complex system. **Chapter** 6 illustrates that a highly simplified conceptual model can give rise to interesting predictions, which in principle can be tested in various empirical systems. In particular, we derive the following testable predictions:

1. strong and weak competitors differ systematically in their habitat preferences;
2. strong competitors will accumulate on resource-rich habitat patches;
3. in stable environments, heritable differences in competitive ability cannot persist;
4. spatiotemporal variation in the environment foster the coexistence of strong and weak competitors;
5. strong competitors flourish in periods of environmental stasis; weak competitors flourish in periods of environmental change.

Moreover, the model provides some surprising new insights. For example, the spatial distribution of competitors crucially depends on how individuals make patch choice decisions and the process of movement to reach IFD itself. Additionally details of individual patch occupation matters: whether individuals fill up an initially empty habitat or whether they are already present and later redistribute according to their preferences. This implies that virtually all current ideal free models have to be treated with care, as they make the simplifying assumptions on how the distribution is obtained and even when they do, usually consider only two patches. These insights may be viewed as a word of warning: the insights obtained from conceptual models may look general and robust, but they can easily be overturned by a new generation of slightly more sophisticated conceptual models.

From a more general perspective, the model in **Chapter 6** provides a new mechanism for the evolutionary emergence and stability of individual (personality) differences. Spatiotemporal variation in the environment has often been cited as a potential driver of personality variation, both from a theoretical (Dingemanse et al. 2010; Wolf and Weissing 2010; Dall et al. 2012) and an empirical perspective (Duckworth et al. 2015; Nicolaus et al. 2022). But to my best knowledge, our model in **Chapter 6** is the first demonstration that (and how) such variation can induce personality differences. Yet, in view of the underlying assumptions of 'ideal' and 'free' individuals, care should be taken when interpreting the results. Before extrapolating the results, it seems imperative to first consider more realistic variants of the model that consider limited information on the environment and the whereabouts of other individuals and constraints on (or costs of) movement.

Models tailored to empirical systems

The second modelling approach not so much strives for general insights but rather aims at a sound understanding of a particular empirical system. The starting point is often a simple conceptual model that is expanded in a step-wise manner, thus tailoring the model more and more to the system under scrutiny. While conceptual models strive for general, qualitative predictions, the aim is now to derive detailed quantitative predictions. Often this is done for a whole suite of models; subsequently, model selection techniques (Burnham and Anderson 2002) are applied to find out which model explains the empirical data best.

In the initial phase of the project, I employed such a step-wise modelling approach to obtain a better understanding of partial migration and migration syndromes. To this end, I developed a suite of eco-evolutionary models of increasing complexity, which were studied by means of individual-based evolutionary simulations (as in **Chapter** 6). As this part of my work has never been finalized, I here briefly sketch the approach taken and my main findings.

1. In the simplest model, the individuals only differed in their decision on whether to migrate or to stay at home; in all other respects, they were identical. The probability to migrate was an inherited (and, hence, evolvable) strategy. Technically speaking, this is a mixed strategy (corresponding to a bet-hedging strategy). We hypothesised that partial migration is driven by competition avoidance in the winter months due to seasonal reduction in resource availability. The presence of migrants makes selection frequency-dependent: if the number of migrants is small, competition 'at home' will be intense, making migration advantageous; if most individuals migrate, it may be advantageous to stay at home, as there are only a few competitors left. Under these circumstances, one would expect the evolution of a single mixed strategy, leading to a fixed proportion of migrants and residents. This is exactly what I found in my simulation.

2. Individuals in a population are rarely identical. Furthermore, in sticklebacks and other fish, migrating individuals tend to have a larger body size. In a second model, we therefore considered a population of fish differing in body size. In this model variant, body size variation was purely environmental and not heritable. We assumed that, all other things being equal, larger fish have a higher probability to survive migration than smaller fish. Now, we allowed for the evolution of condition-dependent strategies, where the migration decision of an individual is made dependent on the individual's body size. In the individual-based simulations, such strategies did indeed evolve: large individuals had a high tendency to migrate, while small individuals had a low tendency to migrate. This is the first step toward the evolution of a migration syndrome, as body size gets associated with migration tendency.

3. In a third model, the distribution of body sizes was not random but determined by a growth strategy. Fast-growing individuals reached on average a larger body size than slow-growing individuals. Faster growth comes with enhanced risks related to acquiring more resources and hence exposure to risks, but the resulting larger body size provides the survivors with a migration advantage or a competitive advantage when staying at home. In addition to the growth strategy, the individuals also harboured a migration strategy (as in model (2)). In this model variant, a mixed growth strategy evolved (with a fixed proportion of fast-growers), together with a condition-dependent migration strategy (where the larger individuals had a higher tendency to migrate).

4. In model variant (3), the growth decision comes first, and the migration decision is then based on the implications of the growth decision. There are some indications that in real populations, the situation may be different: the migration decision can be taken very early in life, and it determines the developmental trajectory (including the growth rate), which is very different for migrants than for residents (e.g., body size and threshold to migrate are both potentially genetically determined in Atlantic salmons, *Salmo salar*, Páez et al. 2011). To model this, we now considered the evolution of two-stage strategies: in the first stage an evolvable mixed strategy determined the migration decision (as in model (1)), and in the second stage two growth strategies (one for the migrants and one for the residents) determined the growth trajectory. Again, partial migration and migration syndromes emerged in the evolutionary simulations. A fixed proportion of the population decided early in life to migrate, and these individuals tended to take the fast-growth option, leading to larger body size.

5. Finally, we combined models (3) and (4), in order to see whether one of the two types of strategy (model (3): decide first on growth, later on migration; model (4): decide first on migration, later on growth) is advantageous. To our surprise, all simulations resulted in a type (4) strategy, where the migration decision is more basal than the growth decision. We do not have a good explanation for this outcome. Yet, this prediction is interesting, as it may be testable by systematically manipulating the developmental environment.

Although the modelling outcomes are intriguing from a conceptual perspective, we never reached the point to actually test the model predictions with our stickleback system (as we did not get sound evidence for partial migration in our field populations). We therefore abandoned our study and never completed it.

Modeling moving forward

Moving forward, I think both modelling strategies are important as they help us get a strong foundational and conceptual footing and enable us to test hypotheses flexibly in an empirical system. I am especially fascinated by the second strategy of confronting suites of specifically tailored models with empirical data. A similar approach is taken when analysing empirical data using statistical models. However, the mathematical framework underlying statistical models is much more simplistic than the more ‘biological’ model structures sketched above. In particular, statistical models cannot easily incorporate mechanistic detail, and they cannot easily cope with tailored null models and complex alternatives. For instance, a null model for the density-dependent movement of animals between patches cannot easily be implemented by the inclusion of a parameter determining the degree of density dependence, as the local density changes with each movement decision of an animal. For comparing null models of such dynamical processes, one would better use a theoretical modelling approach and parametrise them using empirical data to have tailored predictions. In a project with MSc student Tirza Moerman, we used such an individual-based simulation approach to create null hypotheses and employed model fitting and model selection techniques, to test whether stickleback habitat preference depends on individual personality and the current density of competing conspecifics.

Closing remarks

This thesis is titled ‘Animal personalities on the move’ to indicate two general insights I obtained over the course of my PhD.

1. **Animal personalities emerge readily in the context of movement.** The puzzling characteristics that form the core of enquiry in animal personality research are individual variation, consistency of these variation over time and behavioural syndromes leading to integrated suites of behaviours. In a movement context, studies including this thesis show that personalities readily emerge.
   (a) Individual variation in movement emerges through bet-hedging(as explained in **Chapter 1**) from competition avoidance (density-dependent selection as in the case of our model (1) above) and from negative frequency dependent selection in a spatiotemporally varying environment (**Chapter 6**).
   (b) Consistency in movement, not explicitly tested in this thesis, can emerge if certain types of movement (for eg. in the context of foraging) are associated with space-use and search strategies associated with foraging movement that are honed by frequent use. In such a situation, frequent and repeated movements may increase familiarity and experience (reducing costs of movement over time), leading to a consistent movement strategy.
   (c) Syndromes also arise naturally, as different require a different degree of ’responsiveness’ to the environment, different ways and degrees of information gathering and processing, and different physiological (e.g. metabolic) states. Moreover, they expose the animal to different challenges and different types of environment, which require different strategies for coping with them. All these other factors, associated with differences in movement, can then feedback on movement, potentially enhancing variation in movement strategies.
2. **The field of animal personality itself is on the move.** In this thesis, we have attempted to study animal personalities through an integrative approach,

regarding (a) research questions (eco - evo - devo) and (b) research methodologies (field, lab, mesocosm, theory). This is in part due to the fact that the different disciplines within behavioural sciences have embraced and incorporated ’personality-thinking’ leading to to seemingly diverging developments that require reunification. For example, behavioural physiology interprets a variety of behaviours related to stress responses as facets of a ’coping style’ syndrome (**?**); similarly, life history theory views risk-related behaviour as part of a syndrome, because the principle of life-history consistency predicts that individuals that are risk-prone (resp. risk-averse) in one context should also be risk-prone (resp. risk-averse) in other contexts (Wolf et al. 2007). An interesting and inevitable intersection is if life-history strategies shape risk-related behaviours by also having impact on the underlying coping style mechanisms. In such a way, personality can be approached different perspectives, and integrating these perspectives is moving the field forward. Such an attempt was made in this thesis, but my trajectory also shows that many more years and PhD theses are required to fully capture the many facets of animal personality.

Summary

The diversity of animal movement and its link to personality

Movement is a key factor connecting an organism with its environment. Movement can be induced by environmental conditions, and it can lead to a change in these conditions. Movement requires more than the morphological and physical abilities that are necessary for a change in location – it also requires sensory and cognitive abilities for navigation, behavioural tendencies such as novelty-seeking or boldness, and social capabilities allowing the coordination with conspecifics. As movement is an important determinant of organismal survival and reproduction (and, hence, organismal fitness), one would expect that movement and all its underlying features are jointly shaped by natural selection. But this does not imply that, in a given environment, evolution will result in a single fitness-maximising movement strategy. In fact, it is recently becoming clear that, even within a population, animals exhibit a wide array of movement types. Moreover, various other morphological, physiological, and behavioural traits are associated with movement, forming a so-called 'syndrome'. All this raises several questions: Why is there variation in movement types? What drives the evolutionary emergence and stable persistence of different movement types? Which factors determine the evolution of movement-related syndromes?

To address these questions, I studied the movement behaviour of three-spined sticklebacks in the wild, in the lab, and in a semi-natural system of connected ponds. Moreover, I conducted some theoretical studies on the evolution of movement types and behavioural syndromes.

Migration in sticklebacks - a natural experiment

In the north of the Netherlands, three-spined sticklebacks breed in inland freshwaters in spring and early summer. In autumn, the juveniles of the year migrate to the sea where they grow to adult size during winter, before returning to the freshwater in the next spring. In the last 50 years, man-made barriers (such as pumping stations and sluices) have been extensively built in rivers to maintain water levels below sea level, with the consequence that some of the side water drainages are cut off from the main river channel. As a result, several populations of 'resident' sticklebacks are trapped in freshwater for their whole lifecycle. This leads to two types of stickleback populations in the Netherlands: one part still able to migrate to the sea ('migrant'), and a 'resident' counterpart. This unique situation can be viewed as an unintended large-scale replicate experiment, where we can compare several populations of residents and migrants, allowing us to study the implications of restricted movement. In particular, we can use the system to ask if 50 years of isolation are enough to shape a new movement type and, potentially, a new movement syndrome.

As a first step, we caught wild sticklebacks from migratory and nearby resident populations and measured several traits (including body size, lateral plates on the body and behaviours such as movement tendency, shoaling, exploration in a novel environment, response to a predator and so on). We found that migratory sticklebacks are bigger, have more armament (lateral plates) and differ from residents in virtually all behavioural traits we measured (**Chapter 2**). Specifically, migrants show a higher movement tendency and a higher tendency to be in a group (shoaling), behaviours that are both important for a migratory lifecycle. However, as the measurements were made on wild fish that have spent their lives under different environmental conditions, it is unclear whether the observed differences in morphology and behaviour reflect a response to the environment (developmental plasticity) or genetic differentiation between migrant and resident populations.

In **Chapter 3**, we address this point by conducting a 'common-garden' experiment – we made four crossings (migrant x migrant, resident x resident, migrant x resident, resident x migrant) and raised the offspring under the same environmental conditions. It turned out that some of the conspicuous differences between migrants and residents that we observed in Chapter 2 (e.g. the large size difference and the armament in migrants) did not occur in the F1 offspring. Apparently, these differences were caused by the environmental differences experienced by the wild-caught fish in Chapter 2. However, some differences reappeared, despite the fact that the F1 offspring had experienced identical rearing conditions. Most notably, movement and shoaling tendencies, crucial behaviours related to migration, were highest in migrant x migrant offspring and lowest in resident x resident offspring, indicating that 50 years of isolation were sufficient to produce substantial genetic differentiation between neighbouring resident and migrant populations.

Mesocosm system - a stepping stone between the lab and the field

Ideally, questions such as those tackled by my thesis should be studied under natural conditions. This, however, is a major challenge. Tracking individuals in the wild is often technologically demanding and for a small fish such as the stickleback, it is virtually impossible to track groups in the wild. Therefore, field studies on fish are often complemented by aquarium studies in the lab. We undertook this as the first step in Chapters 2 3. Lab studies have the advantage of offering control over confounding variables and recording high-resolution behavioural data but they hardly represent the situation in the wild in terms of the complexity of the environment. For this reason, we developed a system of connected ponds that allowed us to study the sticklebacks in considerable detail under much more natural conditions. This ‘mesocosm’ system was connected to a nearby freshwater ditch allowing natural water, nutrients etc. to be pumped in. We also allowed the growth of plants and algae and water flow, mimicking the ditches through which stickleback in the Netherlands usually move and which they use for breeding. We saw that sticklebacks readily used the mesocosm, moving between the ponds through the corridors and readily started breeding in spring, indicating that the mesocosm indeed mimics the natural environment for these fish and hence can be used as a stepping stone between lab and field studies. We equipped the ponds with antennas between the corridors and within the ponds such that we can remotely record when a tagged fish is detected in the vicinity of an antenna. Thus we are able to remotely track large numbers of fish, over longer duration of time while allowing a semi-natural environment.

In **Chapter 4**, we asked if groups of migrant and resident sticklebacks differ in their movement tendencies in the ponds. We did not find differences in short-scale movement (within ponds), but, as expected, migrants moved much more than residents over larger scales (between ponds), confirming that the two types have indeed diverged in their movement tendencies. In order to explore this in more detail, we also tested the fish under various ecological conditions such as different water flows and group sizes. We found that, irrespective of these conditions, migrants consistently exhibit higher larger-scale movement tendencies than residents.

Behavioural tendencies can be strongly affected by the social environment. The effect of the social environment is, however, usually difficult to quantify, due to the difficulty of tracking all individuals of a social group and of testing the same individuals in different social groups. Our mesocosm allows us to track not only focal fish but also groups of fish, in different group compositions. In **Chapter 5**, we did this by following resident and migrant individuals in different group contexts (different percentages of migrant and resident fish). To our surprise, we found that residents and migrants maintained their inherent movement tendencies across different social contexts, indicating that these movement tendencies are not affected by the social environment.

Insights from individual-based models

In addition to my empirical work, I also participated in two theoretical studies that intend to provide insights into the question of how individual differences in characteristics like competitive ability affect the movement of individuals and the distribution of individuals in space. In an **Intermezzo** and in **Chapter 6**, we ran individual-based evolutionary simulations to study movement in the context of foraging on a resource landscape. When the resource landscape is relatively stable throughout the lifetime of individuals, individual differences in competitive ability quickly disappear in the course of evolution. This changes considerably when the resource distribution is reshuffled repeatedly. Now, a broad spectrum of competitive types evolves from an initially homogeneous population. We could explain this novel mechanism for the evolutionary emergence of individual differences by the fact that strong competitors have a fitness benefit under stable environmental conditions, while weak competitors profit from environmental change. Hence, the spatiotemporal variation of the environment is key to the evolution of individual differences.

Finally, I conclude my thesis with a general discussion where I reflect on the question "Where are we now - where should we go next?" with regards to the questions and methodologies pertaining to the stickleback system and also more broadly the field of animal personality research.

Nederlandse samenvatting

Het verband tussen bewegingsstrategieën en persoonlijkheid

Beweging (de verplaatsing van individuele dieren naar een andere locatie) is een sleutelfactor die een organisme met zijn omgeving verbindt. Beweging kan worden uitgelokt door omgevingsomstandigheden, en kan leiden tot een verandering van deze omstandigheden. Beweging vereist meer dan de morfologische en fysieke vaardigheden die nodig zijn voor een verandering van plaats - zij vereist ook zintuiglijke en cognitieve vaardigheden voor navigatie, gedragsneigingen zoals het zoeken naar nieuwigheden of stoutmoedigheid, en sociale capaciteiten die de coördinatie met soortgenoten mogelijk maken. Aangezien beweging in belangrijke mate bepalend is voor overleving en voortplanting (en dus voor het reproductieve succes van het organisme), zou men verwachten dat beweging en alle onderliggende kenmerken daarvan gezamenlijk door natuurlijke selectie worden bepaald. Maar dit impliceert niet dat, in een gegeven omgeving, evolutie zal resulteren in één enkele optimale bewegingsstrategie. De laatste tijd is duidelijk geworden dat dieren, zelfs binnen een populatie, een breed scala aan bewegingstypes vertonen. Bovendien worden verschillende andere morfologische, fysiologische en gedragskenmerken geassocieerd met beweging, waardoor een zogenaamd "syndroom" ontstaat. Dit alles roept verschillende vragen op: Waarom is er variatie in bewegingstypes? Wat is de drijvende kracht achter het evolutionaire ontstaan en de stabiele persistentie van verschillende bewegingstypes? Welke factoren bepalen de evolutie van bewegingsgerelateerde syndromen?

Om deze vragen te beantwoorden, bestudeerde ik het bewegingsgedrag van driedoornige stekelbaarsjes in het wild, in het lab, en in een semi-natuurlijk systeem van verbonden vijvers. Bovendien voerde ik enkele theoretische studies uit over de evolutie van bewegingstypes en gedragssyndromen.

Migratie bij stekelbaarsjes - een natuurlijk experiment

In het noorden van Nederland broeden driedoornige stekelbaarsjes in het voorjaar en de vroege zomer in zoete binnenwateren. In de herfst migreren de jonge stekelbaarsjes naar zee, waar ze in de winter uitgroeien tot volwassen exemplaren, alvorens in het volgende voorjaar terug te keren naar het zoete water. In de afgelopen 50 jaar zijn in de rivieren op grote schaal kunstmatige barrières (zoals gemalen en sluizen) gebouwd om het waterpeil onder de zeespiegel te houden, met als gevolg dat sommige zijwaterafvoeren van de hoofdgeul van de rivier zijn afgesneden. Het gevolg is dat verschillende populaties 'residente' stekelbaarsjes gedurende hun hele levenscyclus in zoet water gevangen zitten. Dit leidt tot twee soorten stekelbaarspopulaties in Nederland: een deel dat nog kan migreren naar zee ('migranten'), en een 'residente' tegenhanger. Deze unieke situatie kan worden gezien als een onbedoeld grootschalig replicatie-experiment, waarbij we verschillende populaties van residenten en migranten met elkaar kunnen vergelijken, zodat we de implicaties van beperkte verplaatsing kunnen bestuderen. In het bijzonder kunnen we het systeem gebruiken om ons af te vragen of 50 jaar isolatie voldoende is om een nieuw bewegingstype en, mogelijk, een nieuw bewegingssyndroom te vormen.

Als eerste stap vingen we wilde stekelbaarsjes van migrerende en naburige residente populaties en maten we verschillende eigenschappen (waaronder lichaamsgrootte, beschermende platen op het lichaam en gedragingen zoals bewegingsneiging, scholingsdrang, exploratie in een nieuwe omgeving, reactie op een predator, enzovoort). We ontdekten dat migrerende stekelbaarzen groter zijn, meer beschermende platen hebben en verschillen van residenten in vrijwel alle gedragskenmerken die we hebben gemeten (**hoofdstuk 2**). Meer specifiek, migrerende stekelbaarsjes vertonen een grotere bewegingsneiging en een grotere neiging om in een groep te zitten (scholingsdrang), gedragingen die beide belangrijk zijn voor een migrerende levenscyclus. Aangezien de metingen werden verricht bij wilde vissen die hun leven onder verschillende milieuomstandigheden hebben doorgebracht, is het echter onduidelijk of de waargenomen verschillen in morfologie en gedrag een reactie op het milieu (ontwikkelingsplasticiteit) of een genetische differentiatie tussen migrerende en residente populaties weerspiegelen.

In **hoofdstuk 3** gaan we op dit punt in door een 'common-garden' experiment uit te voeren - we maakten alle kruisingen tussen migranten en residenten en brachten de nakomelingen groot onder dezelfde milieuomstandigheden. Het bleek dat sommige van de opvallende verschillen tussen migranten en residenten die we in hoofdstuk 2 hadden waargenomen (bijv. het verschil in grootte) niet voorkwamen bij de F1-nakomelingen. Blijkbaar werden deze verschillen veroorzaakt door de milieuverschillen die de in het wild gevangen vissen in hoofdstuk 2 ondervonden. Sommige verschillen kwamen echter terug, ondanks het feit dat de F1 nakomelingen identieke opkweekomstandigheden hadden ondergaan. Met name de bewegings- en scholingsdrang , cruciale gedragingen die verband houden met migratie, waren het grootst bij nakomelingen van migranten x migranten en het kleinst bij nakomelingen van residenten x residenten, wat erop wijst dat 50 jaar isolatie voldoende was om een aanzienlijke genetische differentiatie teweeg te brengen.

Een semi-natuurlijk systeem (mesokosmos systeem) als springplank tussen het lab en het veld

Idealiter zouden vragen zoals die in mijn proefschrift onder natuurlijke omstandigheden moeten worden bestudeerd. Dit is echter een grote uitdaging. Het volgen van individuen in het wild is vaak technologisch veeleisend en voor een kleine vis als de stekelbaars is het vrijwel onmogelijk om groepen in het wild te volgen. Daarom worden veldstudies op vissen vaak aangevuld met aquariumstudies in het lab. In de hoofdstukken 2 en 3 hebben wij dit als eerste stap gedaan. Laboratoriumstudies hebben het voordeel dat ze controle bieden over verstorende variabelen en dat ze gedragsgegevens met een hoge resolutie registreren. Echter zijn ze nauwelijks representatief voor de situatie in het wild, gezien de complexiteit van het milieu. Daarom hebben wij een systeem van met elkaar verbonden vijvers ontwikkeld, dat ons in staat stelde de stekelbaarsjes in veel meer detail te bestuderen onder veel natuurlijker omstandigheden. Dit 'mesokosmos'-systeem was verbonden met een nabijgelegen zoetwatersloot, waardoor natuurlijk water, voedingsstoffen etc. konden worden binnengepompt. We lieten ook planten en algen groeien en water stromen, waarmee we de sloten nabootsten waar stekelbaarsjes in Nederland gewoonlijk doorheen trekken en die ze gebruiken om zich voort te planten. We zagen dat stekelbaarsjes de mesokosmos gemakkelijk gebruikten, zich via de gangen tussen de vijvers verplaatsten en in het voorjaar gemakkelijk begonnen met broeden, wat erop wijst dat de mesokosmos inderdaad de natuurlijke omgeving voor deze vissen nabootst en dus gebruikt kan worden als opstapje tussen lab- en veldstudies. We hebben de vijvers uitgerust met antennes in de tussengangen en in de vijvers, zodat we op afstand kunnen registreren wanneer een vis met een RFID chip in de nabijheid van een antenne wordt waargenomen. Op die manier kunnen we grote aantallen vissen op afstand volgen, over langere perioden, en in een semi-natuurlijke omgeving.

In **hoofdstuk 4** vroegen we of groepen migrerende en residente stekelbaarsjes verschillen in hun verplaatsingstendensen in de vijvers. We vonden geen verschillen in verplaatsingen over korte afstanden (binnen vijvers), maar, zoals verwacht, verplaatsten migranten zich veel meer dan bewoners over grotere afstanden (tussen vijvers), wat bevestigt dat de twee soorten inderdaad van elkaar verschillen in hun verplaatsingstendensen. Om dit nader te onderzoeken, testten we de vissen ook onder verschillende ecologische omstandigheden, zoals verschillende waterstromen en groepsgroottes. We ontdekten dat, ongeacht deze omstandigheden, migranten consistent een grotere neiging tot bewegen vertonen dan bewoners.

Gedragstendensen kunnen sterk worden beïnvloed door de sociale omgeving. Het effect van de sociale omgeving is echter meestal moeilijk te kwantificeren, omdat het moeilijk is alle individuen van een sociale groep te volgen en dezelfde individuen in verschillende sociale groepen te testen. Ons mesokosmos stelt ons in staat niet alleen individuele vissen te volgen, maar ook groepen vissen, in verschillende groepssamenstellingen. In **hoofdstuk 5** hebben we dit gedaan door residente en migrerende individuen te volgen in verschillende groepscontexten (verschillende percentages migrerende en residente vissen). Tot onze verrassing vonden we dat residenten en migranten hun inherente bewegingstendensen behielden in verschillende sociale contexten, wat erop wijst dat deze bewegingstendensen niet beïnvloed worden door de sociale omgeving.

Inzichten uit op het individu gebaseerde simulaties

Naast mijn empirische werk heb ik ook meegewerkt aan twee theoretische studies die inzicht moeten verschaffen in de vraag hoe individuele verschillen in kenmerken zoals competitief vermogen de beweging van individuen en de verdeling van individuen in de ruimte beïnvloeden. In een **Intermezzo** en in **hoofdstuk 6** bespreken we de uitkomst van evolutionaire, op individu gebaseerde, simulaties. In deze modellen worden individuen gesimuleerd, om beweging te bestuderen in de context van foerageergedrag. Wanneer de verdeling van het voedsel relatief stabiel is gedurende het leven van individuen, leidt evolutie tot één competitiestrategie. Dit verandert aanzienlijk wanneer de voedselverdeling herhaaldelijk verandert. In dat geval evolueert een breed spectrum aan competitiestrategieën. Met andere woorden, onder fluctuerende omstandigheden ontstaan verschillende ‘persoonlijkheden’. We konden dit nieuwe mechanisme voor het ontstaan van individuele verschillen verklaren door het feit dat competitief sterke individuen een voordeel hebben onder stabiele milieuomstandigheden, terwijl competitief zwakke individuen profiteren van milieuveranderingen.

Tenslotte sluit ik mijn proefschrift af met een algemene discussie waarin ik reflecteer op de vraag "Waar staan we nu en waar moeten we naartoe?" wat betreft de vragen en methodologieën die betrekking hebben op het stekelbaarssysteem en ook meer in het algemeen op het gebied van persoonlijkheidsonderzoek bij dieren.

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About the author

Aparajitha (Apu) Ramesh, was born in Chennai, India on July 25, 1993. In 2011, Apu started an integrated bachelor's and master's studies in Biology at the Indian Institute of Science Education and Research, Thiruvananthapuram (IISER-TVM). In 2017, she was awarded an Adaptive Life scholarship from the University of Groningen to pursue her PhD in the groups of Franjo Weissing (Theoretical Research in Evolutionary Life Sciences), Marion Nicolaus (Conservation Ecology) and Ton Groothuis (Evolutionary Genetics, Development and Behaviour). She has broad research interests, including individual differences in behaviour, animal movement and migration, and competition. Her eventual career goal is an academic position studying the evolution of animal behaviour, using a combination of theoretical and empirical approaches, operating at the nexus of different disciplines with a strong affinity to teaching.

Publications

1. Netz, C., **Ramesh, A.**, Gismann, J., Gupte, P. R., & Weissing, F. J. (2022). Details matter when modelling the effects of animal personality on the spatial distribution of foragers. Proceedings of the Royal Society B, 289(1970), 20210903.

2. **Ramesh, A.**, Groothuis, T. G., Weissing, F. J., & Nicolaus, M. (2022). Habitat fragmentation induces rapid divergence of migratory and isolated sticklebacks.

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3. **Ramesh, A.**, Domingues, M. M., Stamhuis, E. J., Groothuis, T. G. G., Weissing, F. J., & Nicolaus, M. (2021). Does genetic differentiation underlie behavioral divergence in response to migration barriers in sticklebacks? A common garden experiment. Behavioral Ecology and Sociobiology, 75(12), 1-12.

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5. **Ramesh, A.***, Gismann, J.*, Groothuis, T. G., Weissing, F. J., & Nicolaus, M. (2022). Mesocosm experiments reveal the loss of migratory tendencies in a recently isolated population of three-spined sticklebacks. bioRxiv. https://doi.org/10.1101/2022.05.09.491155.

6. Netz, C., **Ramesh, A.**, & Weissing, F. J. (2022). Ideal free distribution of unequal competitors: spatial assortment and evolutionary diversification of competitive ability. bioRxiv. https://doi.org/10.1101/2022.06.27.497704.

Author affiliations

Aparajitha Ramesh
Groningen Institute for Evolutionary Life Sciences, University of Groningen, Nijenborgh 7, 9747 AG, Groningen, The Netherlands.

Prof. dr. Franz J. Weissing
Groningen Institute for Evolutionary Life Sciences, University of Groningen, Nijenborgh 7, 9747 AG, Groningen, The Netherlands.

Dr. Marion Nicolaus
Groningen Institute for Evolutionary Life Sciences, University of Groningen, Nijenborgh 7, 9747 AG, Groningen, The Netherlands.

Prof. dr. Ton G. G. Groothuis
Groningen Institute for Evolutionary Life Sciences, University of Groningen, Nijenborgh 7, 9747 AG, Groningen, The Netherlands.

Prof. dr. Eize J. Stamhuis
Department of Oceans Ecosystems, Energy and Sustainability Research Institute Groningen, University of Groningen, Nijenborgh 7, 9747 AG, Groningen, The Netherlands.

Mariana M. Domingues
Groningen Institute for Evolutionary Life Sciences, University of Groningen, Nijenborgh 7, 9747 AG, Groningen, The Netherlands.

Jakob Gismann
Groningen Institute for Evolutionary Life Sciences, University of Groningen, Nijenborgh 7, 9747 AG, Groningen, The Netherlands.

Christoph Netz
Groningen Institute for Evolutionary Life Sciences, University of Groningen, Nijenborgh 7, 9747 AG, Groningen, The Netherlands.