Learning and exploration in the Trinidadian guppy: investigations into plasticity and constraints

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and the end of all our exploring
will be to arrive where we started
and know the place for the first time.

T.S. ELIOT

Contents

Abstract

The conditions in an environment can change rapidly within a lifetime. Animals can adapt to such changes by shifting behaviour in response to environmental cues, a phenomenon known as behavioural plasticity. However, behaviour is not infinitely plastic and proximate and/or ultimate constraints may inhibit plasticity. In this thesis I capitalize on the ecology and behaviour of the Trinidadian guppy to examine whether behavioural plasticity may be constrained as a result of presumed historical reward contingencies, consistency in individual behaviour ('animal personality') and/or behavioural trait correlations ('behavioural syndromes'). In **Chapter 2**, I investigated whether learned shifts in preferences for coloured foraging items was unconstrained by giving guppies an object learning task with two colours thought to differ in their ecological relevancy. After rewarding guppies for foraging from either green or blue objects, I found that learned preferences for a green object became stronger than those for a blue object. I go on to hypothesise that this constraint may reflect the evolutionary importance of detecting green algae, an important foraging resource for guppies. Having established that object preferences can be flexibly shaped I went on to examine whether novel object exploration could be shifted independently of responses to novelty in another context (spatial exploration). Guppies tend to exhibit an 'exploration syndrome', that is, a guppy that prefers novel objects also prefers novel mates and is also more likely to be exploratory in a novel area. Whether these individual differences and their cross-contextual correlations reflect constraints on behavioural plasticity (*i.e.,* changes in one context carryover to another context) is unclear, but would have adaptive consequences if for example the costs and benefits of exploratory propensities differ between novelty contexts. In **Chapter 3**, I investigated whether individual propensities for exploratory behaviour are fixed or flexibly shaped by experience. I show that preferences for novel objects can be shifted by manipulating experienced rewards, but this shift does not influence spatial exploration. This suggests that an individual's behavioural responses to other novelty contexts need not impose strong constraints on plasticity as is commonly hypothesised. Moreover, I find consistency in exploratory tendencies only appears after rewarding experiences with either novel or familiar stimuli. In sum, I find that while there can be constraints on behavioural plasticity, they are likely to be context specific. Such constraints may be more likely to arise due to an evolutionary history involving ecologically important cues biasing which behavioural changes are more readily made rather than constraints arising from cross-contextual behavioural syndromes.

Résumé

Les conditions dans un environnement peuvent changer rapidement au cours d'une vie. Les animaux peuvent s'adapter à de tels changements en modifiant leur comportement en réponse à des signaux environnementaux, un phénomène connu sous le nom de plasticité comportementale. Cependant, le comportement n'est pas infiniment plastique et des contraintes proches et / ou ultimes peuvent inhiber la plasticité. Dans cette thèse, je capitalise sur l'écologie et le comportement du guppy trinidadien pour examiner si la plasticité comportementale peut être limitée en raison de contingences de récompenses historiques présumées, de la cohérence du comportement individuel (‘personnalité animale’) et / ou des corrélations des traits comportementaux (‘comportemental syndromes’). Dans le **Chapitre 2**, j'ai examiné si les changements appris dans les préférences pour les articles de recherche de nourriture colorés n'étaient pas limités en donnant aux guppys une tâche d'apprentissage d'objets avec deux couleurs censées différer par leur pertinence écologique. Après avoir récompensé les guppys pour s'être nourris d'objets verts ou bleus, j'ai découvert que les préférences apprises pour un objet vert devenaient plus fortes que celles pour un objet bleu. Je continue en émettant l'hypothèse que cette contrainte peut refléter l'importance évolutive de la détection des algues vertes, une ressource alimentaire importante pour les guppys. Après avoir établi que les préférences d'objet peuvent être façonnées de manière flexible, j'ai continué à examiner si l'exploration d'objets nouveaux pouvait être déplacée indépendamment des réponses à la nouveauté dans un autre contexte (exploration spatiale). Les guppys ont tendance à présenter un «syndrome d’exploration», c’est-à-dire qu’un guppy qui préfère les objets nouveaux préfère également les nouveaux compagnons et est également plus susceptible d’être explorateur dans un domaine nouveau. La question de savoir si ces différences individuelles et leurs corrélations trans-contextuelles reflètent des contraintes sur la plasticité comportementale (c'est-à-dire les changements dans un contexte reporté dans un autre contexte) n'est pas claire, mais aurait des conséquences adaptatives si, par exemple, les coûts et les avantages des propensions exploratoires différaient entre les contextes de nouveauté. Au **Chapitre 3**, j'ai cherché à savoir si les propensions individuelles au comportement exploratoire étaient fixées ou modelées de manière flexible par l'expérience. Je montre que les préférences pour les objets nouveaux peuvent être modifiées en manipulant des récompenses expérimentées, mais ce changement n'influence pas l'exploration spatiale. Cela suggère que les réponses comportementales d’un individu à d’autres contextes de nouveauté n’ont pas besoin d’imposer de fortes contraintes à la plasticité, comme on l’hypothèse communément. De plus, je trouve que la cohérence des tendances exploratoires n'apparaît qu'après des expériences enrichissantes avec des stimuli nouveaux ou familiers. En résumé, je trouve que s'il peut y avoir des contraintes sur la plasticité comportementale, elles sont susceptibles d'être spécifiques au contexte. De telles contraintes peuvent être plus susceptibles de survenir en raison d'une histoire évolutive impliquant des indices importants sur le plan écologique qui biaisent les changements de comportement plus faciles à faire plutôt que des contraintes découlant de syndromes comportementaux inter-contextuels.

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I'd like to first thank my supervisors, Simon Reader and Rachel Page for all their help throughout the course of this degree. Simon took a chance on me and gave me the opportunity to go to graduate school. Thank you for the practical and financial support, feedback, providing an environment where I can experiment and learn from mistakes, giving me the opportunity to pursue additional interests and develop a varied skillset, supporting the additional avenues I have wanted to pursue in media, programming, and genetics all of which were well beyond the initial scope of this MSc but for which my training as a scientist has been made richer.

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One of the joys of this degree has been the opportunity to gain teaching experience by mentoring undergraduates conducting research projects. The two undergraduate students I supervised for Honours/independent research projects gave me valuable pedagogical experience in addition to performing useful research projects. Alex and Beatriz thank you (and sorry!) for being my guinea pigs! Beatriz I'm so proud of how much you were able to innovate during your Honour's project. You turned a vague hazy idea I had thought up early in the pandemic into a fully realized project and did it better than I could have hoped to. I would also like to thank Mina Araya-Yohannes for her help on a follow-up experiment in Chapter 2. Without you I could not have strengthened that study.

My final thanks are to mama and papa. It doesn't seem so long ago that I was complaining about not having enough time on the GameCube in our little rented space in Stewartown. I don't know what's next but I'm thankful that I know you'll be there to encourage me.

Contribution to original knowledge

This thesis is manuscript based, with all chapters containing original scholarship. All chapters have either been submitted or intend to be submitted to peer-reviewed journals. In **Chapter 2**, I wanted to determine whether the shift in preference for coloured objects would be unconstrained in Trinidadian guppies given that colours vary in their ecological importance for guppies in the wild. Green and blue objects are thought to differ greatly in their ecological importance. I found that foraging preferences for green shifted much more readily than foraging preferences for blue, possibly reflecting an evolutionary predisposition for green foraging items in guppies, though more work across more guppy populations will be needed to support this conclusion. In **Chapter 3**, I wanted to determine whether shifts in exploratory behaviour would be unconstrained (*i.e.,* free to shift independently in different contexts) in Trinidadian guppies, where exploratory behaviour tends to be correlated across contexts. I find that novel object exploration can be upregulated as a consequence of manipulating the rewards of novelty in the environment and that this shift does not lead to a concordant shift in spatial exploration. This reveals that exploratory behaviours across novelty contexts are likely free to change independently and need not be constrained by the costs and benefits of exploratory behaviour in a separate context.

Contribution of authors

Chapter 1: General introduction
M. Wyatt Toure wrote the chapter with feedback from Simon M. Reader.

Chapter 2: Colour biases in learned foraging preferences in Trinidadian guppies
M. Wyatt Toure and Simon M. Reader conceived and designed the experiment and wrote the paper. M. Wyatt Toure collected and analysed the data with practical support, input and supervision from Simon M. Reader.

Manuscript in revision with *Ethology*.

Chapter 3: Experimental manipulation of novelty rewards reveals associative learning can underpin variation in exploratory behaviour
M. Wyatt Toure and Simon M. Reader conceived and designed the experiment and wrote the paper. M. Wyatt Toure collected and analysed the data with practical support, input and supervision from Simon M. Reader.

Manuscript in prep for *Proceedings of the Royal Society B*

Chapter 4: General Discussion
M. Wyatt Toure wrote the chapter with feedback from Simon M. Reader.

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Chapter 1 General Introduction

“I speculated whether a species very liable to repeated and great changes of conditions might not assume a fluctuating condition ready to be adapted to either condition.”

— Darwin, from a letter to
Karl Semper in 1881

A chief tenet of the modern synthesis is that adaptive evolution occurs via natural selection on genetically based variation (Huxley 1942). The explanatory power of this genetic approach is considerable. With this, evolutionary biologists were able to establish the key mechanisms of evolution, namely mutation, gene flow, natural selection and genetic drift. Since natural selection has no lasting evolutionary consequences without inheritance, and inheritance is largely genetic[1], phenotypes with an established genetic basis were prioritized in investigations of adaptation. However, while this prioritization allowed considerable progress, researchers such as Mary Jane West-Eberhard argue it led to environmentally induced changes in phenotype, known as phenotypic plasticity, being given less prominence, citing investigators that described phenotypic plasticity as simply odd by-products of development with little adaptive relevance (West-Eberhard 2003). This was despite phenotypic plasticity being discussed with respect to its role in evolution for quite some time (e.g. Semper, 1881, Baldwin, 1896). Time has shown that phenotypic plasticity can be adaptive and have considerable ecological and evolutionary implications (Bradshaw 1974; Torres-Dowdall *et al.* 2012; Handelsman *et al.* 2014; Hendry 2016; Donelan *et al.* 2019; Ducatez *et al.* 2020).

Phenotypic plasticity is often presented as an alternative to genetic adaptation[2]. Rather than adapting over generations to the environment via evolved genetic changes that impact phenotypes, phenotypes can be tuned to environmental conditions within the lifetime of an individual. A fixed gene-phenotype mapping that is nonadaptive typically requires natural selection to correct the mismatch between the trait of interest and the environment. However, via phenotypic plasticity organisms can produce adaptive phenotypes if the

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[1] Notable exceptions are epigenetic and cultural inheritance, however at the time of the modern synthesis these were not as well understood within an evolutionary framework.

[2] Though this is likely to be a false dichotomy. The degree of plasticity, and constraints on plasticity, can themselves be evolved traits.

environment changes. Phenotypic plasticity is thus important for an animal to meet the changing contemporary demands of an environment. Behaviour is often distinguished from other phenotypic dimensions by being highly plastic on relatively short, within-lifetime, timescales — from seconds to minutes compared to traits like morphology which can take days to weeks to respond to environmental cues (Piersma & Drent 2003). This rapid shift in phenotypic expression allows organisms to address environmental change on fine temporal scales more rapidly than is possible through genetic adaptation or morphological plasticity. While behavioural plasticity allows organisms to rapidly respond to a number of environmental changes, the value of behavioural plasticity will vary based on how predictably changeable the environment is. If environmental changes are highly predictable, then behavioural responses may be more likely to become genetically encoded since there are notable costs to behavioural plasticity (Brown 2001; Mery & Kawecki 2003; Niven *et al.* 2007; Burger *et al.* 2008; Boyer *et al.* 2010; McCabe *et al.* 2015). Moreover, if environmental changes are highly unpredictable then plasticity resulting in enduring changes may also not be advantageous since past environmental cues will do little to reduce ecological uncertainty. In environmental conditions where ecological uncertainty about changes in the environment can be reliably reduced for the benefit of future responses, behavioural plasticity may be more likely to be favourable.

Dealing with ecological uncertainty

Managing ecological uncertainty through space and time is a major fitness challenge many species face (Dall *et al.* 2005). At any given moment animals have only incomplete information regarding local environment conditions. They do not have complete knowledge of what foods are edible, where the best foraging patches are, or where mates are located. Animals can manage this uncertainty by using information gained about stimuli present in the environment to shift their behaviour in a phenotypically plastic fashion. Two important processes by which organisms may reduce ecological uncertainty are learning and exploration. Learning can be defined as the change in behaviour in response to specific stimuli as a result of prior experience with those specific or similar stimuli. Exploratory behaviour refers to an individual's behavioural response to novelty (Réale *et al.* 2007). Exploring the stimuli within an environment and learning about the stimuli that have been explored within the environment provides animals with crucial information about conditions within the environment and informs their behavioural response to them. Learning and exploration can thus interact with each other to contribute to the reduction of an individual's ecological uncertainty (Renner 1988; Mettke-Hofmann *et al.* 2006; Cohen *et al.* 2007; Bousquet *et al.* 2015; Griffin *et al.* 2017; Rojas-Ferrer *et al.* 2020). A situation which often introduces ecological uncertainty, for which learning and exploration can be solutions, is the encounter with novelty in the environment.

Novelty is involved in many aspects of an animal's life. An animal can encounter novelty due to a number of ecological processes, such as seasonal changes introducing new biota (*e.g.,* new fruits or vegetation) or dispersal introducing animals to new habitats (Greenberg & Mettke-Hofmann 2001). Before an individual can learn about a novel stimulus, *e.g.,* how beneficial or distasteful a novel food is, it typically must be approached and explored[3]. Early in the history of learning studies this was a dilemma for investigators because it became apparent that individual animals differed in their propensities to engage with novelty. This variation in exploratory tendency was thought to be noise one needed to account for through controls or habituation. In behavioural ecology, as with plasticity in evolutionary biology, it has again become apparent to investigators that this "noise" might indeed be itself meaningful (Sih *et al.* 2004; Réale *et al.* 2007).

Exploration as a 'personality' trait

There has been a surge of research interest in individual variation in the expression of particular behavioural patterns, and the consistency of this variation across time, contexts and situations (Sih *et al.* 2004; Réale *et al.* 2007). An example would be conspecific prey foraging before and during the arrival of predators: all individuals reduce foraging activity, but relative differences in foraging activity are maintained across both situations (Sih *et al.* 2003). These consistent among-individual differences in average behaviour across repeated observations in separate times and/or contexts have come to be called animal personality and, while the value of this research approach has proven controversial

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[3] Notably social learning is an exception to this, but I restrict my conversation of learning to individual learning

(Beekman & Jordan 2017), it has certainly led to an eruption of investigations into the causes and consequences of individual variation in animal behaviour (Bell 2007).

Consistent individual differences in behaviour are taxonomically widespread, having been documented in mammals (Svartberg *et al.* 2005), birds (Nicolaus *et al.* 2012), amphibians (López *et al.* 2005), fishes (Conrad *et al.* 2011), and invertebrates (Udino *et al.* 2017) proving to be a seemingly general phenomenon in animals (Bell *et al.* 2009). While consistent individual differences have been documented in several taxa, one of the critiques of personality studies is that they are often descriptive with many studies taking a correlational approach *i.e.,* studies typically take a measure of a personality trait and correlate it to another trait or simply document consistency in behaviour (Jungwirth *et al.* 2017). This has led to findings on associations between personality and traits of evolutionary, ecological, and cognitive importance, such as learning (Guillette *et al.* 2009; Trompf & Brown 2014), behavioural innovation (Griffin & Guez 2014; Berdal *et al.* 2018), dispersal (Dingemanse *et al.* 2003), dominance (Colléter & Brown 2011), survival (Smith & Blumstein 2010), pair-bonding (Firth *et al.* 2018), and fitness (Cote *et al.* 2008; Smith & Blumstein 2008; Ballew *et al.* 2017) which warrant further investigation. However, investigations into mechanisms and direct experimental manipulations, are not as common, leading to criticisms over the field's lack of mechanistic insight and strongly descriptive nature (DiRienzo & Montiglio 2015; Beekman & Jordan 2017). How 'personality' develops in nonhuman animals and what mechanisms are implicated in their presence remains comparatively understudied (Stamps & Groothuis 2010).

Exploratory behaviour has often come to be studied within the context of animal personality (Réale *et al.* 2007). The existence and consistency of individual variation in exploratory behaviour has been established in several species (Verbeek *et al.* 1994; Drent *et al.* 2003; Miller *et al.* 2018). Some species engage with novelty more readily than others and the conditions under which a species evolves can contribute to these responses (Tebbich *et al.* 2009). For example, a large comparative study of 61 parrot species found that species which live in more complex habitats and habitats with more fruits, fruits that can be difficult to find, are more likely to explore novel objects (Mettke-Hofmann *et al.* 2002). Additionally, species that fed on potentially dangerous resources such as insects were less exploratory. However, the environment can also influence the expression of exploratory responses on within-lifetime scales (Candler and Bendar 2015). Rats living in variable environments have a lower level of food neophobia than those which live in stable environments (Modlinska *et al.* 2015; Modlinska & Stryjek 2016). How individuals come to develop these propensities tends to be unclear. When attempting to assess developmental influences on individual behaviour, personality studies often sample or place animals in environments that differ in some ecological variable and then assay the exploratory behaviour of individuals (Miranda *et al.* 2013; Thompson *et al.* 2018; Breck *et al.* 2019; Grunst *et al.* 2019). While differences may be detected, this approach can suffer from the pitfalls of a 'black box' approach. It remains unclear what processes underly these behavioural differences. Understanding the mechanisms at play is important since alternative mechanisms will have different evolutionary consequences and have implications for our understanding of why behavioural variation is maintained (Aubin-Horth & Renn 2009; Bell & Aubin-Horth 2010; Bell & Dochtermann 2015). Often when investigating mechanisms of individual variation, investigators focus on molecular or hormonal agents (Bell & Aubin-Horth 2010) however another potential source of observed variation in 'personality' is learning (Frost *et al.* 2007). Direct experience with novelty may be a mechanism producing the divergent responses to novelty we observe in individuals from different environments however, it remains unclear how pervasive this is. I directly address this in **Chapter 3** by manipulating the value of novelty in the environment to see whether learning is a process which may explain divergence in exploratory behaviour. Moreover, it is unclear whether the presence of a 'personality' suggests that there are constraints in the plasticity an individual animal is able to express. An open question then is whether personalities represent the manifestation of biological mechanisms which predispose individual to behave in certain ways and not others.

Are there constraints on behavioural plasticity?

"The strong bounds that nature places on diversity provide our best starting point for a study of limits"

— Stephen J. Gould,
The Evolutionary Biology of Constraint, p. 39

Just as organisms are subject to constraints that prevent evolved or plastic shifts in morphology, such as the size of insects being limited by oxygen levels in the environment (Harrison *et al.* 2010), behavioural plasticity may be constrained by physical, mechanistic, and/or evolutionary constraints. Evolution works with an existing mechanistic architecture. As a result of historical or mechanistic contingencies there may be constraints in the ability of phenotypes to shift in response to environmental variation or selection. For example, the squinting bush brown butterfly, *Bicyclus anynana,* has two wing spots on the forewing that can differ based on size and colour. Artificial selection on these two wing spots can change their sizes but not colours independently, showing there is a lack of constraint on wing spot size shifts but a constraint on colour shifts. Artificial selection can make both eyespots become more black or both eyespots become more yellow but it is not possible to make one eyespot black and the other eyespot yellow (Beldade *et al.* 2002; Allen *et al.* 2008). This is due to a mechanistic constraint, namely a genetic correlation between eyespot colours, underlying the development of colour in both eyespots. Such constraints are thought to also act on behavioural traits. Observed correlations across behavioural traits, termed behavioural syndromes, have been hypothesised to constrain independent evolutionary and/or plastic changes in correlated behavioural traits (Sih *et al.* 2004) in a similar fashion to which *Bicyclus anynana* eyespot colour changes are constrained. However, the extent to which behavioural traits are truly constrained in their plasticity due to the presence of syndromes remains unclear. Behavioural syndromes therefore provide an opportunity to investigate the potential for constraints acting on behavioural plasticity in exploratory behaviour which I examine in **Chapter 3**.

Constraints on exploration and learning

There is evidence that exploratory propensities across different stimuli contexts are correlated. A behavioural syndrome is the correlation among suites of behavioural traits across different ecological contexts such as mating, anti-predator responses, and foraging or within the same behavioural context, *e.g.* an aggression syndrome — aggression towards mates, competitors, and predators (Sih *et al.* 2004). In an exploration syndrome, responses to different novel stimuli (*e.g.* objects, areas, and foods) are correlated with each other. Across several taxa responses to different novel stimuli have been found to be correlated within individuals (Grill *et al.* 2012; Schuett *et al.* 2012). For example, cichlid individuals (*Neolamprologus caudopunctatus*) that touched a novel object more spent more time swimming in a novel environment (Grill *et al.* 2012) and common mynas, *Acridotheres tristis*, that interact more with a novel object more are quicker to explore a novel area (Perals *et al.* 2017).

The presence of syndromes has been argued by some to suggest a constraint to behavioural plasticity. Such a constraint could arise from genetic or hormonal pleiotropies, like testosterone simultaneously influencing aggression and courtship behaviours (Ketterson & Nolan 1999). In several species some of the genes which influence aggression also influence mating behaviour but, in opposite directions depending on the context. The overlap in differentially expressed genes leads to a correlation between mating behaviour and docility (Certel *et al.* 2007; Sanogo & Bell 2016). If common mechanisms predispose suites of behaviours to occur together, then maladaptive behavioural responses could arise in situations or environments where being able to express the behaviours in an independent fashion would be beneficial (Hendry 2016). Moreover, syndromes have the potential to bias evolution due to certain behavioural changes being achieved more readily than others (Fischer *et al.* 2016).

If exploratory tendencies result from general processes that influence responses in several contexts of novelty then experience with one novelty context could carry over into experiences with another novelty context (Reader 2015). However, domain specific phenotypic plasticity of exploratory propensities may exist if the consequences of novelty vary greatly across contexts and therefore require divergent responses. This could lead to the evolution of separate mechanisms mediating the response to novelty across different contexts. In the context of associative learning, a mechanism which could alter the degree to which a syndrome is observed is stimulus generalization. Stimulus generalization is when a learned association between one stimulus and one consequence is generalized to other similar stimuli. With respect to exploration, experiencing positive consequences for engaging with novel objects may generalize to expecting positive consequences for engaging with novelty across different situations such as novel areas or mates. This type of generalization would require animals to have a “concept” of novelty and there exists phylogenetically broad evidence that animals do have a concept of novelty (Macphail & Reilly 1989; Newport *et al.* 2015; Muszynski & Couvillon 2020).

Once organisms overcome the initial hurdle of exploring a stimulus, learning may take place. Learning can be seen as a special case of behavioural plasticity. Learned behaviour implies a shift in behaviour that arises as a result of prior experience, whereas a shift in behaviour need not necessarily be due to prior experience to be called behavioural plasticity. The relevant causal associations among environmental factors will differ broadly and also between species based on their ecology. As a result, not all stimuli have an equal possibility to be associated with a response (Shettleworth 1972). Animals may form associations with particular stimuli much more readily than others due to evolved predispositions for reliably tracking information from important stimuli. One example is food-storing birds. Compared to non-food-storers, food-storing birds display an increased propensity for learning about spatial cues given the importance of such cues in determining the location of previously hidden foods (Clayton & Krebs 1994). The ability for animals to readily evolve such learning predispositions has been demonstrated experimentally with *Drosophila* across two stimulus dimensions (Dunlap & Stephens 2014). *Drosophila* where colours reliably predict a consequence but odours do not, evolved increased sensitivity to learning colour-consequence pairings over 30 generations, while the reverse contingencies had the opposite result. That is, manipulations of the historical association between stimulus and consequence led to differences in the acquisition of behavioural responses. In addition to biases towards information across stimuli dimensions there can be biases for particular stimuli *within* stimulus dimensions. Animals often display clear preferences for particularly coloured stimuli. In vertebrates these are often investigated in the context of unlearned preferences for specific colours (Lythgoe 1979; Honkavaara *et al.* 2002; Osorio *et al.* 2004) — particularly in a mating context (Houde 1997). However, historic associations between colour and reward may go on to shape the ease with which associations are formed between certain colours over others. While historic associations may constrain the unlearned preference for particular colours it remains unclear how strongly *learned* preferences for particular colours can be constrained in vertebrates (Rain *et al.* 2006). Such constraints are examined in **Chapter 2**.

Study System

Trinidadian guppies, *Poecilia reticulata*, provide a valuable study system for answering questions about constraints on plasticity in learning and exploration. Guppies are a small tropical freshwater fish native to Trinidad where they occur in generally distinct populations (though see Blondel et al., 2019 for evidence that populations may not be as separate as is typically assumed). The rivers that are home to guppies experience a broad range of environmental conditions varying in predation risk, primary productivity, population density, and parasite prevalence, providing a natural laboratory with different experimental treatments based on the ecological conditions of the stream (Godin & Smith 1988; Harris *et al.* 2010; Reznick & Travis 2019). These ecological differences have supported extensive study in the wild on guppy morphological (Endler 1980, 1995), life history (Reznick 1983; Reznick *et al.* 1997, 2001; Reznick & Travis 2019), and behavioural (Magurran & Seghers 1990; Magurran *et al.* 1992) evolution. Early guppy behavioural studies, reflecting the trends of studies on adaptation at the time, focused heavily on evolved behavioural differences, rather than focusing on behavioural plasticity, with population differences due to plastic changes removed through common garden experiments (Seghers 1974; Breden & Stoner 1987; Breden *et al.* 1987; Magurran & Seghers 1991; Seghers & Magurran 1995; Seghers *et al.* 1995; Kelley & Magurran 2003). Examples of the behavioural adaptations various ecological difference elicit are increased anti-predator behaviour when sympatric with predators (Magurran *et al.* 1992) or parasite-mediated shifts in shoaling behaviour (Stephenson 2019). However, recent work has shown that behavioural plasticity in guppies can be considerable, vary across environments, and have important ecological and evolutionary consequences. Importantly for this thesis, guppies discriminate behaviourally between novel and familiar stimuli (Lucon-Xiccato & Dadda, 2016), show population variation in exploratory behavior (Burns *et al.* 2016; Jacquin *et al.* 2017) which has consistently been shown to be correlated across several novelty contexts (Daniel *et al.* 2020) and sensitive to environmental conditions (Burns *et al.* 2016; Elvidge *et al.* 2016), suggesting exploratory behaviour may be plastic. Given that exploratory behaviours tend to be correlated across contexts yet also seem to exhibit considerable plasticity, I use guppies to investigate whether plastic shifts in exploratory behaviour can be induced by manipulating the rewards of novelty in a single context and whether experience with reward in a single novelty context leads to changes in other novelty contexts.

To investigate constraints on learning in an ecologically relevant manner we need a stimulus dimension with demonstrable ecological importance for the species in question. For guppies colour is such a stimulus dimension. Reflecting this importance, a wealth of studies have been conducted which aim to understand the ecological, evolutionary, and developmental processes that are involved in colour-based behaviour and morphology in guppies (Rodd *et al.* 2002; Grether *et al.* 2005; White *et al.* 2005; Hoffmann *et al.* 2007; de Serrano *et al.* 2012; Sathyan & Couldridge 2013; Gotanda & Hendry 2014; Sandkam *et al.* 2016). Females generally prefer to mate with males that have more orange colouration (Houde 1987), however this varies by population (Endler & Houde 1995; Sathyan & Couldridge 2013) and depends on lighting and ecological conditions (Endler 1995; Gamble *et al.* 2003). Additionally, in different populations of guppies, males and females are more attracted to particular foraging item colours (Rodd *et al.* 2002). If different colours have different historic associations with reward for which the cognitive and/or sensory systems are particularly tuned there may be differences in colour learning which reflect this evolutionary history. Important resources such as green algae and orange fruit (Rodd *et al.* 2002) are thought to be resources to which guppies are particularly tuned (Dussault & Kramer 1981; Cole *et al.* 2019). Guppies therefore provide a useful system for investigating the factors that shape and/or constrain differences in colour learning.

Thesis aims and outlines

In this thesis I sought to determine whether foraging preferences and exploration propensities respond plastically to experiences within the environment via reinforcement training and whether there are constraints in this plasticity arising from evolutionary and/or developmental processes. I did this primarily by attempting to shift preferences for coloured and novel objects in guppies through reinforcement training. In **Chapter 2**, I investigated whether behavioural plasticity, in the form of learning, is constrained in guppies as a result of stimulus colour, an ecologically important and relevant trait within the guppy system. I then discuss the assumption that constraints have arisen as a result of historic evolutionary associations between reward and colour. In **Chapter 3**, I investigated whether exploratory behaviour is plastic in response to the experience of paired rewards with novelty and the degree to which individual exploratory behaviour is constrained in its plasticity as a result of putative behavioural syndromes. In **Chapter 4**, I synthesize the findings of my experimental chapters and discuss the role of constraint in the evolution of and plasticity in behavioural traits.

References

Allen, C.E., Beldade, P., Zwaan, B.J. & Brakefield, P.M. (2008). Differences in the selection response of serially repeated color pattern characters: standing variation, development, and evolution. *BMC Evol. Biol.*, 8, 94.

Aubin-Horth, N. & Renn, S.C.P. (2009). Genomic reaction norms: using integrative biology to understand molecular mechanisms of phenotypic plasticity. *Mol. Ecol.*, 18, 3763–3780.

Baldwin, J.M. (1896). A new factor in evolution. *Am. Nat.*, 30, 441–451.

Ballew, N.G., Mittelbach, G.G. & Scribner, K.T. (2017). Fitness consequences of boldness in juvenile and adult largemouth bass. *Am. Nat.*, 189, 396–406.

Beekman, M. & Jordan, L.A. (2017). Does the field of animal personality provide any new insights for behavioral ecology? *Behav. Ecol.*, 28, 617–623.

Beldade, P., Koops, K. & Brakefield, P.M. (2002). Modularity, individuality, and evo-devo in butterfly wings. *Proc. Natl. Acad. Sci. U. S. A.*, 99, 14262–14267.

Bell, A.M. (2007). Animal personalities. *Nature*, 447, 539–540.

Bell, A.M. & Aubin-Horth, N. (2010). What can whole genome expression data tell us about the ecology and evolution of personality? *Philos. Trans. R. Soc. B Biol. Sci.*, 365, 4001–4012.

Bell, A.M. & Dochtermann, N.A. (2015). Integrating molecular mechanisms into quantitative genetics to understand consistent individual differences in behavior. *Curr. Opin. Behav. Sci.*, 6, 111–114.

Bell, A.M., Hankison, S.J. & Laskowski, K.L. (2009). The repeatability of behaviour: a meta-analysis. *Anim. Behav.*, 77, 771–783.

Berdal, M.A., Rosenqvist, G. & Wright, J. (2018). Innovation as part of a wider behavioural syndrome in the guppy: the effect of sex and body size. *Ethology*, 124, 760–772.

Blondel, L., Baillie, L., Quinton, J., Alemu, J.B., Paterson, I., Hendry, A.P., *et al.* (2019). Evidence for contemporary and historical gene flow between guppy populations in different watersheds, with a test for associations with adaptive traits. *Ecol. Evol.*, 9, 4504–4517.

Bousquet, C.A.H., Petit, O., Arrivé, M., Robin, J.P. & Sueur, C. (2015). Personality tests predict responses to a spatial-learning task in mallards, *Anas platyrhynchos*. *Anim.*

Behav., 110, 145–154.

Boyer, N., Réale, D., Marmet, J., Pisanu, B. & Chapuis, J.L. (2010). Personality, space use and tick load in an introduced population of Siberian chipmunks *Tamias sibiricus. J. Anim. Ecol.*, 79, 538–547.

Bradshaw, A.D. (1974). Environment and phenotypic plasticity. In: *Basic Mechanisms in Plant Morphogenesis; Report of Symposium,* pp. 75-94.

Breck, S.W., Poessel, S.A., Mahoney, P. & Young, J.K. (2019). The intrepid urban coyote: a comparison of bold and exploratory behavior in coyotes from urban and rural environments. *Sci. Rep.*, 9, 2104.

Breden, F., Scott, M. & Michel, E. (1987). Genetic differentiation for anti-predator behaviour in the Trinidad guppy, *Poecilia reticulata*. *Anim. Behav.*, 35, 618–620.

Breden, F. & Stoner, G. (1987). Male predation risk determines female preference in the Trinidad guppy. *Nature*, 329, 831–833.

Brown, C. (2001). Familiarity with the test environment improves escape responses in the crimson spotted rainbowfish, *Melanotaenia duboulayi*. *Anim. Cogn.*, 4, 109–113.

Burger, J.M.S., Kolss, M., Pont, J. & Kawecki, T.J. (2008). Learning ability and longevity: A symmetrical evolutionary trade-off in *Drosophila*. *Evolution.*, 62, 1294–1304.

Burns, J.G., Price, A.C., Thomson, J.D., Hughes, K.A. & Rodd, F.H. (2016). Environmental and genetic effects on exploratory behavior of high- and low-predation guppies (*Poecilia reticulata*). *Behav. Ecol. Sociobiol.*, 70, 1187–1196.

Certel, S.J., Savella, M.G., Schlegel, D.C.F. & Kravitz, E.A. (2007). Modulation of *Drosophila* male behavioral choice. *Proc. Natl. Acad. Sci.*, 104, 4706–4711.

Clayton, N.S. & Krebs, J.R. (1994). Memory for spatial and object-specific cues in food-storing and non-storing birds. *J. Comp. Physiol. A*, 174.

Cohen, J.D., McClure, S.M. & Yu, A.J. (2007). Should I stay or should I go? How the human brain manages the trade-off between exploitation and exploration. *Philos. Trans. R. Soc. B Biol. Sci.*, 362, 933–942.

Cole, G.L., Lynn, J.C.B., Kranz, A.M. & Endler, J.A. (2019). Colour-based foraging diverges after multiple generations under different light environments. *Ethology*, 125, 212–221.

Colléter, M. & Brown, C. (2011). Personality traits predict hierarchy rank in male rainbowfish social groups. *Anim. Behav.*, 81, 1231–1237.

Conrad, J.L., Weinersmith, K.L., Brodin, T., Saltz, J.B. & Sih, A. (2011). Behavioural syndromes in fishes: A review with implications for ecology and fisheries management. *J. Fish Biol.*, 78, 395–435.

Cote, J., Dreiss, A. & Clobert, J. (2008). Social personality trait and fitness. *Proc. R. Soc. B.*, 275, 2851–2858.

Dall, S.R.X., Giraldeau, L., Olsson, O., Mcnamara, J.M. & Stephens, D.W. (2005). Information and its use by animals in evolutionary ecology. *Trends Ecol. Evol.*, 20, 187–193.

Daniel, M.J., Koffinas, L. & Hughes, K.A. (2020). Mating preference for novel phenotypes can be explained by general neophilia in female guppies. *Am. Nat.*, 196, 414–428.

Dingemanse, N.J., Both, C., Van Noordwijk, A.J., Rutten, A.L. & Drent, P.J. (2003). Natal dispersal and personalities in great tits (*Parus major*). *Proc. R. Soc. B.*, 270, 741–747.

DiRienzo, N. & Montiglio, P.-O. (2015). Four ways in which data-free papers on animal personality fail to be impactful. *Front. Ecol. Evol.*, 3.

Donelan, S.C., Hellmann, J.K., Bell, A.M., Luttbeg, B., Orrock, J.L., Sheriff, M.J., *et al.* (2019). Transgenerational plasticity in human-altered environments. *Trends Ecol. Evol.*, 1–10.

Drent, P.J., Van Oers, K. & Van Noordwijk, A.J. (2003). Realized heritability of personalities in the great tit (*Parus major*). *Proc. R. Soc. B.*, 270, 45–51.

Ducatez, S., Sol, D., Sayol, F. & Lefebvre, L. (2020). Behavioural plasticity is associated with reduced extinction risk in birds. *Nat. Ecol. Evol.*, 4, 788–793.

Dunlap, A.S. & Stephens, D.W. (2014). Experimental evolution of prepared learning. *Proc. Natl. Acad. Sci.*, 111, 11750–11755.

Dussault, G. V. & Kramer, D.L. (1981). Food and feeding behavior of the guppy, *Poecilia reticulata* (Pisces: *Poeciliidae*). *Can. J. Zool.*, 59, 684–701.

Elvidge, C.K., Chuard, P.J.C. & Brown, G.E. (2016). Local predation risk shapes spatial and foraging neophobia patterns in Trinidadian guppies. *Curr. Zool.*, 62, 457–462.

Endler, J.A. (1980). Natural selection on color patterns in *Poecilia reticulata*. *Evolution.*, 34, 1–20.

Endler, J.A. (1995). Multiple-trait evolution and environmental gradients in guppies. *Trends Ecol. Evol.*, 10, 22–29.

Endler, J. A., & Houde, A. E. (1995). Geographic variation in female preferences for male traits in *Poecilia reticulata*. *Evolution*, 49, 456–468

Firth, J.A., Cole, E.F., Ioannou, C.C., Quinn, J.L., Aplin, L.M., Culina, A., *et al.* (2018). Personality shapes pair bonding in a wild bird social system. *Nat. Ecol. Evol.*, 2, 1696–1699.

Fischer, E.K., Ghalambor, C.K. & Hoke, K.L. (2016). Plasticity and evolution in correlated suites of traits. *J. Evol. Biol.*, 29, 991–1002.

Frost, A.J., Winrow-Giffen, A., Ashley, P.J. & Sneddon, L.U. (2007). Plasticity in animal personality traits: does prior experience alter the degree of boldness? *Proc. R. Soc. B.*, 274, 333–339.

Gamble, S., Lindholm, A.K., Endler, J.A. & Brooks, R. (2003). Environmental variation and the maintenance of polymorphism: the effect of ambient light spectrum on mating behaviour and sexual selection in guppies. *Ecol. Lett.*, 6, 463–472.

Godin, J.G.J. & Smith, S.A. (1988). A fitness cost of foraging in the guppy. *Nature*, 333, 69–71.

Gotanda, K.M. & Hendry, A.P. (2014). Using adaptive traits to consider potential consequences of temporal variation in selection: male guppy colour through time and space. *Biol. J. Linn. Soc.*, 112, 108–122.

Greenberg, R. & Mettke-hofmann, C. (2001). Ecological aspects of neophobia and neophilia in birds. In: *Current Ornithology, Volume 16*. Springer US, Boston, MA, pp. 119–178.

Grether, G.F., Kolluru, G.R., Rodd, F.H., De La Cerda, J. & Shimazaki, K. (2005). Carotenoid availability affects the development of a colour-based mate preference and the sensory bias to which it is genetically linked. *Proc. R. Soc. B.*, 272, 2181–2188.

Griffin, A.S. & Guez, D. (2014). Innovation and problem solving: a review of common mechanisms. *Behav. Processes*, 109, 121–134.

Griffin, A.S., Netto, K. & Peneaux, C. (2017). Neophilia, innovation and learning in an urbanized world: a critical evaluation of mixed findings. *Curr. Opin. Behav. Sci.*, 16, 15–22.

Grill, J., Blum, C., Wagner, R.H., Mann, M., Urban, D., Schaedelin, F.C., *et al.* (2012). Exploring novelty: a component trait of behavioural syndromes in a colonial fish. *Behaviour*, 149, 215–231.

Grunst, A.S., Grunst, M.L., Pinxten, R. & Eens, M. (2019). Personality and plasticity in neophobia levels vary with anthropogenic disturbance but not toxic metal exposure in urban great tits: Urban disturbance, metal pollution and neophobia. *Sci. Total Environ.*, 656, 997–1009.

Guillette, L.M., Reddon, A.R., Hurd, P.L. & Sturdy, C.B. (2009). Exploration of a novel space is associated with individual differences in learning speed in black-capped chickadees, *Poecile atricapillus*. *Behav. Processes*, 82, 265–270.

Handelsman, C.A., Ruell, E.W., Torres-Dowdall, J. & Ghalambor, C.K. (2014). Phenotypic plasticity changes correlations of traits following experimental introductions of Trinidadian guppies (*Poecilia reticulata*). *Integr. Comp. Biol.*, 54, 794–804.

Harris, S., Ramnarine, I.W., Smith, H.G. & Pettersson, L.B. (2010). Picking personalities apart: estimating the influence of predation, sex and body size on boldness in the guppy *Poecilia reticulata*. *Oikos*, 119, 1711–1718.

Harrison, J.F., Kaiser, A. & VandenBrooks, J.M. (2010). Atmospheric oxygen level and the evolution of insect body size. *Proc. R. Soc. B.*, 277, 1937–1946.

Hendry, A.P. (2016). Key questions on the role of phenotypic plasticity in eco-evolutionary dynamics. *J. Hered.*, 107, 25–41.

Hoffmann, M., Tripathi, N., Henz, S.R., Lindholm, A.K., Weigel, D., Breden, F., *et al.* (2007). Opsin gene duplication and diversification in the guppy, a model for sexual selection. *Proc. R. Soc. B.*, 274, 33–42.

Honkavaara, J., Koivula, M., Korpimäki, E., Siitari, H. & Viitala, J. (2002). Ultraviolet vision and foraging in terrestrial vertebrates. *Oikos*, 98, 505–511.

Houde, A.E. (1987). Mate choice based upon naturally occurring color-pattern variation in a guppy population. *Evolution.*, 41, 1–10.

Houde, A.E. (1997). *Sex, color, and mate choice in guppies*. Princeton University Press. Princeton, NJ

Huxley, J. (1942). Evolution. The modern synthesis. *Evol. Mod. Synth.*

Jacquin, L., Dybwad, C., Rolshausen, G., Hendry, A.P. & Reader, S.M. (2017). Evolutionary and immediate effects of crude-oil pollution: depression of exploratory behaviour across populations of Trinidadian guppies. *Anim. Cogn.*, 20, 97–108.

Jungwirth, A., Wedell, N., Bshary, R. & Milinski, M. (2017). Animal personalities: an empty placeholder feigning understanding: a comment on Beekman and Jordan. *Behav.*

Ecol., 28, 629–630.

Kelley, J.L. & Magurran, A.E. (2003). Effects of relaxed predation pressure on visual predator recognition in the guppy. *Behav. Ecol. Sociobiol.*, 54, 225–232.

Ketterson, E.D. & Nolan, V. (1999). Adaptation, exaptation, and constraint : a hormonal perspective. *Am. Nat.*, 154, S4–S25.

López, P., Hawlena, D., Polo, V., Amo, L. & Martín, J. (2005). Sources of individual shy-bold variations in antipredator behaviour of male Iberian rock lizards. *Anim. Behav.*, 69, 1–9.

Lucon-Xiccato, T. & Dadda, M. (2016). Guppies show behavioural but not cognitive sex differences in a novel object recognition test. *PLoS One*, 11, e0156589.

Lythgoe, J.N. (1979). *Ecology of vision*. Clarendon Press.

Macphail, E.M. & Reilly, S. (1989). Rapid acquisition of a novelty versus familiarity concept by pigeons (*Columba livia*). *J. Exp. Psychol. Anim. Behav. Process.*, 15, 242–252.

Magurran, A.E. & Seghers, B.H. (1990). Population differences in predator recognition and attack cone avoidance in the guppy *Poecilia reticulata*. *Anim. Behav.*, 40, 443–452.

Magurran, A.E. & Seghers, B.H. (1991). Variation in schooling and aggression amongst guppy (*Poecilia reticulata*) populations in Trinidad. *Behaviour*, 118, 214–234.

Magurran, A.E., Seghers, B.H., Carvalho, G.R. & Shaw, P.W. (1992). Behavioural consequences of an artificial introduction of guppies (*Poecilia reticulata*) in N. Trinidad: evidence for the evolution of anti-predator behaviour in the wild. *Proc. R. Soc. B Biol. Sci.*, 248, 117–122.

McCabe, C.M., Reader, S.M. & Nunn, C.L. (2015). Infectious disease, behavioural flexibility and the evolution of culture in primates. *Proc. R. Soc. B Biol. Sci.*, 282, 20140862.

Mery, F. & Kawecki, T.J. (2003). A fitness cost of learning ability in *Drosophila melanogaster. Proc. R. Soc. B Biol. Sci.*, 270, 2465–2469.

Mettke-Hofmann, C., Rowe, K.C., Hayden, T.J. & Canoine, V. (2006). Effects of experience and object complexity on exploration in garden warblers (*Sylvia borin*). *J. Zool.*, 268, 405–413.

Mettke-Hofmann, C., Winkler, H. & Leisler, B. (2002). The significance of ecological factors for exploration and neophobia in parrots. *Ethology*, 108, 249–272.

Miller, A.J., Page, R.A. & Bernal, X.E. (2018). Exploratory behavior of a native anuran species with high invasive potential. *Anim. Cogn.*, 21, 55–65.

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

Modlinska, K. & Stryjek, R. (2016). Food neophobia in wild rats (*Rattus norvegicus*) inhabiting a changeable environment-a field study. *PLoS One*, 11, 2–13.

Modlinska, K., Stryjek, R. & Pisula, W. (2015). Food neophobia in wild and laboratory rats (multi-strain comparison). *Behav. Processes*, 113, 41–50.

Muszynski, N.M. & Couvillon, P.A. (2020). Category difference facilitates oddity learning in honeybees (*Apis mellifera*). *J. Comp. Psychol.*, 134, 266–279.

Newport, C., Wallis, G. & Siebeck, U.E. (2015). Same/different abstract concept learning by archerfish (*Toxotes chatareus*). *PLoS One*, 10, e0143401.

Nicolaus, M., Tinbergen, J.M., Bouwman, K.M., Michler, S.P.M., Ubels, R., Both, C., *et al.* (2012). Experimental evidence for adaptive personalities in a wild passerine bird. *Proc. R. Soc. B.*, 279, 4885–4892.

Niven, J.E., Anderson, J.C. & Laughlin, S.B. (2007). Fly photoreceptors demonstrate energy-information trade-offs in neural coding. *PLoS Biol.*, 5, 828–840.

Osorio, D., Smith, A.C., Vorobyev, M. & Buchanan-Smith, H.M. (2004). Detection of fruit and the selection of primate visual pigments for color vision. *Am. Nat.*, 164, 696–708.

Perals, D., Griffin, A.S., Bartomeus, I. & Sol, D. (2017). Revisiting the open-field test: what does it really tell us about animal personality? *Anim. Behav.*, 123, 69–79.

Piersma, T. & Drent, J. (2003). Phenotypic flexibility and the evolution of organismal design. *Trends Ecol. Evol.*, 18, 228–233.

Raine, N. E., Ings, T. C., Dornhaus, A., Saleh, N., & Chittka, L. (2006). Adaptation, genetic drift, pleiotropy, and history in the evolution of bee foraging behavior. *Advances in the Study of Behavior*, *36*, 305–354.

Reader, S.M. (2015). Causes of individual differences in animal exploration and search. *Top. Cogn. Sci.*, 7, 451–468.

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

Renner, M.J. (1988). Learning during exploration: The role of behavioral topography during exploration in determining subsequent adaptive behavior. *Int. J. Comp. Psychol.*, 2, 43–56.

Reznick, Butler & Rodd. (2001). Life-history evolution in guppies. VII. the comparative ecology of high- and low-predation environments. *Am. Nat.*, 157, 126.

Reznick, D. (1983). The structure of guppy life histories: the tradeoff between growth and reproduction. *Ecology*, 64, 862–873.

Reznick, D.N., Shaw, F.H., Rodd, F.H. & Shaw, R.G. (1997). Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). *Science*, 275, 1934–1937.

Reznick, D.N. & Travis, J. (2019). Experimental studies of evolution and eco-evo dynamics in guppies (*Poecilia reticulata*) . *Annu. Rev. Ecol. Evol. Syst.*, 50, 335–354.

Rodd, F.H., Hughes, K.A., Grether, G.F. & Baril, C.T. (2002). A possible non-sexual origin of mate preference: are male guppies mimicking fruit? *Proc. R. Soc. B.* , 269, 475–481.

Rojas-Ferrer, I., Thompson, M.J. & Morand-Ferron, J. (2020). Is exploration a metric for information gathering? Attraction to novelty and plasticity in black-capped chickadees. *Ethology*, 126, 383–392.

Sandkam, B.A., Deere-Machemer, K.A., Johnson, A.M., Grether, G.F., Helen Rodd, F. & Fuller, R.C. (2016). Exploring visual plasticity: dietary carotenoids can change color vision in guppies (*Poecilia reticulata*). *J. Comp. Physiol. A Neuroethol. Sensory, Neural, Behav. Physiol.*, 202, 527–534.

Sanogo, Y.O. & Bell, A.M. (2016). Molecular mechanisms and the conflict between courtship and aggression in three-spined sticklebacks. *Mol. Ecol.*, 25, 4368–4376.

Sathyan, R. & Couldridge, V.C.K. (2013). Female preference for blue in Japan blue guppies (*Poecilia reticulata*). *Environ. Biol. Fishes*, 96, 953–959.

Schuett, W., Laaksonen, J. & Laaksonen, T. (2012). Prospecting at conspecific nests and exploration in a novel environment are associated with reproductive success in the jackdaw. *Behav. Ecol. Sociobiol.*, 66, 1341–1350.

Seghers, B.H. (1974). Geographic variation in the responses of guppies (*Poecilia reticulata*) to aerial predators. *Oecologia*, 98, 93–98.

Seghers, B.H. & Magurran, A.E. (1995). Population differences in the schooling behaviour of the Trinidad guppy, *Poecilia reticulata* : adaptation or constraint? *Can. J. Zool.*, 73, 1100–1105.

Seghers, B.H., Shaw, P.W. & Carvalho, G.R. (1995). The behavioral diversity and evolution of guppy, *Poecilia reticulata,* populations in Trinidad. *Adv. Study Behav.*, 24, 155–202.

Semper, C. (1881). *Animal life as affected by the natural conditions of existence. By Karl Semper ... With two maps and one hundred and six woodcuts.* D. Appleton and Co., New York,.

de Serrano, A.R., Weadick, C.J., Price, A.C. & Rodd, F.H. (2012). Seeing orange: prawns tap into a pre-existing sensory bias of the Trinidadian guppy. *Proc. R. Soc. B Biol. Sci.*, 279, 3321–3328.

Shettleworth, S.J. (1972). Constraints on learning. *Adv. Study Behav.*, 4, 1–68.

Sih, A., Bell, A. & Johnson, J.C. (2004). Behavioral syndromes : an ecological and evolutionary overview. *Trends Ecol. Evol.*, 19.

Sih, A., Kats, L.B. & Maurer, E.F. (2003). Behavioural correlations across situations and the evolution of antipredator behaviour in a sunfish-salamander system. *Anim. Behav.*, 65, 29–44.

Smith, B.R. & Blumstein, D.T. (2008). Fitness consequences of personality: A meta-analysis. *Behav. Ecol.*, 19, 448–455.

Smith, B.R. & Blumstein, D.T. (2010). Behavioral types as predictors of survival in Trinidadian guppies (*Poecilia reticulata*). *Behav. Ecol.*, 21, 919–926.

Stamps, J. & Groothuis, T.G.G. (2010). The development of animal personality: relevance, concepts and perspectives. *Biol. Rev.*, 85, 301–325.

Stephenson, J.F. (2019). Parasite-induced plasticity in host social behaviour depends on sex and susceptibility. *Biol. Lett.*, 15.

Svartberg, K., Tapper, I., Temrin, H., Radesäter, T. & Thorman, S. (2005). Consistency of personality traits in dogs. *Anim. Behav.*, 69, 283–291.

Tebbich, S., Fessl, B. & Blomqvist, D. (2009). Exploration and ecology in Darwin's finches. *Evol. Ecol.*, 23, 591–605.

Thompson, M.J., Evans, J.C., Parsons, S. & Morand-Ferron, J. (2018). Urbanization and individual differences in exploration and plasticity. *Behav. Ecol.*, 29, 1415–1425.

Torres-Dowdall, J., Handelsman, C.A., Reznick, D.N. & Ghalambor, C.K. (2012). Local adaptation and the evolution of phenotypic plasticity in Trinidadian guppies (*Poecilia*

Reticulata). *Evolution.*, 66, 3432–3443.

Trompf, L. & Brown, C. (2014). Personality affects learning and trade-offs between private and social information in guppies, *Poecilia reticulata*. *Anim. Behav.*, 88, 99–106.

Udino, E., Perez, M., Carere, C. & d'Ettorre, P. (2017). Active explorers show low learning performance in a social insect. *Curr. Zool.*, 63, 555–560.

Verbeek, M.E.M., Drent, P.J. & Wiepkema, P.R. (1994). Consistent individual differences in early exploratory behaviour of male great tits. *Anim. Behav.*, 48, 1113–1121.

West-Eberhard, M.J. (2003). *Developmental plasticity and evolution*. Oxford University Press, Oxford (UK).

White, E.M., Church, S.C., Willoughby, L.J., Hudson, S.J. & Partridge, J.C. (2005). Spectral irradiance and foraging efficiency in the guppy, *Poecilia reticulata*. *Anim. Behav.*, 69, 519–527.

Chapter 2

Colour biases in learned foraging preferences in Trinidadian guppies

M. Wyatt Toure, Simon M. Reader

In revision with *Ethology*

Abstract

Learning allows animals to adaptively adjust their behaviour in response to variable but predictable environments. Stable aspects of the environment may result in evolved or developmental biases in the systems impacting learning, allowing for improved learning performance according to local ecological conditions. Guppies (*Poecilia reticulata*), like many animals, show striking colour preferences in foraging and mating contexts, and guppy artificial selection experiments have found the form and progress of evolved responses to coloured stimuli differ depending on stimulus colour. Blue colouration is thought to typically be a relatively unimportant food cue in guppies. This raises the possibility that learned foraging associations with blue objects are formed less readily than with other colours. Here, guppies were rewarded for foraging at a green or blue object. Guppies readily foraged from these objects, but learning performance differed with rewarded object colour. With equal amounts of training, the preference for a green object became stronger than the preference for a blue object. These differences in performance were not attributable to differences in initial preferences or to foraging success during training. These findings suggest that associative pairings within a single sensory modality that do not have a historic relevancy can be more difficult for animals to learn even when there is no clear initial bias present.

Keywords: Learning, plasticity, constraint, colour learning, mate choice, fish

1. Introduction

Animals forage in variable environments and reliable cues of resource availability or quality can optimize resource acquisition. Many stimuli properties can provide reliable cues, and one property animals commonly use is colour. Colour is a virtually ubiquitous property of the environment and has become widely exploited as a cue by a large diversity of animals (Cuthill et al., 2017; Honkavaara, Koivula, Korpimäki, Siitari, & Viitala, 2002). Learning which colour cues predict valuable food sources can increase foraging efficiency and thus impact fitness. For example, sunbirds increase their efficiency at foraging on flowers by learning to use colour as a cue for nectar quality, leading to demonstrable energetic benefits via reduced traveling and handling times (Whitfield, Köhler, & Nicolson, 2014).

However, not all colours may be equally useful as foraging cues. Some colours may consistently predict valuable forage over multiple generations, in which case we would expect evolution to favour consistent preferences for these colours. Similarly, colours rarely predicting valuable forage would be expected to become less preferred. For example, there is evidence that the exploitation of coloured food items shaped the evolved visual sensitivities of primates (Surridge, Osorio, & Mundy, 2003). Developmental processes may also result in similar consistent preferences for or against certain colours. Notably, processes responsible for behavioural change within an individual's lifetime, such as learning, can themselves be biased by evolutionary and developmental processes—resulting in learning biases or predispositions (Dukas, 2004; Sherry, Jacobs, & Gaulin, 1992; Shettleworth, 1998). Noted potential illustrations of this phenomenon are the rapid acquisition of learned aversive responses to long-term threats (snake predators or brood parasites) after being exposed to conspecifics responding to these stimuli, while similar conspecific responses to control stimuli results in little learning (Cook & Mineka, 1990; Davies & Welbergen, 2009). In *Drosophila*, experimental manipulation of cue reliability over 40 generations resulted in evolved learning biases for colour versus odour depending on which cue was more reliable (Dunlap & Stephens, 2014). Thus, we might expect biases in learning to be widespread where cue reliability differs. Biases in cue preferences or learning may also develop or evolve due to their reliability in other contexts, such as a carryover between mating and foraging contexts, or have arisen for unrelated or non-adaptive reasons (*e.g.* Sandkam et al., 2016). A variety of processes, from peripheral to central, could underlie biases towards, or learning about. particular colours (Avarguès-Weber & Giurfa, 2014; Cole & Endler, 2015; Sandkam, Young, & Breden, 2015).

Colour is a particularly salient aspect of guppy ecology. It impacts survival, reproduction, and fitness: colour influences how likely a guppy is to be predated, what a guppy will eat, and who a guppy will mate with (Endler, 1980; Houde, 1997; Kodric-Brown, 1989). Putatively unlearned colour preferences in foraging and mating contexts are frequently observed (Rodd, Hughes, Grether, & Baril, 2002; Rowden, 1994; Sathyan & Couldridge, 2013; White, Church, Willoughby, Hudson, & Partridge, 2005), guppies outperform zebrafish in colour discrimination experiments when two domestic strains are compared (Gatto, Lucon-Xiccato, Bisazza, Manabe, & Dadda, 2020; Lucon-Xiccato, Manabe, & Bisazza, 2019), and artificial selection studies have found evolved responses to coloured stimuli differ depending on stimulus colour (Cole & Endler, 2015, 2018a). These two selection studies compared evolved responses to red and blue stimuli, one selecting on optomotor responses in fish from a wild population, the other on spot chasing behaviour in fish from a feral population. Evolved responses to both colours were observed, but in both studies responses to blue were weaker than those to red (Cole & Endler, 2015, 2018a). Given that blue foods are rare in the native Trinidadian ranges of guppies (Cole & Endler 2015), there may be a lack of a historic evolutionary association with blue food. In comparison, green food such as algae makes up a sizeable proportion of many guppy's diets (Dussault & Kramer, 1981). Indeed, preferences for foraging on green versus other colours (including blue) was robust across feral guppies raised for several generations under different light conditions (Cole, Lynn, Kranz, & Endler, 2019). Thus, learned associations may differ between blue and green food. These biases may reflect, and go on to shape, what guppies learn about their foraging and broader environment. For instance, males in some guppy populations have green and/or blue spots and females in some populations may prefer males with those colours (Endler and Houde 1995).

In this experiment we rewarded individual guppies for foraging from either a blue or green object for 20 trials. We found that object preferences shifted, but learning performance differed with rewarded object colour such that the shift in preference for the blue object was significantly weaker than that for the green object at the end of training.

2. Methods

2.1. Subjects

We used 16 wild-derived, laboratory-reared female guppies originating from the “Houde” Paria tributary in Trinidad that had been outbred in laboratory conditions in the Rodd Laboratory at the University of Toronto for several generations (at least 13 years) before being moved to similar rearing and housing conditions in our laboratory at least 6 months before the current study. During the experiment, we housed fish in pairs in 5-gallon home tanks (*l* 40 cm *w* 20 cm *h* 25 cm; water depth 20 cm) to avoid any isolation stress. Tanks were fitted with a heater and filter, with water maintained at 25±1ºC, with a gravel floor and terra cotta pots for shelter and a transparent plastic lid. Water parameters (pH, hardness, nitrites, nitrates, ammonia) were measured weekly to ensure optimum conditions. Every week 30% of the water was replaced with fresh conditioned water of the same temperature using a fine siphon. Guppies were kept under a 12:12 light-dark cycle, and prior to the experiment were fed commercial tropical fish flakes (TetraMin, Tetra, Germany) daily and re-hydrated, decapsulated brine shrimp eggs (Brine Shrimp Direct Inc., Utah, USA) three times per week. The repeated measures design required individuals to be identified so to avoid invasive marking procedures we placed females of discernibly different sizes and/or gravid spot colouration in each tank. At the end of the experiment fish were returned to breeding populations at McGill University.

2.2. Training and Testing

We trained female guppies individually in a 50.8 cm by 25.4 cm test tank with a water depth of 8 cm. The tank had white corrugated plastic walls and a laminated white paper under the base to increase contrast for positional data collection via computer vision. Guppies were primarily fed in the test tank for the duration of the experiment to encourage foraging motivation with additional flake food (TetraMin) given in the home tank every 3 days to ensure all fish received sufficient food. The objects were two similarly sized but differently shaped plastic Lego® blocks, one blue and one green (Supplementary Material figure S1), placed in the test tank 37 cm from one another, midway from front to back, and 6 cm from the short edge of the tank. Half the guppies were trained by rewarding the blue object and the other half by rewarding the green object to form two experimental treatments: green-rewarded and blue-rewarded. We chose blue and green objects to avoid colours previously found to be strongly preferred by guppies (Rodd *et al.* 2002).

The food reward was a plastic strip strip with a width of 1.5 cm and a height of 0.5 cm and a mixture of gelatin, flake food and decapsulated brine shrimp egg attached to it. Gelatine has been previously used in feeding experiments on guppies (Griffiths, 1996; Magurran & Seghers, 1991; Snijders et al., 2019) and other teleost fish (Rubio, Sánchez-Vázquez, & Madrid, 2003). This food reward was placed behind the object such that the food reward was not visible to the guppy until it swam behind the object. Our reasoning for this was that if food is readily visible from the start of the trial, then the possibility of associating an object with a food reward may be decreased since there is no need to make a decision based on the object's appearance if the food is immediately visible. Nothing was placed behind the unrewarded object during training. Fresh strips were used for each subject on each trial to avoid any residual odour cues. After a two-minute habituation period inside a 20 cm tall, open-ended glass holding cylinder with a 7 cm diameter, in the centre of the tank, guppies were given five minutes daily in the test tank to move about the tank and feed, before being returned to their housing tank. Once a guppy had been released from the holding cylinder the experimenter (MWT) left the room and thus was out of sight during experiments. On the first day, guppies were placed in the test tank with both objects but empty food strips to measure initial preferences at a baseline. Training then occurred once daily between 10h00 to 16h00 for 20 days. During training the location of the rewarding object (left or right) was randomized across days and individuals. That is, only the object's appearance provided a reliable cue to food location. On the 21st day an unreinforced 'probe' test was given where new duplicates of the objects were presented with empty plastic strips attached to them. This unreinforced trial was used to assess whether learning had occurred. The objects were presented with no food to ensure that guppies were not being guided by the smell of food, and new objects were used to avoid any residual odour cues from training. This unreinforced trial was used to assess whether learning had occurred. At test, the number of individuals were presented with the formerly rewarded object on the left and right side was equal. Individuals were trained and tested in the same order per day throughout the experiment.

2.3. Behavioural measures

Fish movement was recorded by a camcorder (Sony FDR-AX100 4K Ultra HD Camcorder) mounted above the tank. EthoVision XT motion tracking software (version 11.5, Noldus *et al.* 2001) was used to quantify object preference metrics and activity metrics from video footage. Our object preference metric was the time spent within 4 cm (about 1 to 2 body lengths) of one object subtracted from the time spent within 4 cm of the other object. Increased preference for an object was expected to be expressed behaviourally by an increased relative amount of time spent near that particular object over the other object. This metric was selected as guppies will often spend more time near areas where food is expected, *e.g.,* the top of the water column during feeding times.

2.4. Statistical analyses

Data were analysed using R statistical software (Version 3.6.2, R Core Team, 2019). All model residuals were checked they met model assumptions with the DHARMa R package (Hartig, 2018). Data and R code to reproduce this analysis are available at FigShare. We ran four statistical models.

Model 1: Preference for the green object before training

We first examined whether there was a difference in the initial preference for the green or blue object with an intercept-only linear model. Here we ask whether, over all guppies, there is a significant preference for the green object over the blue object during the initial trial before training began. This linear model has the response variable 'green object preference', the time spent near (within 4 cm) the green object subtracted from the time spent near the blue object. Here the green object preference is being tested against zero. In this initial trial fish had not yet been reinforced for visiting either object and no food was present. It is not likely that these laboratory reared fish would have previously associated green or blue objects with food, although, similar to other guppy colour studies (*e.g.,* Rodd et al., 2002), they were raised on multicoloured food flakes.

Model 2: Preference for the rewarding object during training

To examine responses during training we fit a linear mixed effects model to ask whether the preference for the rewarded object changed throughout training, and whether this differed between the treatments. The response variable here was 'rewarding object preference', the time spent near the rewarded object subtracted from the time spent near the unrewarded object. A positive value thus indicates more time spent near the rewarded object compared to the unrewarded object. The rewarding object colour depends upon the treatment (blue-rewarded or green-rewarded). Trial was coded as an integer in this model and we analysed data from the 20 training trials. A random effect of individual ID was used to account for repeated measures.

Model 3: Change in preference for the rewarded object after training

Here, we compared the preference at the initial test before training (trial 0) to the final test conducted after training (trial 21). We fit a generalized linear mixed effects model with a Gaussian distribution with fixed effects of trial (initial test versus. final test), rewarding object colour (green versus blue), the interaction between trial and rewarding object colour, a random effect of individual identity, and a response variable of rewarding object preference. We thus ask whether the preference for the rewarding object changed between the initial and final test and whether this differs with rewarded object colour.

Model 4: Is there a difference in feeding attempts during training between treatments?

During training we noticed some variation in how consistently subjects fed. Since differences in reinforcement between treatments may influence performance on the final preference test we compared the number of trials in which an individual fish ate throughout training and between treatments. To do this we fit a generalized linear model with a negative binomial distribution and a fixed effect of rewarding object colour. Here the response variable is 'feeding count' which is a sum of the number of trials in which a guppy ate throughout training. A guppy was considered to have fed if it pecked at the food on the food strip.

3. Results

3.1 Preference for the green object before training (Model 1)

Before training began, there was no significant difference in the time spent near the green versus the blue object across all guppies ($p = 0.193$). Similarly, there was no significant difference in object preference between fish destined to be rewarded for approaching the green object versus those destined to be rewarded for approaching the blue object ($p = 0.459$; Table 1).

3.2 Preference for the rewarding object during training (Model 2)

During training there was a significant effect of trial ($p < 0.001$). Over the 20 training trials, guppies in the two treatments increased their relative preference for their respective rewarded objects by 11 seconds on average each trial (Figure 1). There was also a significant effect of rewarded-object colour ($p = 0.013$): during training green-rewarded guppies expressed a stronger preference for their rewarded object (the green object) than did blue-rewarded guppies did for the blue object. However, there was no significant interaction effect between rewarding object colour and trial ($p = 0.348$), *i.e.*, the change in object preference over trials did not significantly differ between the treatments.

3.3 Change in preference for the rewarded object after training (Model 3)

When comparing the initial and final preference test, both conducted without food rewards present, we found a significant interaction effect between test and rewarding object colour ($p < 0.001$; Table S1). Guppies that had been green-rewarded had a shift in their rewarded object preference that was on average 84 seconds stronger than the shift in rewarded object preference of guppies trained to blue (Figure 2). These results were unaffected by the removal of one fish that did not feed during training.

Post-hoc comparisons (Table 1) reveal that initially, before training, there was no significant difference in the strength of preference for the rewarded object between the treatments (p

= 0.459). The shift in rewarded object preference between the initial and final preference tests was significant for green-trained guppies but not for blue-trained guppies: green trained guppies increased their preference for the green object by 104 seconds ($p < 0.001$) from initial to final test, whereas blue-trained guppies increased their preference for the blue object by 20 seconds, an effect that was not statistically significant ($p = 0.413$). At final test, green-rewarded guppies had a significantly stronger preference for the previously rewarded object compared to the blue-rewarded guppies ($p=0.002$).

3.4 Is there a difference in feeding attempts during training between treatments (Model 4)

We found no significant difference in the number of trials individuals fed between green-rewarded and blue-rewarded fish (Figure S1, $p = 0.873$). We also incorporated feeding count as a covariate in Model 3 and found the same pattern of results described in Model 3 (Supplementary Material tables S1 and S2).

4. Discussion

We found that guppies readily foraged from two differently coloured objects, blue and green, and detected no initial preference for one colour over the other. However, the strength of learning differed between blue and green objects, with clear evidence that training resulted in a learned preference for the green but not the blue object. We observed no evidence that these differences were due to differential foraging success during training. Although the objects also differed in shape, the shape difference was minor and guppies have been found to learn more readily about colour than shapes (Lucon-Xiccato, Manabe, & Bisazza, 2019). Thus, we conclude object colour impacted learning speed.

Given that fish were raised under laboratory conditions, our findings are most likely explained by a genetic predisposition impacting colour learning. We cannot rule out developmental influences of the standardized rearing environment shaping the learning predisposition we observed, but we note robust preferences to peck on green over blue objects were observed in a feral guppy population raised across three different light environments (Cole et al., 2019). We studied only one guppy population, fish originating from the Paria river, meaning that we cannot make inferences about guppies broadly. However, our data adds to previous findings in guppies, including work on the same origin population, which finds limits in evolved behavioural responses to blue when compared to ecologically important carotenoid colours (Cole & Endler, 2015, 2018b; Ehlman, Sandkam, Breden, & Sih, 2015; Rodd et al., 2002). Similar to our results, the African cichlid *Neolamprologus pulcher* showed superior learning performance when rewarded for foraging at yellow over blue objects, though this difference was not maintained on a reversal learning task (Culbert et al., 2020). Surprisingly, we found no evidence for a green over blue colour preference prior to training, only during and after training. Perhaps this was because the initial object exposure was perceived as being outside a foraging context, or preferences were only revealed during learning. Our results emphasize that colour biases may be only revealed under certain conditions, a finding with implications for studies of learning which use coloured stimuli across different contexts (Avarguès-Weber & Giurfa, 2014).

Our results suggest that a bias in the perceptual, cognitive, and/or motivational systems of the guppy may constrain acquisition of learned foraging preferences for blue objects. The difference in colour learning performance we observed could reflect an adaptation to foraging conditions. Green algae are an important food source which can enhance sexual ornamentation and growth for guppies (Bassar et al., 2012; Dussault & Kramer, 1981; Grether et al. 1999; Karino & Haijima, 2004) thereby influencing survival and reproduction in both males (enhanced sexual ornamentation) and females (enhanced growth and fecundity). Being able to quickly detect and consume such food may be particularly advantageous for guppies in low predation-regime streams, such as the Paria population our subjects originated from, where population densities, and thus competition, tend to be high (Reznick, Butler, & Rodd, 2001). Our study does not allow us to conclude whether the learning bias we observe is adaptive without testing additional guppy populations (or closely related Poeciliid species) in which the colours associated with foraging rewards are different. Such an approach has been taken with bumblebees (Raine, Ings, Dornhaus, Saleh, & Chittka, 2006). Quantifying the relative abundances of food colours in the environment or, of foods consumed by guppies, and examining links to colour learning would be a useful extension to our work. Moreover, an experimental evolution approach would help determine whether historic associations with stimuli within a single sensory modality can shape learning predispositions (Liefting, Hoedjes, Le Lann, Smid, & Ellers, 2018). Often, learning biases are thought to be advantageous because they protect individuals from learning associations unlikely to be productive. However, we should also be open to non-adaptive explanations for our findings, and for the possibility that the biases we observe are ancestral to guppies. For example, since blue foods are relatively rare in nature (Newsome et al. 2014), and foods such as green algae are widely beneficial to many fish taxa, a bias of green over blue may have been inherited from a guppy ancestor rather than actively being selected for in guppies specifically. If this were the case, we should expect broad-scale biases for learning about green over blue across Poeciliids or even deeper in their phylogenetic history.

The mechanisms underlying the observed bias in colour learning and impacts of this bias on other behaviour patterns are interesting avenues for further work. Guppy's colour preferences during foraging may have impacts on other contexts, such as mate choice. The sensory drive hypothesis proposes that males have evolved phenotypic features which match the detectability biases of females (Endler, 1992; Ryan, 1990). The orange colouration of male guppies is thought to be one such trait: population differences in preference for orange foraging items correlates with female preferences for male orange colouration, and it has been suggested that male guppies are benefiting from a pre-existing foraging preference for orange in females (Rodd et al., 2002). Predators may also capitalize on this colour preference (de Serrano, Weadick, Price, & Rodd, 2012). Moreover, artificial selection on guppies for a red foraging preference leads to the subsequent evolution of red spectra colouration in males (Cole & Endler, 2018a). If the learned foraging colour preference we observe in this experiment can similarly shape mate choice this raises the possibility of plasticity-led evolution of male colouration. A learned colour preference could potentially carry over to influence mate colour preferences which in turn causes genetically-based changes in male colouration, just as experimentally evolved colour preferences have been demonstrated to do (Cole & Endler, 2018a). The active role of learning in shaping evolution has received research attention in recent times (Lachlan & Servedio, 2004; Laland et al., 2015). Natural variation in male colouration is considerable (Endler & Houde, 1995). If the rewards for foraging on particular colours change repeatedly and differ between locations, this may promote diversity in female colour preferences and thus colour diversity in males within and amongst populations. Over evolutionary time such processes can contribute to speciation (Jablonka, Lamb, & Zeligowski, 2014). Interestingly, the males of the Paria population of guppies we used in this experiment have much less blue colouration compared to fish from another nearby drainage, the Marianne (Gotanda & Hendry, 2014). There also exists a peculiar Japanese feral population of guppies in which the males are almost entirely blue and the females have a documented preference for blue males (Sathyan & Couldridge, 2013). This variation in colouration and colour preference raises the possibility for investigating carryovers between learning about mate and food colours. Overall, we find biases in colour learning performance that may be linked to ecological foraging conditions and are likely to allow guppies to readily learn preferentially about food sources that have historically been rewarding.

Ethics statement

Methods were approved by the Animal Care Committee of McGill University (Protocol 2015-7708) and were conducted in accordance with the ethical guidelines of the Canadian Council on Animal Care and ABS/ASAB.

Data accessibility

Data and R scripts to produce all analyses, figures, and residual diagnostic plots are available at https://github.com/wyatt-toure/guppy-colour-learning and will be permanently archived on FigShare or a similar repository upon publication.

Authors' contributions

MWT and SMR wrote the paper and conceived and designed the experiment. MWT collected and analysed the data with practical support, input and supervision from SMR.

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References

Avarguès-Weber, A., & Giurfa, M. (2014). Cognitive components of color vision in honey bees: How conditioning variables modulate color learning and discrimination. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology*, *200*, 449–461. doi: 10.1007/s00359-014-0909-z

Bassar, R. D., Ferriere, R., López-Sepulcre, A., Marshall, M. C., Travis, J., Pringle, C. M., & Reznick, D. N. (2012). Direct and indirect ecosystem effects of evolutionary adaptation in the Trinidadian guppy (*Poecilia reticulata*). *American Naturalist*, *180*, 167–185. doi: 10.1086/666611

Cole, G. L., & Endler, J. A. (2015). Artificial selection for food colour preferences. *Proceedings of the Royal Society B: Biological Sciences*, *282*, 20143108. doi: 10.1098/rspb.2014.3108

Cole, G. L., & Endler, J. A. (2018a). Change in male coloration associated with artificial selection on foraging colour preference. *Journal of Evolutionary Biology*, *31*, 1227–1238. doi: 10.1111/jeb.13300

Cole, G. L., & Endler, J. A. (2018b). Courtship diverges with foraging behaviour in artificially selected populations. *Animal Behaviour*, *144*, 9–15. doi: 10.1016/j.anbehav.2018.07.016

Cole, G. L., Lynn, J. C. B., Kranz, A. M., & Endler, J. A. (2019). Colour-based foraging diverges after multiple generations under different light environments. *Ethology*, *125*, 212–221. doi: 10.1111/eth.12847

Cook, M., & Mineka, S. (1990). Selective associations in the observational conditioning of fear in rhesus monkeys. *Journal of Experimental Psychology: Animal Behavior Processes*, *16*, 372–389. doi: 10.1037//0097-7403.16.4.372

Culbert, B. M., Talagala, S., Barnett, J. B., Stanbrook, E., Smale, P., & Balshine, S. (2020). Context-dependent consequences of color biases in a social fish. *Behavioral Ecology*, *31*, 1410–1419. doi: 10.1093/beheco/araa099

Cuthill, I. C., Allen, W. L., Arbuckle, K., Caspers, B., Chaplin, G., Hauber, M. E., … Caro, T. (2017). The biology of color. *Science*, *357*, eaan0221. doi: 10.1126/science.aan0221

Davies, N. B., & Welbergen, J. A. (2009). Social transmission of a host defense against cuckoo parasitism. *Science*, *324*, 1318–1320. doi: 10.1126/science.1172227 de Serrano, A. R., Weadick, C. J., Price, A. C., & Rodd, F. H. (2012). Seeing orange: Prawns tap into a pre-existing sensory bias of the Trinidadian guppy. *Proceedings of the Royal Society B: Biological Sciences*, *279*, 3321–3328. doi: 10.1098/rspb.2012.0633

Dukas, R. (2004). Evolutionary biology of animal cognition. *Annual Review of Ecology, Evolution, and Systematics*, *35*, 347–374. doi: 10.1146/annurev.ecolsys.35.112202.130152

Dunlap, A. S., & Stephens, D. W. (2014). Experimental evolution of prepared learning. *Proceedings of the National Academy of Sciences*, *111*, 11750–11755. doi: 10.1073/pnas.1404176111

Dussault, G. V., & Kramer, D. L. (1981). Food and feeding behavior of the guppy, *Poecilia reticulata* (Pisces: *Poeciliidae*). *Canadian Journal of Zoology*, *59*, 684–701. doi: 10.1139/z81-098

Ehlman, S. M., Sandkam, B. A., Breden, F., & Sih, A. (2015). Developmental plasticity in vision and behavior may help guppies overcome increased turbidity. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology*, *201*, 1125–1135. doi: 10.1007/s00359-015-1041-4

Endler, J. A. (1980). Natural selection on color patterns in *Poecilia reticulata*. *Evolution*, *34*, 76. doi: 10.2307/2408316

Endler, J. A. (1992). Signals, signal conditions, and the direction of evolution. *The American Naturalist*, *139*, S125–S153. doi: 10.1086/285308

Endler, J. A., & Houde, A. E. (1995). Geographic variaiton in female preferences for male traits in *Poecilia reticulata*. *Evolution*, *49*, 456–468. doi: 10.1111/j.1558-5646.1995.tb02278.x

Gatto, E., Lucon-Xiccato, T., Bisazza, A., Manabe, K., & Dadda, M. (2020). The devil is in the detail: zebrafish learn to discriminate visual stimuli only if salient. *Behavioural Processes*, *179*, 104215. doi: 10.1016/j.beproc.2020.104215

Gotanda, K. M., & Hendry, A. P. (2014). Using adaptive traits to consider potential consequences of temporal variation in selection: male guppy colour through time and space. *Biological Journal of the Linnean Society*, *112*, 108–122. doi: 10.1111/bij.12261

Grether, G. F., Hudon, J., & Millie, D. F. (1999). Carotenoid limitation of sexual coloration along an environmental gradient in guppies. Proceedings of the Royal Society of London. Series B: Biological Sciences, 266, 1317–1322.

https://doi.org/10.1098/rspb.1999.0781

Griffiths, S. W. (1996). Sex differences in the trade-off between feeding and mating in the guppy. *Journal of Fish Biology*, *48*(5), 891–898. https://doi.org/10.1111/j.1095-8649.1996.tb01484.x

Hartig, F. (2018). *DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models. R package v. 0.2.0*.

Honkavaara, J., Koivula, M., Korpimäki, E., Siitari, H., & Viitala, J. (2002). Ultraviolet vision and foraging in terrestrial vertebrates. *Oikos*, *98*, 505–511. doi: 10.1034/j.1600-0706.2002.980315.x

Houde, A. E. (1997). *Sex, color, and mate choice in guppies*. Princeton, NJ: Princeton University Press. doi: 10.1046/j.1439-0310.2000.00565.x

Jablonka, E., Lamb, M. J., & Zeligowski, A. (2014). *Evolution in four dimensions: Genetic, epigenetic, behavioral, and symbolic variation in the history of life* (Revised edition, pp. 153–188). Cambridge, Massachusetts ; London, England: A Bradford Book, The MIT Press.

Karino, K., & Haijima, Y. (2004). Algal-diet enhances sexual ornament, growth and reproduction in the guppy. *Behaviour*, *141*, 585–601. doi: 10.1163/1568539041166672

Kodric-Brown, A. (1989). Dietary carotenoids and male mating success in the guppy: an environmental component to female choice. *Behavioral Ecology and Sociobiology*, *25*, 393–401. doi: 10.1007/BF00300185

Lachlan, R. F., & Servedio, M. R. (2004). Song learning accelerates allopatric speciation. *Evolution*, *58*, 2049–2063. doi: 10.1111/j.0014-3820.2004.tb00489.x

Laland, K. N., Uller, T., Feldman, M. W., Sterelny, K., Müller, G. B., Moczek, A., … Odling-Smee, J. (2015). The extended evolutionary synthesis: its structure, assumptions and predictions. *Proceedings of the Royal Society B: Biological Sciences*, *282*, 20151019. doi: 10.1098/rspb.2015.1019

Liefting, M., Hoedjes, K. M., Le Lann, C., Smid, H. M., & Ellers, J. (2018). Selection for associative learning of color stimuli reveals correlated evolution of this learning ability across multiple stimuli and rewards. *Evolution*, *72*, 1449–1459. doi: 10.1111/evo.13498

Lucon-Xiccato, T., Manabe, K., & Bisazza, A. (2019). Guppies learn faster to discriminate between red and yellow than between two shapes. *Ethology*, *125*, 82–91. doi: 10.1111/eth.12829

Magurran, A. E., & Seghers, B. H. (1991). Variation in schooling and aggression amongst guppy (*Poecilia reticulata*) populations in Trinidad. *Behaviour*, *118*, 214–234. https://doi.org/10.1163/156853991X00292

Newsome, A. G., Culver, C. A., & van Breemen, R. B. (2014). Nature's palette: The search for natural blue colorants. *Journal of Agricultural and Food Chemistry*, *62*, 6498–6511. https://doi.org/10.1021/jf501419q

Noldus, L. P. J. J., Spink, A. J., & Tegelenbosch, R. A. J. (2001). EthoVision: A versatile video tracking system for automation of behavioral experiments. *Behavior Research Methods, Instruments, and Computers*, *33*, 398–414. doi: 10.3758/BF03195394

R Core Team. (2019). *R: A language and environment for statistical computing.* R Foundation for statiscal computing.

Raine, N. E., Ings, T. C., Dornhaus, A., Saleh, N., & Chittka, L. (2006). Adaptation, genetic drift, pleiotropy, and history in the evolution of bee foraging behavior. *Advances in the Study of Behavior*, *36*, 305–354. doi: 10.1016/S0065-3454(06)36007-X

Reznick D. N., Butler M. J., & Rodd F. H., (2001). Life-history evolution in guppies. VII. The comparative ecology of high- and low-predation environments. *The American Naturalist*, *157*, 126. doi: 10.2307/3079254

Rodd, F. H., Hughes, K. A., Grether, G. F., & Baril, C. T. (2002). A possible non-sexual origin of mate preference: are male guppies mimicking fruit? *Proceedings of the Royal Society B: Biological Sciences*, *269*, 475–481. doi: 10.1098/rspb.2001.1891

Rowden, J. (1994). The effects of visual experience on female choice in guppies (*Poecilia reticulata*). *Journal of the Elisha Mitchell Scientific Society*, *110*, 17–22.

Ryan, M. J. (1990). Sexual selection, sensory systems and sensory exploitation. *Oxford Surveys in Evolutionary Biology*, *7*, 157–195.

Rubio, V. C., Sánchez-Vázquez, F. J., & Madrid, J. A. (2003). Macronutrient selection through postingestive signals in sea bass fed on gelatine capsules. *Physiology & Behavior*, *78*, 795–803. https://doi.org/10.1016/S0031-9384(03)00082-9

Sandkam, B. A., Deere-Machemer, K. A., Johnson, A. M., Grether, G. F., Helen Rodd, F., & Fuller, R. C. (2016). Exploring visual plasticity: dietary carotenoids can change color vision in guppies (*Poecilia reticulata*). *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology*, *202*, 527–534. doi: 10.1007/s00359-016-1097-9

Sandkam, B. A., Young, C. M., & Breden, F. (2015). Beauty in the eyes of the beholders: Colour vision is tuned to mate preference in the Trinidadian guppy (*Poecilia reticulata*). *Molecular Ecology*, *24*, 596–609. doi: 10.1111/mec.13058

Sathyan, R., & Couldridge, V. C. K. (2013). Female preference for blue in Japan blue guppies (*Poecilia reticulata*). *Environmental Biology of Fishes*, *96*, 953–959. doi: 10.1007/s10641-012-0091-5

Sherry, D. F., Jacobs, L. F., & Gaulin, S. J. C. (1992). Spatial memory and adaptive specialization of the hippocampus. *Trends in Neurosciences*, *15*, 298–303. doi: 10.1016/0166-2236(92)90080-R

Shettleworth, S. J. (1998). Cognition, evolution, and behavior (2nd ed.). New York, NY, US: Oxford University Press.

Snijders, L., Kurvers, R. H. J. M., Krause, S., Tump, A. N., Ramnarine, I. W., & Krause, J. (2019). Females facilitate male food patch discovery in a wild fish population. *Journal of Animal Ecology*, *88*, 1950–1960. https://doi.org/10.1111/1365-2656.13086

Surridge, A. K., Osorio, D., & Mundy, N. I. (2003). Evolution and selection of trichromatic vision in primates. *Trends in Ecology and Evolution*, *18*, 198–205. doi: 10.1016/S0169-5347(03)00012-0

White, E. M., Church, S. C., Willoughby, L. J., Hudson, S. J., & Partridge, J. C. (2005). Spectral irradiance and foraging efficiency in the guppy, *Poecilia reticulata*. *Animal Behaviour*, *69*, 519–527. doi: 10.1016/j.anbehav.2004.05.011

Whitfield, M., Köhler, A., & Nicolson, S. W. (2014). Sunbirds increase foraging success by using color as a cue for nectar quality. *Behavioral Ecology*, *25*, 328–334. doi: 10.1093/beheco/art115

Table 1: Table of post-hoc tests with a multivariate-t adjustment for multiple comparisons of a selected set of means. ‘Initial’ represents the initial test before training and ‘Final’ the final test after training. The colour corresponds to the identity of the object rewarded during training (blue for blue-rewarded guppies, green for green-rewarded guppies). Values are all rounded to 3 decimal places. Significant p-values are bolded. CL = confidence limit.

Contrast	**Estimate**	**Lower CL**	**Upper CL**	**df**	**P Value**
Final blue - Initial blue	19.586	-16.091	55.262	18	0.413
Final green - Initial green	103.926	54.892	152.961	18	< .001
Final green - Final blue	92.766	34.336	151.195	18	0.002
Initial green - Initial blue	8.425	-7.8	24.649	18	0.459

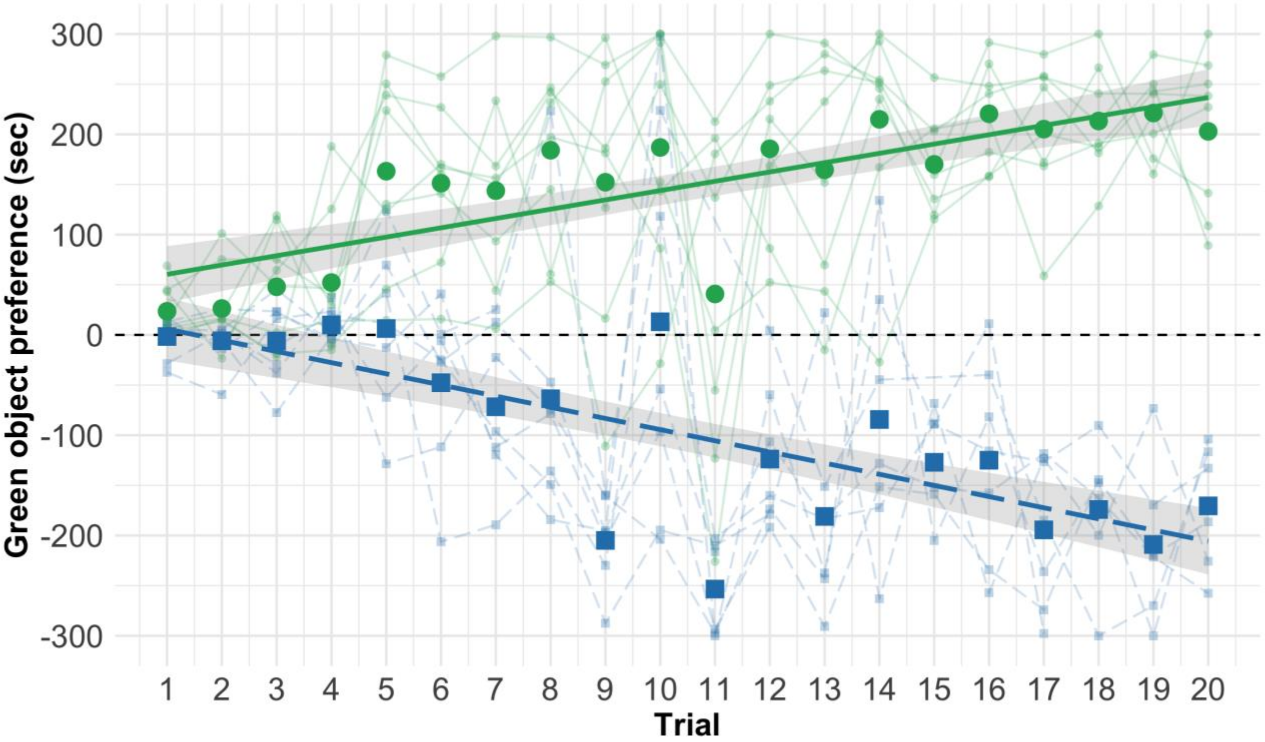

Figure 1: Relative preference for the green object in both treatments during training trials (trials 1-20). Negative values represent more time spent with the blue object; positive values indicate more time spent with the green object. Light lines connect individuals across trials and bold lines represents a linear fit with 95% CI (grey shading). Subjects were consistently rewarded for approaching the blue object (blue squares and dashed blue lines) or the green object (green circles and solid green lines).

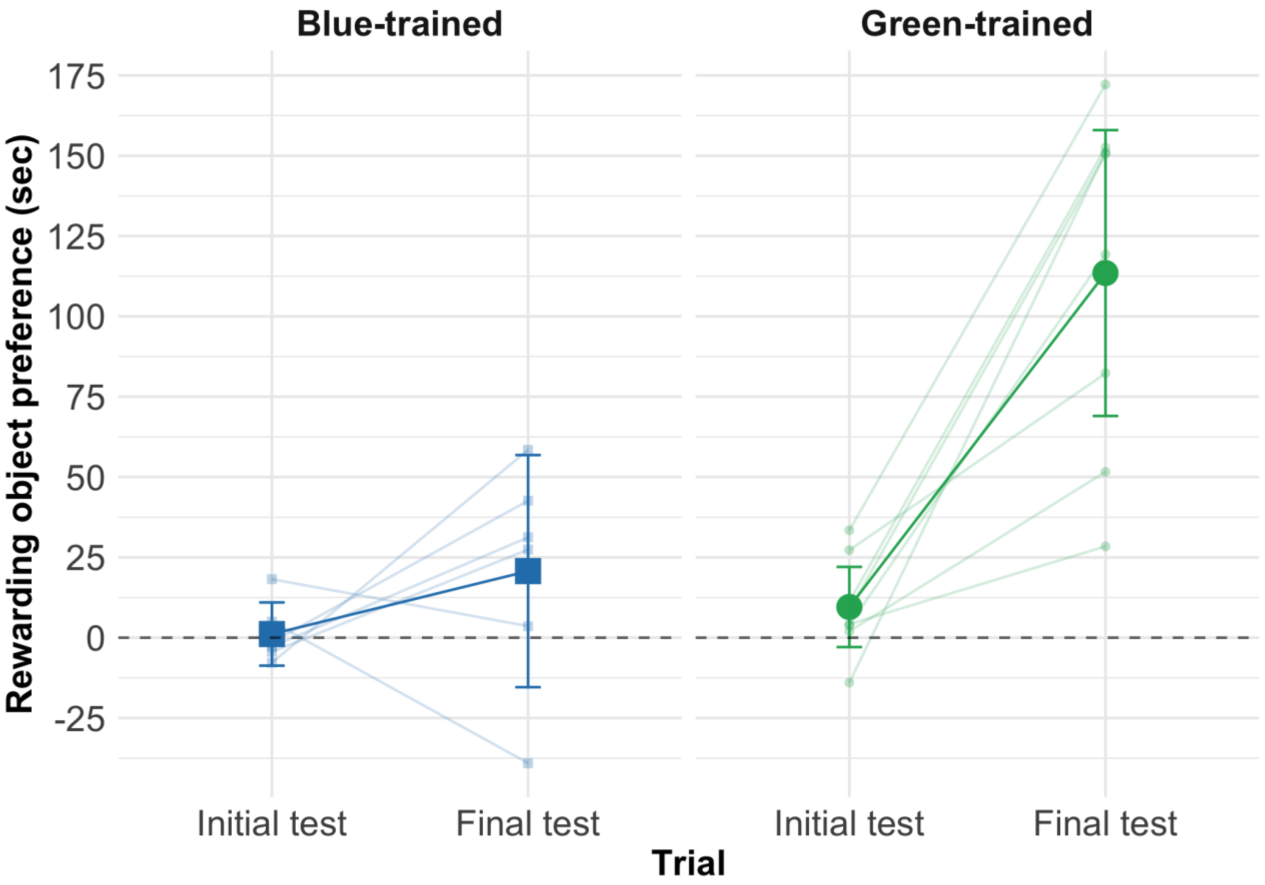

Figure 2: Changes in object preference from an initial test before training to a final test after training. During training, fish were rewarded for approaching the blue object (blue squares and lines) or the green object (green squares and lines). At test, no food reward was present. The dashed line represents an equal preference for either object. Data are means ± 95% CI; lighter points and lines are data for each individual.

Supplementary material for 'Colour biases in learned foraging preferences in Trinidadian guppies'

Supplementary Figures

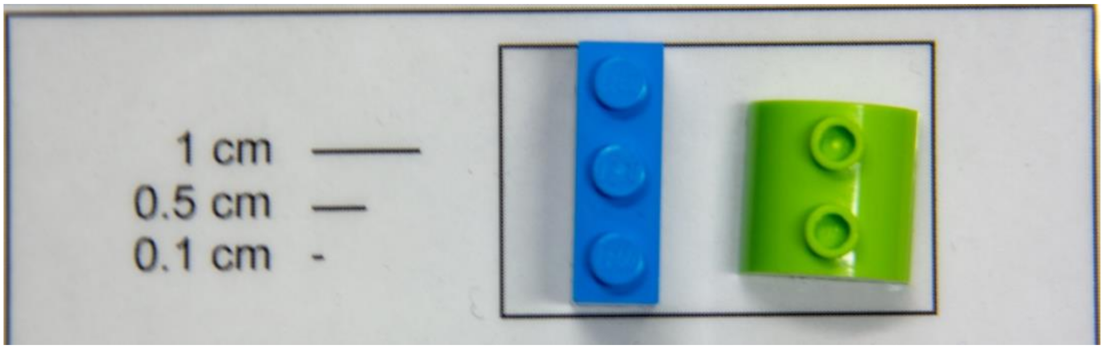

Figure S2: The two objects used in the experiment. Blue-trained guppies were trained to the left object and green-trained guppies were trained to the right object. The manufacturer's colour name for the blue object is 'dark azur' (hex #078BC9) and for the green object is 'bright yellowish green' (hex # BBE90B).

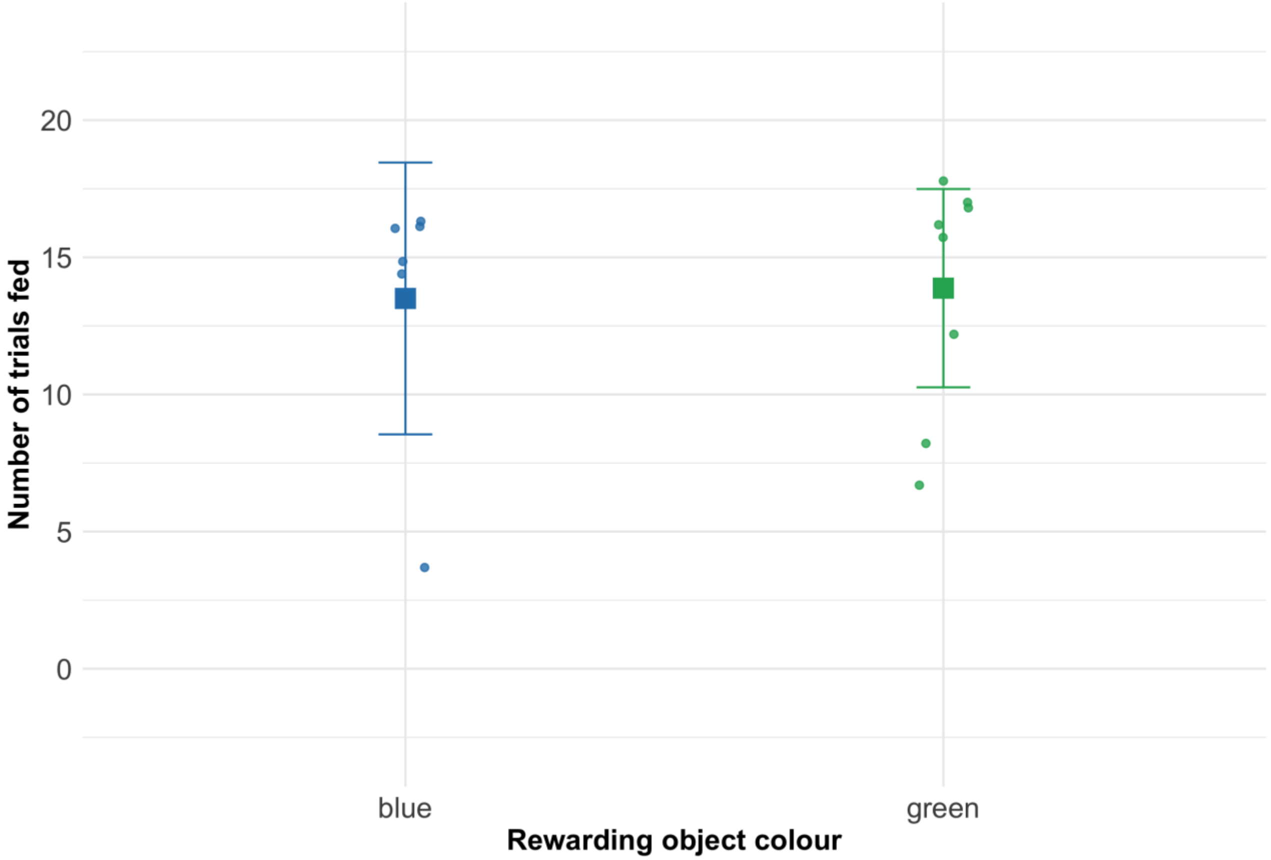

Figure S3: Average number of trials in which a fish fed during training. Data are means ± 95% confidence intervals.

Supplementary Tables

Table S1: Summary table for model 3 in the main text. Estimates ± standard error (SE) of the effects of trial and rewarding object colour on the rewarding object preference from the generalized linear mixed effect model containing the effects Trial, Rewarding object colour, and their interaction effect (Trial X Rewarding object colour). Significant p values are bolded.

Effect	Estimate	SE	T statistic	P value
Trial	19.586	13.295	1.473	0.141
Rewarding object colour	8.425	6.046	1.393	0.163
Rewarding object colour X Trial	84.341	22.598	3.732	< .001

Table S2: Summary table for a modification of model 3 in the main text. This model is the same as that described in Table S1 except it includes feeding count as a covariate. Estimates ± standard error (SE) of the effects of trial and rewarding object colour of the rewarding object colour from the generalized linear mixed effect model containing the effects (Trial, Rewarding object colour, and their interaction effect, as well as feeding count). Significant p values are bolded.

Effect	Estimate	SE	t	P value
Trial	19.583	13.428	1.458	0.145
Rewarding object colour	8.463	6.017	1.407	0.16
Feeding count	-0.102	0.684	-0.149	0.881
Rewarding object colour X Trial	84.346	22.04	3.827	< .001

Linking statement to chapter 3

In **Chapter 2** I established that, while there are some notable constraints based on colour, preferences for specific objects can be shifted via food reinforcement confirming that an instrumental conditioning paradigm can work well within guppies. Having confirmed that guppies can recognize and respond behaviourally to novelty (in an experiment not described in this thesis) and that manipulations of reward can shift preferences for specific objects I went on to ask in **Chapter 3** whether a similar manipulation could shift preferences for *novel* objects. Here rather than the stimulus property of interest being colour, it is novelty. Using the same training paradigm and fish population I investigated whether exploratory behaviour in one context is malleable and whether this carries over to other novelty contexts given the observation that exploratory behaviours tend to be correlated across several novelty contexts.

Chapter 3
Experimental manipulation of novelty rewards reveals associative learning can underpin variation in exploratory behaviour

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Abstract

Exploratory behaviour describes how individuals react to novel situations and appropriate novelty responses have important fitness consequences: novelty can be rewarding but also risky. Exploratory behaviour is often studied under an animal personality framework with a common observation that individuals consistently differ in their exploratory propensities. However, the precise mechanisms which can produce individual differences in exploratory behaviour remain unclear. By manipulating the value of engaging with novelty in Trinidadian guppies we show that the propensity to engage with novel over familiar objects can be shaped by experience. We found that while object exploration was increased by pairing novelty with reward, this did not carryover to spatial exploration. Furthermore, we find that consistency in the propensity to explore novel objects was not present initially but was induced as a consequence of experience. Our results demonstrate that personality traits can exhibit notable plasticity independent of other novelty response behaviours, in contrast to hypotheses of constraints imposed by behavioural syndromes. Moreover, by directly manipulating the trait of interest via instrumental conditioning we reveal that simple associative learning mechanisms can be sufficient to induce variation in personality. Given the virtually ubiquitous distribution of associative learning across animals, these findings suggest that learning likely plays an underappreciated role as a key mechanism in the development of personality differences across the animal kingdom.

Keywords: Learning, Fish, Animal Personality, Exploration, Novelty, Carryover, *Poecilia reticulata*

1. Introduction

Novelty permeates essentially all aspects of animal life. Individuals regularly encounter novel foods, novel mates, novel odours, and/or novel locations. Exploratory behaviour refers to an animal's behavioural response to novelty (Verbeek *et al.* 1994; Dingemanse *et al.* 2002) and how willing individuals are to engage in exploratory behaviour and investigate novel stimuli—their exploratory propensity—has demonstrable fitness consequences (Bergeron *et al.* 2013; Nicolaus *et al.* 2016). The observation that individuals consistently differ in their exploratory behaviour and that exploratory behaviours tend to correlate across contexts has led exploratory behaviour to often be studied within the context of animal personality and behavioural syndromes, where investigators seek to understand the causes and consequences of individual variation and behavioural trait correlations (Sih *et al.* 2004; Réale *et al.* 2007; Dochtermann & Dingemanse 2013).

Exploratory behaviour allows animals to potentially learn about and discriminate between beneficial and detrimental novelty in the environment. Indeed, information gleaned from exploration has been shown to allow learning of escape routes for fleeing when under threat (Renner 1988) or landscape features for efficient travel to a hive (Degen *et al.* 2016). However, there can be considerable costs to exploration. Novel foods may be unpalatable or toxic. Individuals can be exposed to predation while investigating novelty and if entering a novel area, then there may be limited knowledge of escape routes (Brown 2001). Additionally, exploring novel stimuli in an environment can expose an organism to additional pathogens which may be evolutionarily and/or contemporarily unfamiliar and thus more difficult to mount an immune response towards (Boyer *et al.* 2010; McCabe *et al.* 2015). Moreover, exploration is not guaranteed to lead to a resource to exploit or meaningful information gain, leading to an opportunity cost. Given these potential outcomes, whether to explore or exploit may reflect individual experience and/or the current or past ecological environment (Reader 2015). Therefore, the value of exploratory behaviour will often vary with environmental conditions.

When attempting to establish what environmental conditions promote or reduce the propensity for exploration, individuals from different environments are often assayed. An example would be assaying the response to novelty in urban and non-urban conspecifics (Miranda *et al.* 2013; Thompson *et al.* 2018; Breck *et al.* 2019; Grunst *et al.* 2019). Typically, urban individuals are found to be more exploratory, but there are conflicting results (Griffin *et al.* 2017). A limitation of such studies is that it remains unclear what specific stimuli or environmental processes animals are responding to. Understanding these processes may help resolve while different studies find contradictory results. One possibility is that environments differ in the costs and benefits of exploration and thus responses to local conditions are learned and differ based on the reward contingencies relating to novelty in that particular environment (Morand-Ferron & Giraldeau 2010), however the costs and benefits of exploration are rarely explicitly known. A known understanding of the relationship between novelty and reward is necessary to understand what specific processes produce variation in personality can help illuminate what ecological factors organisms are responding to.

Associative learning as a result of direct experiences in the environment may help explain variation in exploratory behaviour. Associative learning describes the process whereby animals develop an association between a stimulus or behaviour and another stimulus or a particular consequence (Thorndike 1898; Pavlov 1927). If personal experience in the environment plays a large role in shaping individual exploration, then this may explain a portion of the non-genetic variation observed in the exploratory tendencies of individuals across different environmental conditions (Dingemanse *et al.* 2007; Jacquin *et al.* 2017; Moran *et al.* 2017; Johnson *et al.* 2020).

Populations of Trinidadian guppies, *Poecilia reticulata*, inhabit environments that differ in a suite of environmental traits, potentially leading to different consequences of engaging with novelty. Indeed, novelty plays a demonstrably important role in guppy ecology. Accurately assessing how to respond to novel heterospecifics can have considerable fitness consequences in the context of novel predator recognition (Magurran & Seghers 1990; Brown *et al.* 2013; Crane & Ferrari 2017). In the wild guppies feed on several food types in varied locations with potential food items falling from the canopy (Dussault & Kramer 1981; Rodd *et al*. 2002). How exploratory a guppy is can therefore have consequences for how much of the food in an environment it can exploit. A novel object may be a possible food item and a novel area could harbour increased foraging opportunities. Quicker engagement with these forms of novelty could then lead to better exploitation of novel resources. This may be particularly important in low predation sites where canopy cover is increased and population densities tend to be higher, leading to lower food availability (Reznick *et al.* 2001). Foraging success is crucial for female guppies since food acquisition is directly related to fecundity (Hester 1964; Reznick 1983). Exploration of novelty can also be of direct reproductive benefit to males. Novel males are preferred by female guppies across several distinct populations (Dargent *et al.* 2018; Valvo *et al.* 2019) and males with locally rare phenotypes have higher mating success (Hughes *et al.* 2013; Graber *et al.* 2015). Males that travel to novel areas more often could therefore benefit from increased mating success which may serve as a positive reinforcer for increased novel area use. However, there are also potential costs of exploration. An exploring guppy may increase its predation risk (Brown 2001) which is a particularly unforgiving environmental pressure in streams located below waterfalls in Trinidad which tend to contain voracious guppy predators (Millar *et al.* 2006).

If experience in the local environment plays a large role in how animals respond to novelty, then we might expect that animals from populations that differ environmentally would have different responses to novelty but that these differences would diminish in a common garden. In guppies, across four matched pairs of low- and high-predation population sites Burns *et al.* 2016 found that high-predation guppies were less exploratory, as measured by an open-field test, than low-predation guppies. However, these differences do not persist after rearing guppies in a common garden, suggesting environmental sensitivity of exploratory behaviour in guppies. Additionally, correlations have been observed between novel area exploration and novel object preference (De Serrano *et al.* 2016), novel area exploration and novel mate preference (Lucon-Xiccato *et al.* 2019), and in a large correlative study, correlations were observed across eight different novelty contexts comprising of novel patterns, males, objects, environments, fruits, insects, females, and juveniles (Daniel *et al.* 2020). Birds (Schuett *et al.* 2012) and mammals (Dulawa *et al.* 1999) have demonstrated correlated responses to novel stimuli suggesting correlated novelty responses may reflect a phylogenetically conserved underlying mechanism across vertebrates (Gosling & John 1999). It has been hypothesized that such cross-contextual correlations, termed behavioural syndromes, are the result of overlapping proximate mechanisms that create the potential for shifts in one behavioural trait to give rise to changes in correlated behavioural traits (Lande & Arnold 1983). If overlapping mechanisms produce the correlation observed between behaviours across novelty contexts, then one might expect the response to experience in one behaviour to lead to changes in mechanistically linked behaviours. This may prove detrimental to fitness if the costs and benefits of multiple novelty contexts differ but the underlying mechanisms force changes in one context to carry over to another context (Hendry 2016).

Here we tested two hypotheses. First, that experiences with novelty in the environment can shape future behavioural responses to novelty. If this first hypothesis is correct, then we would expect positive experiences with novel objects will lead to increased exploratory behaviour towards other novel objects. Second, that responses to novel area contexts will generalize from experiences with object novelty. If this second hypothesis is correct, then positive experiences with novel objects should lead to a cross contextual increase in spatial exploration. To test our hypotheses, we first conducted initial assays of exploration propensities across two contexts, spatial and object novelty. Then through reinforcement training, we rewarded guppies for approaching either novel or familiar objects. Finally, we re-tested their exploratory propensities to assess whether positive reinforcement for approaching novel objects led to changes in object exploration and concordant changes in novel area exploration.

2. Methods

Individual guppies were given two consecutive open field tests to facilitate habituation to a novel test tank environment. Individuals were then given alternating initial object and spatial exploration assays (three object assays and two spatial assays) to provide initial measurements of exploratory behaviour. Individual guppies were then rewarded over 20 training trials for foraging from either novel or familiar objects, forming the ‘novelty-rewarded’ and ‘familiar-rewarded’ treatments. After training spatial and object exploration propensities were re-assessed in the same manner as before training. Figure 1 provides a timeline of the experiment and Supplementary Video 1 a video demonstration of the behavioural assays.

2.1. Subjects

We used 45 wild-derived, originating from the "Houde" Paria tributary in Trinidad that had been outbred in laboratory conditions in the Rodd Laboratory at the University of Toronto for several generations (at least 13 years) before being moved to similar rearing and housing conditions in our laboratory at least 6 months before the current study. These fish had not been used in prior experiments and were naïve to the objects we used in the experiment. During the experiment, we housed fish in pairs in 19L (5-gallon) home tanks (*l* 40 cm *w* 20 cm *h* 25 cm; water depth 20 cm) to avoid any isolation stress. Tanks were fitted with a heater and filter, with water maintained at 25±1ºC, with a gravel floor and plants for shelter and a transparent plastic lid (i.e., the same as our laboratory housing conditions). Water parameters (pH, hardness, nitrites, nitrates, ammonia) were measured weekly to ensure optimum conditions. Every week 30% of the water was replaced with fresh conditioned water of the same temperature using a fine siphon. Guppies were kept under a 12:12 light-dark cycle, and prior to the experiment were fed commercial tropical fish flakes (TetraMin, Tetra, Germany) daily and re-hydrated, decapsulated brine shrimp eggs (Brine Shrimp Direct Inc., Utah, USA) three times per week. The repeated measures design required individuals to be identified so to avoid invasive marking procedures we placed females of discernibly different sizes and/or gravid spot colouration in each tank. At the end of the experiment fish were returned to breeding populations at McGill University.

2.2 Objects

We used plastic Lego® blocks to obtain a large number of distinct objects (Figure 2) so as to be able to train for novelty. Lego blocks are available in a large number of unique, standardised structures and have been used as novel objects in past research and elicit behavioural variation in novel object responses (Antunes & Biala 2012; Frost *et al.* 2013; White *et al.* 2013; Hamilton *et al.* 2017). Since guppies have been demonstrated to be able to discriminate between shapes and between colours (Lucon-Xiccato & Bisazza 2014, 2016), an object was classed as novel if it differed in shape and/or colour from any other Lego block previously experienced by the guppy.

We used 42 different block designs in total (Figure 2). During training we had a set of 10 blocks which served as familiar objects and a set of 20 blocks which served as the novel objects: familiar objects were used twice and novel objects once during training. Three familiar objects and three novel objects were used for the 3 initial test trials a different six objects for the 3 final test trials. Thus, subjects saw novel objects only once, and different objects were used for test trials and training. Any learning of the individual exemplars used during training should therefore not impact behaviour at test.

2.3 Behavioural assays

Open field test

We habituated guppies to the 19L (5-gallon) test tank environment by giving them two consecutive open field tests at the start of the experiment. Individual guppies were netted from their home tank and brought to a test tank via a white opaque transfer cup. Guppies were placed in an empty open field tank filled to a water depth of 8 cm and a laminated white paper under the base to increase contrast for automated video tracking as well as white corrugated plastic walls. On this and all subsequent assays (unless otherwise stated) fish were allowed to habituate for 2 min within a transparent cylinder at the centre of the tank and were released by slowly lifting the cylinder, after which the experimenter (MWT) left the room and the test lasted 10 minutes, at which point the guppy was recaptured with the transfer cup and returned to its home tank

Object exploration

We assayed novel versus familiar object exploration by presenting guppies with two objects, one familiar and one novel, in the same 19L test tank the open field was conducted in. The tank was filled to a water depth of 8 cm and had white corrugated plastic walls with a laminated white paper under the base. We familiarized guppies to their familiar object for the entire 24 hours before measuring object preferences by placing a copy of the object in their housing tank. For training trials, the familiar and novel object pairs were rotated among tanks such that all guppies experienced all object pairs but in different orders. For test trials, all guppies experienced the same familiar and novel object pairs at the same time. Then, during the next day's training session, a novel object from the set of novel objects was placed in the test tank opposite to a duplicate of the familiar object. A duplicate was used to avoid the possibility that odour cues remained on the object from the housing tank.

In the object exploration assays more exploratory individuals are those that spend more time near novel objects, an established way of measuring novel object responses in guppies and teleost fish in general (De Serrano *et al.* 2016; May *et al.* 2016; Fior *et al.* 2018; Kirsten *et al.* 2018). Our object preference metric was the time spent within 4 cm (about 1 to 2 body lengths) of the object. Increased preference for an object was expected to be expressed behaviourally by an increased relative amount of time spent near that particular object over the other object.

Spatial exploration

We assayed spatial exploration by counting the number of unique compartments visited by guppies in a complex maze. This maze was constructed within a 177L (39-gallon) tank with white walls, white laminated paper under the base, and 10 cm of conditioned water with a number of walls to form 16 maze compartments (see supplementary video). Similar to other studies on teleost fish spatial exploration, exploratory individuals were expected to visit more unique compartments (Chapman *et al.* 2010; Jacquin *et al.* 2017; Berdal *et al.* 2018).

2.4. Training and testing

We trained guppies individually in the same 19L test tank in which they had been given open field tests and novel object preference assays. Guppies were primarily fed in the test tank for the duration of the experiment to encourage foraging motivation with additional flake food (TetraMin) given in the home tank every 3 days to ensure all fish received sufficient food. The familiar and novel objects were placed in the test tank 15 cm from one another, midway from front to back, and 3 cm from the short edge of the tank.

The food reward was a 1.5 cm x 0.5 cm (*w x h*) plastic strip with a mixture of gelatine, flake food and decapsulated brine shrimp egg attached to it. This food reward was placed behind the object such that the food reward was not visible to the guppy until it swam behind the object, while an empty strip was placed behind the unrewarded object. Fresh strips were used for each subject on each trial. After a two-minute habituation period inside a 20 cm tall, open-ended glass holding cylinder with a 7 cm diameter, in the centre of the tank, guppies were given five minutes daily in the test tank to move about the tank and feed, before being returned to their housing tank. Once a guppy had been released from the holding cylinder the experimenter (MWT) left the room and thus was out of sight during experiments. Training occurred once daily between 10h00 to 16h00 for 20 trials. During training the location of the rewarding object (left or right) was randomized across days and individuals. That is, only the object's familiarity or novelty provided a reliable cue to food location.

After 20 training trials three final assays of object exploration and two assays of spatial exploration were given as described in the object and spatial exploration assay sections. These unreinforced trials were used to assess whether exploratory propensities had shifted. During final assays, equal number of individuals were presented with the formerly rewarded object on the left and right side. Individuals were trained and tested in the same order per day throughout the experiment. At test during novel object trials the objects were presented with new, never used food strips with no food to ensure that guppies were not being guided to particular objects by the odour of food.

2.5. Behavioural data collection

Fish movement was recorded by a Basler camera (model acA1920-150uc - ace) mounted above the tank. EthoVision XT motion tracking software (version 11.5, Noldus *et al.* 2001) was used to quantify object preference metrics and activity metrics from video footage.

2.6 Statistical Analysis

Data were analysed using linear and generalised linear mixed effect models with R statistical software (Version 3.6.2, R Core Team, 2019). Model residuals were checked they met model assumptions with the DHARMa R package (Hartig 2018). All raw data and R scripts to reproduce the analyses and residual diagnostic plots will be freely available at FigShare.

Model 1 – Initial preference for familiar or novel objects

To see whether there was a difference in the initial preference for novel over familiar objects, we used an intercept only linear mixed effect model with novel object preference as the response variable. The novel object preference is calculated by taking the time spent near the novel object and subtracting it from the time spent near the familiar object. Since there are three initial baseline tests we included random effects of trial and of individual ID.

Model 2 – Preference for the rewarding object during training

To see whether the preferences for familiar and novel objects changed throughout training we used a linear mixed effects model with rewarding object preference (time spent near the object that is rewarded minus the time spent near the object that is unrewarded) as the response variable and fixed effects of trial and treatment. Trial is coded as an integer in this model and contains data from the 20 training trials. We additionally included individual ID as a random effect to account for repeated measures.

Model 3 – Shift in preference for the novel object at test trials

To see whether there was a change in preference for novel objects between initial and final tests as a result of training we fit a linear mixed effects model with a response variable of novel object preference and fixed effects of ‘trial type’ (initial versus final tests) and ‘treatment’ (familiar-rewarded versus novelty-rewarded) and their interaction. We additionally included random effects of individual ID and trial to account for repeated measures across multiple initial and final test trials.

Model 4 – Feeding activity between treatments during training

To ensure groups did not differ in the amount of feeding reinforcement during training we fit a generalized linear model with a negative binomial distribution with a response variable of ‘feeding count’ which was the number of trials in which an individual fish ate throughout all of training and a fixed effect of ‘treatment’ (familiar-trained versus novelty-trained).

Relationship between spatial and object exploration

To determine whether spatial and object exploration were correlated we determined the Pearson correlation between the mean number of unique compartments visited in spatial exploration trials and the mean preference for novelty in object exploration trials.

Repeatability

Repeatability (also known as the intraclass correlation coefficient, R) was used to establish the degree to which behaviours are consistent (Nakagawa & Schielzeth 2010). Repeatability represents the proportion of variance explained by individual identity and is calculated as the ratio of between-individual variance and the sum of between-and-within individual variance. Behavioural meta-analyses have concluded that values of $R < 0.2$ are marginally repeatable, $0.2 < R < 0.4$, are moderately repeatable, and $R > 0.4$ are highly repeatable (Bell *et al.* 2009; Garamszegi *et al.* 2012), so we used these designations when referring to repeatability results. We used the R package rptR (Stoffel *et al.* 2017) to calculate repeatabilities.

3. Results

3.1 Novel object preference at initial test (Model 1)

During the initial baseline tests we find no evidence for a significant preference for novel over familiar objects ($p = 0.564$). Moreover, as our post-hoc comparisons in model 3 reveal, there is no evidence for a difference between treatments initially in their preference for a novel object ($p = 0.87$).

3.2 Preference for the rewarding object during training (Model 2)

Throughout training, over the 20 trials, guppies increased their relative preference for the rewarded object (trial effect: $p < .001$) and there was no significant effect of treatment on rewarding object preference throughout training (treatment effect: $p = 0.886$), that is whether a guppy was familiar-trained or novelty-trained did not influence how much time it spent near the rewarding object during training.

3.3 Preference for novel objects during testing based on treatment (Model 3)

We compared the novel object preference of guppies at initial test trials and their novel object preferences after training at final test trials (Figure 3). There was a significant interaction effect between trial type (initial versus final) and treatment (familiar-rewarded versus novelty-rewarded). Guppies that were in the novelty-rewarded treatment had an increase in novel object preference that was stronger than the change in novel object preference of guppies in the familiar-rewarded treatment ($p = 0.048$).

Post-hoc comparisons reveal that initially, before training, there was no significant difference in the strength of preference for the rewarding object between the two treatments ($p = 0.87$). The shift in novel object preference between initial and final preference tests was significant for novelty-trained guppies but not for familiar-trained guppies. Novelty-trained guppies increased their preference for novel objects ($p = 0.042$) from initial to final test, whereas familiar-trained guppies do not change their relative preference for novel objects ($p = 0.998$). Since novel object preference is obtained by spending time near the novel object subtracted by time near the familiar object, this indicates that familiar-trained guppies did not increase their preference for familiar objects after training either. During the final test, novelty-trained guppies had a stronger preference for novel objects than familiar-trained guppies ($p = 0.046$).

3.4 Feeding attempts between treatments (Model 4)

We found no difference in the number of feeding attempts during training between novelty-trained guppies and familiar-trained guppies (treatment effect: $p = 0.648$).

3.5 Relationship between spatial and object exploration

Spatial and object exploration were not correlated at baseline ($R = -0.118$, $p = 0.618$) or at re-test ($R = -0.455$, $p = 0.08$).

3.6 Repeatabilities

Open field tests

During the open field tests general activity was highly repeatable ($R = 0.406$, 95% CI = [0.111, 0.657], $p = 0.003$) and time spent in the center of the tank was highly repeatable ($R = 0.508$, 95% CI = [0.222, 0.733], $p < 0.001$).

Novel object exploration

The time spent near a novel object was not repeatable initially ($R = 0.021$, 95% CI = [0, 0.176], $p = 0.441$). However, at re-test the time with spent near the novel object became highly repeatable ($R = 0.421$, 95% CI = [0.254, 0.571], $p < 0.001$) and was present when looking at both familiar-trained ($R = 0.415$, CI = [0.054, 0.605], $p = 0.002$) and novelty-trained guppies separately ($R = 0.428$, CI = [0.061, 0.611], $p = 0.005$). Activity was repeatable during the novel object initial assays ($R = 0.539$, 95% CI = [0.382, 0.705], $p < 0.001$) and at re-test ($R = 0.537$, 95% CI = [0.281, 0.713], $p < 0.001$).

Spatial exploration

Our measure of spatial exploration, the number of visits to unique compartments, was not repeatable in either the initial assays ($R = 0.174$, 95% CI = [0, 0.443], $p = 0.137$) or the final assays ($R = 0.086$, 95% CI = [0, 0.264], $p = 0.316$). Activity in the maze, however, was highly repeatable initially ($R = 0.581$, 95% CI = [0.326, 0.79], $p = 0.002$) and at re-test ($R = 0.449$, 95% CI = [0, 0.772], $p = 0.0437$).

4. Discussion

We found that relative novel object preferences can be shifted via positive foraging reinforcement with novel objects in the environment, supporting our hypothesis that direct experiences with novelty can induce changes in the propensity to engage with novel objects. Moreover, we find that consistency is induced in the response to novel objects. However, the induced increase in preference for novel objects does not translate into concordant shifts in the propensity for spatial exploration as we originally hypothesised. Moreover, while there was an increase in novel object preference for novelty-trained guppies, there was not a similar increase in preference for familiar objects in familiar-trained guppies.

The lack of learning to prefer familiar objects may have arisen as a consequence of the experimental design. Since guppies have to be familiarized with the familiar object for 24 hours before training, latent inhibition may inhibit the formation and/or expression of learned preferences. Latent inhibition describes a phenomenon whereby highly familiar stimuli evoke weaker behavioural responses and cause stimulus-consequence associations to be formed more slowly than novel stimuli do (Lubow & Weiner 2010). For novelty-trained guppies, novelty is presented in a ten-minute window and is rewarding for this entire period, whereas for familiar-trained guppies there is a 10-minute window where the familiar object is rewarding but a prior 24-hour period where it is unrewarding. However, latent inhibition or habituation can not explain why novelty-trained guppies increased their novel object exploration. If habituation was sufficient to induce increase in novel object exploration, then both familiar-trained and novelty-trained guppies would have neophilic responses towards novelty at re-test since both groups were exposed to equal numbers of novel objects throughout the experiment. Thus our results are unlikely the consequence of an exposure effect, *i.e.* one group more exposed to novelty over the other. Only the rewards for engaging with novelty differed between the treatments. Additionally, during training, we found no evidence for a difference in feeding rate or time spent near the novel versus familiar object between the groups, so we do not find support for novel or familiar objects being more likely to be approached or fed from as an explanation for our results.

Previous studies on novelty preferences in guppies consistently document general neophilia, except in the case of females preferring familiar females (Kelley *et al.* 1999; Hughes *et al.* 2013; Lucon-Xiccato & Dadda 2016; Dargent *et al.* 2018; Daniel *et al.* 2019, 2020; Lucon-Xiccato *et al.* 2019; Valvo *et al.* 2019). We do not find an initial preference for novelty across our guppies. Expressed responses towards novelty can differ depending on whether novelty is presented in a familiar or unfamiliar setting (Harris & Knowlton 2001) and guppies specifically have been demonstrated to not investigate novel objects if the environment is unfamiliar (Russell 1967). This may be a consequence of the testing environment being stressful. However, we found in previous experiments (see Chapter 2) that preferences for objects guppies have been trained to still emerge in this testing environment, so we suspect stress interfering in behavioural responses to the objects in this experiment are unlikely to explain the results we observed.

In addition to a lack of neophilia, we find an initial lack of consistency in novel object exploration but find that training induces consistency in object exploration propensities. The lack of neophilia contrasts with Daniel, Koffinas, & Hughes (2020) who document persistent conservation of neophilia across several contexts for individual guppies in the absence of training despite our objects being similar sizes. Our difference in results may be because Daniel, Koffinas & Hughes extensively habituated guppies to the novelty assays so that the only novel aspect of the environment was the novel stimuli of interest. We attempted to account for this by initially testing and habituating the fish twice to the empty test tank in an open field assay before presenting them with novel object trials in the same open field. While not explicitly quantified, when the objects that were to become the familiar objects for the next trial were initially placed in the guppy home tanks (a familiar environment), guppies could be seen initially approaching and orientating towards the newly presented object (Toure, pers. obs.) but perhaps experiencing the familiar object in a different context the next day causes dishabituation. We also find that spatial and object exploration were not correlated in our study population and design. Responses to novelty in these two contexts may be completely uncoupled and able to mount independent behavioural responses. Indeed, even assays that appear to be testing the same novelty context can produce little correlation (Vernouillet & Kelly 2020). Arvidsson *et al.* (2017) found no obvious relationship between exploration behaviour scores in a simple spatial exploration test versus those measured in a more complex environment both designed to assess spatial exploration. Thus, while novelty responses can be correlated (Berdal *et al.* 2018) whether these correlations are widespread and whether constraints to plasticity arise as a consequence seems unlikely but would depend largely on the mechanism underlying them.

It has often been hypothesised that syndromes and personalities stem from pleiotropic effects where single loci or hormones affect multiple behaviours (Trillmich *et al.* 2018). Given that we find there can be independent responses to novel objects and novel area use, it seems unlikely to be the case that exploratory behaviours in different contexts are constrained in their plasticity by shared mechanisms. In mice (*Mus musculus*), for which powerful neuronal and molecular tools are readily available, behavioural responses across several novelty contexts were found to be genetically independent (Kliethermes & Crabbe 2006) and distinct, orthogonal neuronal mechanisms have been shown to shape mouse exploration across spatial and social contexts (Fustiñana *et al.* 2021). Indeed, mechanisms producing variation in exploratory propensities are likely to be highly multigenic with many loci contributing to exploratory propensities and their plasticity. As a result, multiple loci can be targeted by selection to produce exploratory behaviour differences (Bendesky *et al.* 2011).

It remains unclear why different axes of novelty response behaviour sometimes correlate and sometimes do not (*e.g.,* colonial fish and crickets; Grill *et al.* 2012; Dochtermann & Nelson 2014). We do not find a correlation between novel object exploration and spatial exploration in our experiment either before or after training, and did not find training on object exploration influenced spatial exploration, while studies of other guppy populations do find correlations across novelty contexts (De Serrano *et al.* 2016; Lucon-Xiccato *et al.* 2019). It may that across different environments the costs and benefits of exploratory behaviour in different contexts are sufficiently decoupled such that selection or drift has allowed the capacity for independent responses to arise (Sih & Bell 2008; Moldoff & Westneat 2017). One way to confirm that there is a basis for coupling or decoupling would be to explore the molecular or hormonal mechanisms underlying differences in exploratory behaviour. If different modalities of novelty elicit very different genomic and/or neuronal responses, then this may explain the lack of correlation seen in certain populations and species. One promising molecular mechanism influencing exploratory behaviour is dopamine transmission.

Dopamine transmission has been implicated in differences in exploration in mammals (Mohebi *et al.* 2019) and guppies specifically (De Serrano *et al.* 2016, 2021). Moreover, dopamine has been implicated in the width of generalization gradients (Kahnt & Tobler 2016). Variation in dopamine receptors shifting the propensity for exploration and/or the degree to which we observe broad generalization of learned associations with novelty across several novelty contexts versus narrow generalizations that do not span across different contexts may be an important mechanism that explains why individuals differ in their exploratory behaviour and why different axes of novelty behaviours sometimes correlate and sometimes do not. In birds, the gene for the dopamine DRD4 receptor is consistently implicated in producing differences in exploratory behaviour (Fidler *et al.* 2007; Korsten *et al.* 2010; Mueller *et al.* 2013; Riyahi *et al.* 2015, 2017). The role of DRD4 in producing exploratory responses to novel objects and novel areas has been functionally validated in mice where knocking out the DRD4 receptor gene reduces both novel object and spatial exploration (Dulawa *et al.* 1999). Importantly, learning is also intimately tied to dopamine transmission (Schultz 2002; Frank 2004; El-Ghundi *et al.* 2007). There can be individual, genetically based, differences in the capacity to learn (Tang *et al.* 1999) that are directly related to physiological differences in the dopamine reward circuit (Frank *et al.* 2007; Flagel *et al.* 2011). When documenting individual variation in plasticity (*e.g.,* bold individuals that remain bold across environmental contexts while shyer individuals are more flexible; Jolles *et al.* 2019) researchers may be documenting individual variation in the capacity to learn. With exploratory behaviour as an example, since dopamine transmission is tied to both learning and exploration those with a higher capacity to learn may have genetic variation in dopamine receptors that predisposes them to be less exploratory but simultaneously more likely to be able to *learn to be more exploratory* should the environment require it. Shedding light on these mechanisms will allow us to better understand the relationships between personality, plasticity in personality, and behavioural syndromes.

Learning and personality are often investigated with respect to how personality predicts variation in learning or in the context of individual differences in learning ability (Katsnelson *et al.* 2011; Trompf & Brown 2014; Bousquet *et al.* 2015; Gibelli & Dubois 2016; Guido *et al.* 2017; Dougherty & Guillette 2018). While plasticity in personality is increasingly becoming a topic of interest, investigations into whether personality arises as a consequence of learning are comparatively rare (Frost *et al.* 2007) and a role for associative learning in driving plastic changes in personality are absent from major discussions of personality plasticity (Dingemanse *et al.* 2010; Stamps & Biro 2016). As we show here, direct experience with the rewards of approaching novelty is a potential explanation for observed variation in exploratory behaviour. If personality traits are in fact largely the result of previous experience, then future work may benefit from taking a feedback loop approach to understanding personality traits such as exploratory behaviour (Sih *et al.* 2015; Cooke *et al.* 2021).

A limitation of our study, as in most other personality studies (Dingemanse & Wright 2020), is that while we assessed repeatability, the temporal scale over which we re-assessed individuals was rather short. Over long timeframes, consistency in exploratory behaviour has been shown to be variable (Kluen & Brommer 2013; Greggor *et al.* 2016). However, given the potential for learning to explain these responses to novelty the persistence of these responses to novelty may be related to memory windows *i.e.,* how long animals retain relevant learned information. If the length of time over which information about novelty tends to be consistent then memory windows may themselves evolve (Smid *et al.* 2007).

Overall, we find that novel object preferences can shift in response to experience. Further investigations will be needed to determine how long induced changes in the response to novelty persist. Given that we observe induced consistency in behaviour as a result of experience, the role of learning in producing observed variation in personality traits should be given stronger emphasis in animal personality studies. Simple associative learning mechanisms may prove sufficient to explain a considerable degree of ‘personality’ variation observed in animals.

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Ethics

Methods were approved by the Animal Care Committee of McGill University (Protocol 2015-7708) and were conducted in accordance with the ethical guidelines of the Canadian Council on Animal Care and ABS/ASAB.

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Data Availability

Data and R scripts to produce all analyses, figures, and residual diagnostic plots will be archived on FigShare or a similar repository upon submission.

References

Antunes, M. & Biala, G. (2012). The novel object recognition memory: neurobiology, test procedure, and its modifications. *Cogn. Process.*, 13, 93–110.

Arvidsson, L.K., Adriaensen, F., van Dongen, S., De Stobbeleere, N. & Matthysen, E. (2017). Exploration behaviour in a different light: testing cross-context consistency of a common personality trait. *Anim. Behav.*, 123, 151–158.

Bell, A.M., Hankison, S.J. & Laskowski, K.L. (2009). The repeatability of behaviour: a meta-analysis. *Anim. Behav.*, 77, 771–783.

Bendesky, A., Tsunozaki, M., Rockman, M. V., Kruglyak, L. & Bargmann, C.I. (2011). Catecholamine receptor polymorphisms affect decision-making in *C. elegans*. *Nature*, 472, 313–318.

Berdal, M.A., Rosenqvist, G. & Wright, J. (2018). Innovation as part of a wider behavioural syndrome in the guppy: the effect of sex and body size. *Ethology*, 124, 760–772.

Bergeron, P., Montiglio, P.-O., Réale, D., Humphries, M.M., Gimenez, O. & Garant, D. (2013). Disruptive viability selection on adult exploratory behaviour in eastern chipmunks. *J. Evol. Biol.*, 26, 766–774.

Bousquet, C.A.H., Petit, O., Arrivé, M., Robin, J.P. & Sueur, C. (2015). Personality tests predict responses to a spatial-learning task in mallards, *Anas platyrhynchos*. *Anim. Behav.*, 110, 145–154.

Boyer, N., Réale, D., Marmet, J., Pisanu, B. & Chapuis, J.L. (2010). Personality, space use and tick load in an introduced population of Siberian chipmunks *Tamias sibiricus. J. Anim. Ecol.*, 79, 538–547.

Breck, S.W., Poessel, S.A., Mahoney, P. & Young, J.K. (2019). The intrepid urban coyote: a comparison of bold and exploratory behavior in coyotes from urban and rural environments. *Sci. Rep.*, 9, 2104.

Brown, C. (2001). Familiarity with the test environment improves escape responses in the crimson spotted rainbowfish, *Melanotaenia duboulayi*. *Anim. Cogn.*, 4, 109–113.

Brown, G.E., Ferrari, M.C.O., Elvidge, C.K., Ramnarine, I. & Chivers, D.P. (2013). Phenotypically plastic neophobia: a response to variable predation risk. *Proc. R. Soc. B Biol. Sci.*, 280, 20122712–20122712.

Burns, J.G., Price, A.C., Thomson, J.D., Hughes, K.A. & Rodd, F.H. (2016).

Environmental and genetic effects on exploratory behavior of high- and low-predation guppies (*Poecilia reticulata*). *Behav. Ecol. Sociobiol.*, 70, 1187–1196.

Chapman, B.B., Morrell, L.J. & Krause, J. (2010). Unpredictability in food supply during early life influences boldness in fish. *Behav. Ecol.*, 21, 501–506.

Cooke, A.C., Davidson, G.L., van Oers, K. & Quinn, J.L. (2021). Motivation, accuracy and positive feedback through experience explain innovative problem solving and its repeatability. *Anim. Behav.*, 174, 249–261.

Crane, A.L. & Ferrari, M.C.O. (2017). Patterns of predator neophobia: a meta-analytic review. *Proc. R. Soc. B Biol. Sci.*, 284, 20170583.

Daniel, M.J., Koffinas, L. & Hughes, K.A. (2019). Habituation underpins preference for mates with novel phenotypes in the guppy. *Proc. R. Soc. B Biol. Sci.*, 286, 20190435.

Daniel, M.J., Koffinas, L. & Hughes, K.A. (2020). Mating preference for novel phenotypes can be explained by general neophilia in female guppies. *Am. Nat.*, 196, 414–428.

Dargent, F., Chen, L., Fussmann, G.F., Ghalambor, C.K. & Hendry, A.P. (2018). Female preference for novel males constrains the contemporary evolution of assortative mating in guppies. *Behav. Ecol.*, 1–12.

Degen, J., Kirbach, A., Reiter, L., Lehmann, K., Norton, P., Storms, M., *et al.* (2016). Honeybees learn landscape features during exploratory orientation flights. *Curr. Biol.*, 26, 2800–2804.

De Serrano, A.R., Fong, C. & Rodd, F.H. (2016). Effects of methylphenidate on responses to novelty in a teleost fish (*Poecilia reticulata*). *Behav. Brain Res.*, 302, 53–59.

De Serrano, A.R., Hughes, K.A. & Rodd, F.H. (2021). Paternal exposure to a common pharmaceutical (Ritalin) has transgenerational effects on the behaviour of Trinidadian guppies. *Sci. Rep.*, 11, 1–13.

Dingemanse, N.J., Both, C., Drent, P.J., Van Oers, K. & Van Noordwijk, A.J. (2002). Repeatability and heritability of exploratory behaviour in great tits from the wild. *Anim. Behav.*, 64, 929–938.

Dingemanse, N.J., Kazem, A.J.N., Réale, D. & Wright, J. (2010). Behavioural reaction norms: animal personality meets individual plasticity. *Trends Ecol. Evol.*, 25, 81–89.

Dingemanse, N.J. & Wright, J. (2020). Criteria for acceptable studies of animal personality and behavioural syndromes. *Ethology*, 126, 865–869.

Dingemanse, N.J., Wright, J., Kazem, A.J.N., Thomas, D.K., Hickling, R. & Dawnay, N.

(2007). Behavioural syndromes differ predictably between 12 populations of three-spined stickleback. *J. Anim. Ecol.*, 76, 1128–1138.

Dochtermann, N.A. & Dingemanse, N.J. (2013). Behavioral syndromes as evolutionary constraints. *Behav. Ecol.*, 24, 806–811.

Dochtermann, N.A. & Nelson, A.B. (2014). Multiple facets of exploratory behavior in house crickets (*Acheta domesticus*): split personalities or simply different behaviors? *Ethology*, 120, 1110–1117.

Dougherty, L.R. & Guillette, L.M. (2018). Linking personality and cognition: a meta-analysis. *Philos. Trans. R. Soc. B Biol. Sci.*, 373, 20170282.

Dulawa, S.C., Grandy, D.K., Low, M.J., Paulus, M.P. & Geyer, M.A. (1999). Dopamine D4 receptor-knock-out mice exhibit reduced exploration of novel stimuli. *J. Neurosci.*, 19, 9550–9556.

Dussault, G. V. & Kramer, D.L. (1981). Food and feeding behavior of the guppy, *Poecilia reticulata* (Pisces: *Poeciliidae*). *Can. J. Zool.*, 59, 684–701.

El-Ghundi, M., O'Dowd, B.F. & George, S.R. (2007). Insights into the role of dopamine receptor systems in learning and memory. *Rev. Neurosci.*, 18.

Fidler, A.E., Van Oers, K., Drent, P.J., Kuhn, S., Mueller, J.C. & Kempenaers, B. (2007). Drd4 gene polymorphisms are associated with personality variation in a passerine bird. *Proc. R. Soc. B Biol. Sci.*, 274, 1685–1691.

Fior, D., Dametto, F., Fagundes, M., Santos da Rosa, J.G., Sander de Abreu, M., Koakoski, G., *et al.* (2018). Divergent action of fluoxetine in zebrafish according to responsivity to novelty. *Sci. Rep.*, 8, 13908.

Flagel, S.B., Clark, J.J., Robinson, T.E., Mayo, L., Czuj, A., Willuhn, I., *et al.* (2011). A selective role for dopamine in stimulus-reward learning. *Nature*, 469, 53–59.

Frank, M.J. (2004). By carrot or by stick: cognitive reinforcement learning in parkinsonism. *Science*, 306, 1940–1943.

Frank, M.J., Moustafa, A.A., Haughey, H.M., Curran, T. & Hutchison, K.E. (2007). Genetic triple dissociation reveals multiple roles for dopamine in reinforcement learning. *Proc. Natl. Acad. Sci. U. S. A.*, 104, 16311–16316.

Frost, A.J., Thomson, J.S., Smith, C., Burton, H.C., Davis, B., Watts, P.C., *et al.* (2013). Environmental change alters personality in the rainbow trout, *Oncorhynchus mykiss*. *Anim. Behav.*, 85, 1199–1207.

Frost, A.J., Winrow-Giffen, A., Ashley, P.J. & Sneddon, L.U. (2007). Plasticity in animal personality traits: does prior experience alter the degree of boldness? *Proc. R. Soc. B Biol. Sci.*, 274, 333–339.

Fustiñana, M.S., Eichlisberger, T., Bouwmeester, T., Bitterman, Y. & Lüthi, A. (2021). State-dependent encoding of exploratory behaviour in the amygdala. *Nature*.

Garamszegi, L.Z., Markó, G. & Herczeg, G. (2012). A meta-analysis of correlated behaviours with implications for behavioural syndromes: mean effect size, publication bias, phylogenetic effects and the role of mediator variables. *Evol. Ecol.*, 26, 1213–1235.

Gibelli, J. & Dubois, F. (2016). Does personality affect the ability of individuals to track and respond to changing conditions? *Behav. Ecol.*, 28, 101–107.

Gosling, S.D. & John, O.P. (1999). Personality dimensions in nonhuman animals: a cross-species review. *Curr. Dir. Psychol. Sci.*, 8, 69–75.

Graber, R.E., Senagolage, M., Ross, E., Houde, A.E. & Hughes, K.A. (2015). Mate preference for novel phenotypes: a fresh face matters. *Ethology*, 121, 17–25.

Greggor, A.L., Jolles, J.W., Thornton, A. & Clayton, N.S. (2016). Seasonal changes in neophobia and its consistency in rooks: the effect of novelty type and dominance position. *Anim. Behav.*, 121, 11–20.

Griffin, A.S., Netto, K. & Peneaux, C. (2017). Neophilia, innovation and learning in an urbanized world: a critical evaluation of mixed findings. *Curr. Opin. Behav. Sci.*, 16, 15–22.

Grill, J., Blum, C., Wagner, R.H., Mann, M., Urban, D., Schaedelin, F.C., *et al.* (2012). Exploring novelty: a component trait of behavioural syndromes in a colonial fish. *Behaviour*, 149, 215–231.

Grunst, A.S., Grunst, M.L., Pinxten, R. & Eens, M. (2019). Personality and plasticity in neophobia levels vary with anthropogenic disturbance but not toxic metal exposure in urban great tits: urban disturbance, metal pollution and neophobia. *Sci. Total Environ.*, 656, 997–1009.

Guido, J.M., Biondi, L.M., Vasallo, A.I. & Muzio, R.N. (2017). Neophobia is negatively related to reversal learning ability in females of a generalist bird of prey, the Chimango Caracara, *Milvago chimango*. *Anim. Cogn.*, 20, 591–602.

Hamilton, T.J., Tresguerres, M. & Kline, D.I. (2017). Dopamine D1 receptor activation leads to object recognition memory in a coral reef fish. *Biol. Lett.*, 13.

Harris, C.E. & Knowlton, F.F. (2001). Differential responses of coyotes to novel stimuli in familiar and unfamiliar settings. *Can. J. Zool.*, 79, 2005–2013.

Hartig, F. (2018). DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models. R package v. 0.2. 0.

Hendry, A.P. (2016). Key questions on the role of phenotypic plasticity in eco-evolutionary dynamics. *J. Hered.*, 107, 25–41.

Hester, F.J. (1964). Effects of food supply on fecundity in the female guppy, *Lebistes reticulatus* (Peters). *J. Fish. Res. Board Canada*, 21, 757–764.

Hughes, K.A., Houde, A.E., Price, A.C. & Rodd, F.H. (2013). Mating advantage for rare males in wild guppy populations. *Nature*, 503, 108–110.

Jacquin, L., Dybwad, C., Rolshausen, G., Hendry, A.P. & Reader, S.M. (2017). Evolutionary and immediate effects of crude-oil pollution: depression of exploratory behaviour across populations of Trinidadian guppies. *Anim. Cogn.*, 20, 97–108.

Johnson, Z. V., Moore, E.C., Wong, R.Y., Godwin, J.R., Streelman, J.T. & Roberts, R.B. (2020). Exploratory behaviour is associated with microhabitat and evolutionary radiation in Lake Malawi cichlids. *Anim. Behav.*, 160, 121–134.

Jolles, J.W., Briggs, H.D., Araya-Ajoy, Y.G. & Boogert, N.J. (2019). Personality, plasticity and predictability in sticklebacks: bold fish are less plastic and more predictable than shy fish. *Anim. Behav.*, 154, 193–202.

Kahnt, T. & Tobler, P.N. (2016). Dopamine regulates stimulus generalization in the human hippocampus. *Elife*, 5, 1–20.

Katsnelson, E., Motro, U., Feldman, M.W. & Lotem, A. (2011). Individual-learning ability predicts social-foraging strategy in house sparrows. *Proc. R. Soc. B Biol. Sci.*, 278, 582–589.

Kelley, J.L., Graves, J.A. & Magurran, A.E. (1999). Familiarity breeds contempt in guppies. *Nature*, 401, 661–662.

Kirsten, K., Fior, D., Kreutz, L.C. & Barcellos, L.J.G. (2018). First description of behavior and immune system relationship in fish. *Sci. Rep.*, 8, 846.

Kliethermes, C.L. & Crabbe, J.C. (2006). Genetic independence of mouse measures of some aspects of novelty seeking. *Proc. Natl. Acad. Sci.*, 103, 5018–5023.

Kluen, E. & Brommer, J.E. (2013). Context-specific repeatability of personality traits in a wild bird: a reaction-norm perspective. *Behav. Ecol.*, 24, 650–658.

Korsten, P., Mueller, J.C., Hermannstädter, C., Bouwman, K.M., Dingemanse, N.J., Drent, P.J., *et al.* (2010). Association between DRD4 gene polymorphism and personality variation in great tits: a test across four wild populations. *Mol. Ecol.*, 19, 832–843.

Lande, R. & Arnold, S.J. (1983). The measurement of selection on correlated characters. *Evolution.*, 37, 1210–1226.

Lubow, R. & Weiner, I. (2010). *Latent inhibition: cognition, neuroscience and applications to schizophrenia*. Cambridge University Press.

Lucon-Xiccato, T. & Bisazza, A. (2014). Discrimination reversal learning reveals greater female behavioural flexibility in guppies. *Biol. Lett.*, 10, 20140206.

Lucon-Xiccato, T. & Bisazza, A. (2016). Male and female guppies differ in speed but not in accuracy in visual discrimination learning. *Anim. Cogn.*, 19, 733–744.

Lucon-Xiccato, T., Bisazza, A. & Pilastro, A. (2019). Exploratory behaviour covaries with preference for unfamiliar males in female guppies. *Anim. Behav.*, 155, 217–224.

Lucon-Xiccato, T. & Dadda, M. (2016). Guppies show behavioural but not cognitive sex differences in a novel object recognition test. *PLoS One*, 11, e0156589.

Magurran, A.E. & Seghers, B.H. (1990). Population differences in predator recognition and attack cone avoidance in the guppy *Poecilia reticulata*. *Anim. Behav.*, 40, 443–452.

May, Z., Morrill, A., Holcombe, A., Johnston, T., Gallup, J., Fouad, K., *et al.* (2016). Object recognition memory in zebrafish. *Behav. Brain Res.*, 296, 199–210.

McCabe, C.M., Reader, S.M. & Nunn, C.L. (2015). Infectious disease, behavioural flexibility and the evolution of culture in primates. *Proc. R. Soc. B Biol. Sci.*, 282, 20140862.

Millar, N.P., Reznick, D.N., Kinnison, M.T. & Hendry, A.P. (2006). Disentangling the selective factors that act on male colour in wild guppies. *Oikos*, 113, 1–12.

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

Mohebi, A., Pettibone, J.R., Hamid, A.A., Wong, J.-M.T., Vinson, L.T., Patriarchi, T., *et al.* (2019). Dissociable dopamine dynamics for learning and motivation. *Nature*, 570, 65–70.

Moldoff, D.E. & Westneat, D.F. (2017). Foraging sparrows exhibit individual differences but not a syndrome when responding to multiple kinds of novelty. *Behav. Ecol.*, 28, 732–743.

Moran, N.P., Mossop, K.D., Thompson, R.M., Chapple, D.G. & Wong, B.B.M. (2017). Rapid divergence of animal personality and syndrome structure across an arid-aquatic habitat matrix. *Oecologia*, 185, 55–67.

Morand-Ferron, J. & Giraldeau, L.A. (2010). Learning behaviorally stable solutions to producer-scrounger games. *Behav. Ecol.*, 21, 343–348.

Mueller, J.C., Korsten, P., Hermannstaedter, C., Feulner, T., Dingemanse, N.J., Matthysen, E., *et al.* (2013). Haplotype structure, adaptive history and associations with exploratory behaviour of the DRD4 gene region in four great tit (*Parus major*) populations. *Mol. Ecol.*, 22, 2797–2809.

Nakagawa, S. & Schielzeth, H. (2010). Repeatability for gaussian and non-gaussian data: a practical guide for biologists. *Biol. Rev.*, 85, 935–956.

Nicolaus, M., Tinbergen, J.M., Ubels, R., Both, C. & Dingemanse, N.J. (2016). Density fluctuations represent a key process maintaining personality variation in a wild passerine bird. *Ecol. Lett.*, 19, 478–486.

Noldus, L.P.J.J., Spink, A.J. & Tegelenbosch, R.A.J. (2001). EthoVision: A versatile video tracking system for automation of behavioral experiments. *Behav. Res. Methods, Instruments, Comput.*, 33, 398–414.

Pavlov, I.P. (1927). *Conditioned reflexes: an investigation of the physiological activity of the cerebral cortex. Cond. reflexes an Investig. Physiol. Act. Cereb. cortex.* Oxford Univ. Press, Oxford, England.

R Core Team. (2019). R: A language and environment for statistical computing.

Reader, S.M. (2015). Causes of individual differences in animal exploration and search. *Top. Cogn. Sci.*, 7, 451–468.

Réale, D., Reader, S.M., Sol, D., McDougall, P.T. & Dingemanse, N.J. (2007). Integrating animal temperament within ecology and evolution. *Biol. Rev.*

Renner, M.J. (1988). Learning During Exploration: The role of behavioral topography during exploration in determining subsequent adaptive behavior. *Int. J. Comp. Psychol.*, 2, 43–56.

Reznick, Butler & Rodd. (2001). Life-history evolution in guppies. VII. the comparative ecology of high- and low-predation environments. *Am. Nat.*, 157, 126.

Reznick, D. (1983). The structure of guppy life histories: the tradeoff between growth and reproduction. *Ecology*, 64, 862–873.

Riyahi, S., Björklund, M., Mateos-Gonzalez, F. & Senar, J.C. (2017). Personality and urbanization: behavioural traits and DRD4 SNP830 polymorphisms in great tits in Barcelona city. *J. Ethol.*, 35, 101–108.

Riyahi, S., Sánchez-Delgado, M., Calafell, F., Monk, D. & Senar, J.C. (2015). Combined epigenetic and intraspecific variation of the DRD4 and SERT genes influence novelty seeking behavior in great tit *Parus major*. *Epigenetics*, 10, 516–525.

Rodd, F. H., Hughes, K. A., Grether, G. F., & Baril, C. T. (2002). A possible non-sexual origin of mate preference: are male guppies mimicking fruit? *Proc. R. Soc. B.*, 269, 475–481.

Russell, E.M. (1967). The effect of experience of surroundings on the response of *Lebistes reticulatus* to a strange object. *Anim. Behav.*, 15, 586–594.

Schuett, W., Laaksonen, J. & Laaksonen, T. (2012). Prospecting at conspecific nests and exploration in a novel environment are associated with reproductive success in the jackdaw. *Behav. Ecol. Sociobiol.*, 66, 1341–1350.

Schultz, W. (2002). Getting formal with dopamine and reward. *Neuron*, 36, 241–263.

Sih, A., Bell, A. & Johnson, J.C. (2004). Behavioral syndromes : an ecological and evolutionary overview. *Trends Ecol. Evol.*, 19.

Sih, A. & Bell, A.M. (2008). Chapter 5 insights for behavioral ecology from behavioral syndromes. In: *Advances in the Study of Behavior*. pp. 227–281.

Sih, A., Mathot, K.J., Moirón, M., Montiglio, P.O., Wolf, M. & Dingemanse, N.J. (2015). Animal personality and state-behaviour feedbacks: a review and guide for empiricists. *Trends Ecol. Evol.*, 30, 50–60.

Smid, H.M., Wang, G., Bukovinszky, T., Steidle, J.L.M., Bleeker, M.A.K., van Loon, J.J.., *et al.* (2007). Species-specific acquisition and consolidation of long-term memory in parasitic wasps. *Proc. R. Soc. B.*, 274, 1539–1546.

Stamps, J.A. & Biro, P.A. (2016). Personality and individual differences in plasticity. *Curr. Opin. Behav. Sci.*, 12, 18–23.

Stoffel, M.A., Nakagawa, S. & Schielzeth, H. (2017). rptR: repeatability estimation and variance decomposition by generalized linear mixed-effects models. *Methods Ecol. Evol.*, 8, 1639–1644.

Tang, Y.P., Shimizu, E., Dube, G.R., Rampon, C., Kerchner, G.A., Zhuo, M., *et al.* (1999). Genetic enhancement of learning and memory in mice. *Nature*, 401, 63–69.

Thompson, M.J., Evans, J.C., Parsons, S. & Morand-Ferron, J. (2018). Urbanization and individual differences in exploration and plasticity. *Behav. Ecol.*, 29, 1415–1425.

Thorndike, E.L. (1898). Animal intelligence: an experimental study of the associative processes in animals. *Psychol. Rev. Monogr. Suppl.*, 2, i.

Trillmich, F., Müller, T. & Müller, C. (2018). Understanding the evolution of personality requires the study of mechanisms behind the development and life history of personality traits. *Biol. Lett.*, 14, 20170740.

Trompf, L. & Brown, C. (2014). Personality affects learning and trade-offs between private and social information in guppies, *Poecilia reticulata*. *Anim. Behav.*, 88, 99–106.

Valvo, J.J., Rodd, F.H. & Hughes, K.A. (2019). Consistent female preference for rare and unfamiliar male color patterns in wild guppy populations. *Behav. Ecol.*, 30, 1672–1681.

Verbeek, M.E.M., Drent, P.J. & Wiepkema, P.R. (1994). Consistent individual differences in early exploratory behaviour of male great tits. *Anim. Behav.*, 48, 1113–1121.

Vernouillet, A. & Kelly, D.M. (2020). Individual exploratory responses are not repeatable across time or context for four species of food-storing corvid. *Sci. Rep.*, 10, 394.

White, J.R., Meekan, M.G., McCormick, M.I. & Ferrari, M.C.O. (2013). A comparison of measures of boldness and their relationships to survival in young fish. *PLoS One*, 8, e68900.

Figure 1 – Timeline of the experiment. Guppies were given 2 consecutive open field tests to habituate them to the test tank (Trials 1 and 2). They were then given alternating initial object and spatial exploration assays (Trials 3 to 7) to serve as initial measures of object and spatial exploration. Next guppies were given 20 training trials (Trials 8 to 27) where they were rewarded for feeding from either novel or familiar objects forming the two treatments, novelty-rewarded and familiar-rewarded. Finally, guppies were again given alternating object and spatial exploration assays to determine whether exploration levels changed from initial propensities as a result of training.

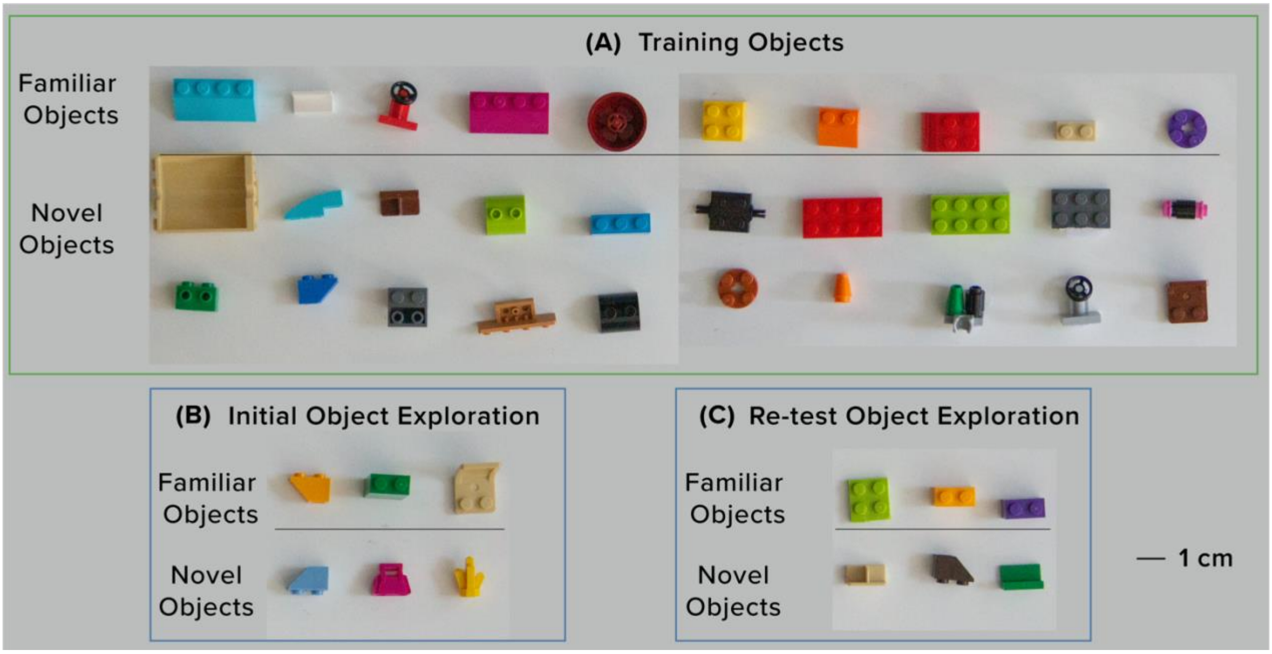

Figure 2 – The set of Legos used throughout the experiment. (A) present the objects used during training. The first row represents the 10 'familiar' objects, objects to which guppies were familiarised for 24 h before a choice test. The second and third rows are the 'novel' objects, objects which guppies experienced for the first time during the choice test. Each familiar object is paired to the two novel objects in its column. Guppies experienced all familiar objects twice during training and all novel objects in (A) once during training. (B) and (C) are the objects used for the initial and re-test object exploration assays. The familiarisation procedure was the same as during training. Thus objects at initial test and re-test were different from one another, and came from a different set of exemplars to those used during training.

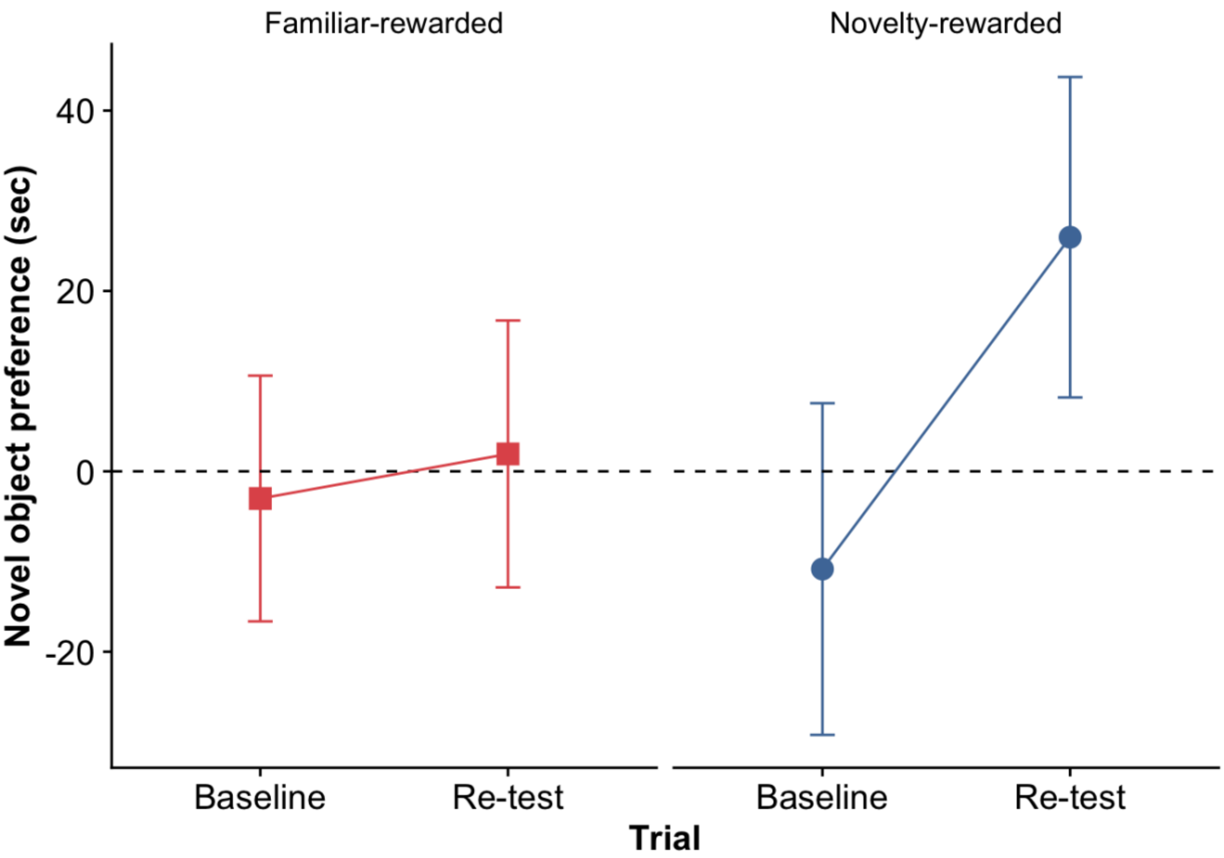

Figure 3 - Changes in novel object preference (the difference between the time spent near the novel object and the time spent near the familiar object) from initial tests (i.e., before training) to final tests (i.e., after training). During training, fish were rewarded for approaching a familiar object (red squares and lines) or a novel object (blue circles and lines). At test, no food reward was present. Dashed line represents an equal preference for either object. Data are means ± 95% CI. The final preference for novel objects increased for novelty-trained guppies but not for familiar-trained guppies.

Chapter 4 General Discussion

In this thesis I have investigated behavioural plasticity in traits relating to foraging preferences and exploratory propensities in Trinidadian guppies. In **Chapter 2**, I found that plasticity in the form of learned behaviour can be constrained by stimulus colour. In **Chapter 3**, I found that the propensity to explore novel objects could be shaped by foraging experience, that consistent individual differences in object exploration could emerge as a product of this experience, and that changes in object exploration were not correlated with changes in spatial exploration. In this general discussion I will synthesize these findings, focusing on their implications for how adaptive learning may be, how we understand 'animal personality' and behavioural syndromes (*i.e.*, consistent individual differences and correlations between personality traits), why it is important to understand the mechanisms underlying behaviour, as well as discussing practical applications, implications for evolution, and draw general conclusions from my results.

Constraints on learning

In **Chapter 2**, I investigated whether colour learning was constrained in a foraging context by comparing the preference developed for colours which vary in their presumed ecological relevancy. Unlearned preferences for colours have been demonstrated across several taxa and such preferences may reflect an evolutionary history of consistent reward and thus help naïve individuals determine what stimuli in the environment are ecologically relevant (Giurfa *et al.* 1995). However, the environment is not always consistent, so there can be variability in reward associations and learning allows animals to overcome the problem of environmental variability. For example, bees can have strong initial preferences for specific colours, but readily learn to forage from alternative colours (Raine & Chittka 2008). One might expect a lack of constraints on learning such that unlearned preferences should be able to be modified and essentially overwritten in the face of experience. However, as I show in **Chapter 2** this is not necessarily the case, even in the absence of an initial preference. Interestingly, I find that during training, once the objects are reinforced, green-rewarded guppies expressed a stronger preference for their rewarded object (the green object) than did blue-rewarded guppies did for the blue object, yet the rate change in preference was not significantly different between the two groups. This would seem to expose a bias towards green objects as soon as the guppy is in a foraging context. However, both groups fed equally from their objects and, despite being given equal levels of reinforcement, guppies trained to green went on to express a stronger preference for the green object at the end of training compared to the preference guppies trained to blue expressed for a blue object. Why such a constraint should be present remains unclear. We may expect constraints on learning when the historic contingency between a particular stimulus and reward is very weak compared to sensory predispositions for truly ecologically important pairings (green and food potentially reflecting the importance of green algae in this case). To determine whether this is the case we need to investigate how colour learning occurs in situations where the historic stimulus-reward contingency is known. Experimental evolution studies are a particularly useful approach to this (Dunlap & Stephens 2009, 2014).

A comparative approach may prove useful in determining the reasons behind learning constraints (Smid *et al.* 2007). With a comparative approach one can determine whether animals which do not share the same environment share the trait in question. Indeed, a limitation of my study is that this was one done in one particular guppy population, derived from the Paria drainage. Whether the minimal preferences for blue in response to blue-rewarded experiences is a general constraint we would observe across the species or if populations vary depending on ecological conditions would help resolve whether this is a constraint arising from a more general mechanistic constraint or one derived from the ecological conditions from which the studied population of guppies originate. I suspect it is unlikely that a mechanistic constraint is at play given male guppy colouration can exhibit high degrees of blue colouration in certain feral populations (Sathyan & Couldridge 2013). Moreover, wild Trinidadian populations can have males with blue spots (Endler & Houde 1995).

The flexibility of ‘personality’

In **Chapter 3**, I investigated whether the propensity to explore novel objects, an often-studied personality trait, could be plastic in response to an environment where interacting with novel objects is manipulated to be beneficial. I additionally wanted to determine whether behavioural syndromes, correlations among suites of behavioural traits across different ecological contexts or within the same behavioural context (Sih *et al.* 2004) would influence whether plastic shifts in exploratory behaviour would carry over to additional novelty contexts. In an exploration syndrome, responses to novel objects, areas, and foods are correlated with each other (Grill *et al.* 2012) and such a syndrome has been documented in guppies across several studies from independent groups (De Serrano *et al.* 2016; Lucon-Xiccato *et al.* 2019; Daniel *et al.* 2020). Given this, I wanted to see if plasticity in one novelty context (object exploration) would lead to a cross contextual change in exploration for another novelty context (spatial exploration): i.e. whether carryover effects would be observed. I found that individuals can shift their propensities for object exploration, and consistency in exploratory behaviour is induced if novelty is rewarded, but that this did not lead to a cross contextual shift in spatial exploration. So, individuals can learn to explore in specific contexts depending on the rewards they experience, in this case in an object context, without impacts on another context. Whether being able to learn to explore is adaptive and context-specific remains an open question. Learning is expected to be adaptive when the environment is not so variable that there is no predictive value in learning but not so stable that unlearned behaviours are sufficient (Dunlap & Stephens 2009). The costs and benefits of investigating or approaching novelty can vary temporally and spatially for species (Greenberg & Mettke-Hofmann 2001) and this is true for guppies where there is pressure to avoid novel predators but also opportunities to exploit novel foods (Brown *et al.* 2013; Elvidge *et al.* 2016). If there is high variability in the costs and benefits of exploring novelty, we may expect learning whether to explore to become favourable. Alternatively, my results may stem from a broad ability to learn categories of objects that are rewarded, rather than adaptive tuning of learning about novelty specifically, although notably I was unable to train guppies to preferentially explore familiar objects.

Whether variability in the costs and benefits of novelty exploration across wild guppy populations maps onto the degree of environmentally sensitive exploratory behaviour present would be a potential avenue for future research. If environmental variability favours plasticity but plasticity has costs, then in conditions where environmental variability is greatly reduced, we should expect a reduction in the degree of plasticity present. The domestication of guppies as a result of their popularity as aquarium pets has introduced them to relatively invariable environments and presents opportunities to test this hypothesis. Several populations of guppies have been exposed to domestication conditions (Balon 2004) multiple times independently around the world. Indeed, consistent with the hypothesis that domestic conditions do not require as much plasticity, laboratory-reared guppies have dramatically smaller brain sizes (a trait that correlates with measures of behavioural plasticity; Kotrschal *et al.* 2014) compared to wild-caught guppies (Burns *et al.* 2009). So, the contexts in which plasticity is maintained may vary. Laboratories which maintain stocks of guppies over several years can compare stock populations to wild populations across novelty contexts to assess the degree to which environmental sensitivity in exploratory behaviour has degenerated or been maintained. It may the case that wild-raised guppies are even more plastic in their exploratory behaviour than the laboratory-reared guppies which I tested in this thesis.

While we performed this experiment with individual guppies, allowing measurement of individual variation, it is important to recognize that guppies, particularly female guppies, can often be found in groups (Seghers 1974; Magurran & Seghers 1991), and the social environment can play an important role in modulating exploratory behaviour. Personality traits have been demonstrated to be sensitive to the social environment (Bevan *et al.* 2018). For example, the exploratory tendencies of zebra finches are predicted by the exploratory type of the foster rather than the genetic parent (Schuett *et al.* 2013). In practice there may be a strong influence of social conditions in shaping the propensity to engage with novelty (Greggor *et al.* 2016), particularly given that guppies often forage in shoals and individual foraging success in the wild is predicted by sociality (Snijders *et al.* 2018). Given that some individuals are more likely to approach novel objects it may be interesting to manipulate group compositions, as has been done in birds (Firth & Sheldon 2015), to determine whether the balance of exploratory versus non-exploratory guppies influences group foraging success. Untangling how processes producing individual-level variation scale up to group-level effects (Jolles *et al.* 2017) remains an important future avenue of research for personality work done in social species.

Critiques of the field of animal personality generally tend to focus on the field's lack of mechanistic insight (Beekman & Jordan 2017). Indeed, studies have been criticised when they simply document repeatability without explicitly testing a hypothesis (Jungwirth *et al.* 2017). I have shown that increased propensities for exploratory behaviour can arise as a consequence of learning. Studies into the processes generating behaviour serve as a demonstration as to why simply documenting the degree of behavioural consistency is problematic. Understanding the processes that generate ‘personality’ influences our interpretation of its causes and consequences. Beekman and Jordan (2017, p. 619) in their critique of animal personality say, “*Once variation, consistency, and correlations among behaviors are examined in the framework of their underlying genetics, life-history, and endocrine mechanisms, these studies necessarily become part of the established fields that existed long before the term “animal personality” emerged”.* My results suggest that associative learning mechanisms can underlie variation in the response to novelty. If, for environmentally sensitive personality traits, we are simply measuring learned responses in behaviour then it seems prudent to place these investigations within learning theory, although notably **Chapter 3** documents a change in responsiveness to a category of objects rather than a single stimulus. Associative learning underlying personality traits can have implications for the number of studies which document environmental sensitivity in the expression of personalities.

In three-spined stickleback, *Gasterosteus aculeatus*, experience with predation led to a cross-contextual change in risk-taking behaviour by inducing a correlation between aggression (aggressive behaviours towards an intruder) and boldness (latency to forage under simulated predation risk), in part through plasticity (Bell & Sih 2007). Brown trout, *Salmo trutta*, that were assayed for their personality, released back into their stream, and then recaptured after experiencing two months in the natural environment were also found to have had a syndrome induced, this time between exploration and aggression (Adriaenssens & Johnsson 2013). Given that I find that rewarding engaging with novel objects induces repeatability in exploratory behaviour, the interpretation of these studies can be approached from a learning framework. Results such as these may be the result of processes arising from associative learning such as instrumental conditioning and stimulus generalization. Learned associations with predation may generalize to additional risk contexts, in the stickleback case boldness influenced by predator threat and going on impact boldness in the face of an intruder, producing a behavioural syndrome. Given that I find there can be constraints on learning different properties of stimuli in **Chapter 2**, similar constraints may influence interpretation of studies which typically fall under the scope of animal personality. Similar to observed constraints in learning about particular stimuli properties, there may be constraints in learning about risk or novelty. Additionally, differences in the long-term consistency in behaviour, as has been documented for exploration, may be a manifestation of differences in memory windows (Kraemer & Golding 1997) and the retention of learned behaviour. This then produces testable hypotheses relating to individual variation and consistency in behaviour we may observe. If we restrict the capacity to learn pharmacologically (Smid *et al.* 2007) or through psychological techniques such as blocking do we then see the capacity for plasticity in 'personality' or consistency in behaviour impacted?

Evolutionary consequences of divergence in exploratory behaviour

I have documented that guppies can upregulate exploratory propensities in specific contexts when it is beneficial to do so and they can do this without this influencing another aspect of exploration, potentially avoiding the costs of a carryover effect. This may enable them to enter new habitats that differ from their current one or deal with seasonal changes, traits which can improve their persistence in novel environments. There is evidence for this being the case in birds where the propensity to respond to novelty in a behaviourally plastic manner is associated with increased survival in novel habitats (Sol *et al.* 2005). The potential for context specific plasticity may play a large role in the success with which guppies have expanded and invaded into new habitats across the globe, having established populations in over 70 countries on 6 continents across a variety of environmental and trophic conditions (Deacon & Magurran 2016). Exposure to novel habitats and responding to novel stimuli may open guppies up to novel selection pressures including ones that favour novelty responses becoming fixed. The Baldwin effect is a process by which environmentally induced phenotypic responses, in this case learned behavioural responses, acquire a genetic basis (Baldwin 1896). As a consequence, shifts in the propensity to explore may scale up to species level effects. If induced changes in the propensity to engage with novelty acquires a genetic basis, then this can have cascading consequences for ecology and evolution.

Engaging with novelty can lead to novel ways in which the environment can be exploited potentially sending animals on different evolutionary trajectories (Wilson 1985). For example, the Barbados bullfinch, *Loxigilla barbadonesis*, is less averse to novelty, approaching novel objects quicker than its closest relative on Barbados the black-faced grassquit, *Tiaris bicolor*, which is comparatively much more averse to novelty (Audet *et al.* 2018). The willingness to approach novel objects has been associated with a propensity to exploit a wide range of novel anthropogenic foods in the exploratory bullfinch. Meanwhile, the conservative grassquit feeds almost entirely on seeds. While this is a paired species comparison and thus constitutes a single replicate, the different responses to novelty are associated with differences in ecology, innovativeness, and neurobiology (Audet *et al.* 2018), illustrating the numerous correlates that may be associated with divergent exploratory tendencies. These can have implications for how we understand speciation processes across closely related taxa with divergent exploratory behaviours.

In sister taxa, over evolutionary time the ecological niches (the suite of biotic and a biotic conditions within which the species can persist) of a pair tend to be conserved due to common ancestry (Peterson *et al.* 1999). However, the exploitation of novel resources due to increased exploratory propensities may provide these species with the ability to cope with or expand into environments that diverge from their ancestral niche. Exploratory taxa being able to diverge from their ancestral niche is supported by studies in birds and mammals showing that exploratory species are more likely to survive in novel environments (Sol *et al.* 2005, 2008). In comparison, individuals that have lower exploratory behaviour should be more likely to track their ancestral niche rather than persist in or exploit novel environments/stimuli (Pyron *et al.* 2015). An enhanced persistence in the face of novelty may allow thus tolerance of or expansion into new ecological niches. To investigate this, it would be useful to determine whether increased exploratory propensities on a species level is associated with species occupying different parts of the environmental space when compared to close relatives. If this is the case, we should see repeated ecological niche divergence in closely related taxa with divergent exploratory propensities. Whether divergence in exploratory behaviour is associated with ecological niche divergence would have implications for how speciation occurs in taxa with documented differences in exploratory behaviour. If niches do diverge it would imply that parapatric speciation is a driving force in the speciation of exploratory taxa. However, if niches do not diverge it would imply that adapting to different environmental variables is not the driving force behind speciation but rather processes such as allopatric speciation (*e.g.,* due to geographic barriers) would be since there would be no environmental reason species could not overlap.

The importance of mechanisms

In **Chapter 3** I discussed dopamine as a candidate molecular mechanism underpinning variation in learning and exploration such as I observed. Dopamine is involved in reward perception, and inhibition of dopamine reduces exploratory behaviour in rats (Ungerstedt 1971). Interestingly, there is increasing evidence for a role for dopamine in the exploratory behaviour of guppies (De Serrano *et al.* 2016, 2021a, b). Associations can be learned but not expressed if there is a lack of dopamine. Dopamine neurons stimulate glutamate ion channels (NMDA receptors) and not receiving glutamate signals properly impairs learning (Zweifel *et al.* 2009). Notably, a divergence in the expression of NMDA receptors is also implicated in the aforementioned suite of correlated differences in exploratory and innovative behaviour in the Barbados bullfinch and black-faced grassquit (Audet *et al.* 2018). Mechanisms related to dopamine transmission therefore have the potential to explain correlations across exploration, innovation and learning across broad phylogenetic distances. Whether dopamine manipulations influence the learning propensities as well as the degree to which learned associations generalize in guppies remains to be seen but may prove a fruitful avenue of future research, particularly because drugs which manipulate dopamine are easily accessible and deliverable to guppies (De Serrano *et al.* 2016, 2021a, b).

Disentangling the mechanisms that produce variation in behaviour can inform how we understand evolutionary processes. For example, in *Heliconius* butterflies a quantitative genetic approach has revealed that the correlation between a behavioural preference for a particular body colour pattern and the expression of that colour pattern arises as a result of physical linkage between the genetic loci controlling the behavioural preference for that colour and the colour itself (Merrill *et al.* 2019). This physical linkage of the reproductive trait of interest and the preference for that trait can cause very rapid speciation during hybridization events, showing how an understanding of genetic mechanisms can aid understanding of evolutionary processes. Exploratory behaviour, like any other phenotype, is the manifestation of environmental and genetic mechanistic influences (Rymer & Pillay 2012). By understanding these influences, we can explain how and why diverse taxa converge on similar behavioural responses to novelty as well as why exploration is often entangled with other traits such as innovation propensities, brain size, and immunocompetence (McCabe *et al.* 2015; Collado *et al.* 2021). Whether pleiotropy or linkage underlies a trait correlation can influence whether that trait correlation can quickly be broken up over evolutionary time or whether the trait correlation will be more difficult for evolution to disentangle, leading to constrained behavioural evolution (Royauté *et al.* 2020). Moreover, by knowing which processes are implicated in shaping exploratory behaviours we can predict and/or explain trait correlations that arise in novelty contexts and apply this mechanistic understanding to applications where practical understanding of how exploration is shaped is useful.

Practical applications of understanding novelty response behaviours

Understanding how individuals respond to novelty through exploratory behaviours is not only of importance for ecology and evolution but can have implications for conservation program efforts, and animal welfare. The extent to which plasticity is expressed in response to novelty will allow populations to adjust to contemporary environmental changes. In the current context of global environmental change and urbanization, responses to novelty will likely be an important determinant of success for more and more organisms (Magory Cohen *et al.* 2020). Conservation programs can benefit from an understanding of the processes producing variation in exploratory behaviour. By understanding what can underlie adaptive responses to novelty we can potentially manipulate them to increase the survival rate of animals released from captivity into the wild. Indeed, **Chapter 3** gives evidence that exploratory tendencies can be changed by experience even in adult individuals, opening up the possibility of training individuals for release is possible for not just specific stimuli but to a broad ecologically important property of stimuli (Greenberg & Mettke-hofmann 2001). This could have impacts on species like the endangered ‘alalā corvid (Greggor *et al.* 2020; Smetzer *et al.* 2021) or hatchery reared fish whose responses to novelty are important for their well-being in the environment. Conservation management programs at times try to reintroduce previously captive animals into wild environments to help threatened populations or species. However, a major issue such efforts face is the large degree of mortality that reintroduced individuals face (Olla *et al.* 1998) in part because captive individuals can differ drastically in their responses to novelty when compared to their wild counterparts (Forss *et al.* 2015). An example is Tasmanian devils whose post-release survival from a captive environment was heavily influenced by how exploratory they were prior to release whereby more exploratory individuals had higher survival (Sinn *et al.* 2014). Upregulating exploratory behaviour to benefit individuals in this case could be done by varying experiences with novelty in captive individuals so that the adaptive behaviour is expressed when in the wild. Applying animal behaviour research to conservation and management can thus help contribute to successful conservation outcomes.

Further questions

Multiple mechanisms for exploration?

Given the lack of a correlation between spatial exploration and object correlation I observed in **Chapter 3**, one interpretation could be that spatial and object exploration are not governed by closely overlapping mechanisms. Notably, a failure to generalize across novelty contexts may still not necessarily mean that different mechanisms underlie the propensity to engage with novel objects versus novel spaces. Rather the two stimuli contexts may be perceived as different. Performance on tasks involving spatial behaviour and spatial cognition can be orthogonal to other behavioural and cognitive tasks such as visual cue discrimination allowing for distinct independently mediated responses to both contexts (Buehlmann *et al.* 2020). Indeed, one of the limitations of my work is that the fish in my experiments are rewarded in contexts with objects but, supported by the finding that there is no correlation between spatial and object exploration, it may be that novel foraging contexts in familiar environments are perceived or processed rather differently than novel areas in the environment. Whether there is a basis for this claim is unclear. Fortunately, there exist rich and exciting new opportunities to explore the mechanistic basis of exploratory behaviour given recent neurobiological and genetic advances that will allow us to resolve why exploratory behaviour traits are sometimes correlated and sometimes not. One could ask whether brain areas activated during a novel object context are different from those activated during a novel area context using techniques such as examination of immediate early gene (IEG) expression or ribosomal protein phosphorylation as markers of neuronal response to the stimulus of interest (Knight *et al.* 2012; Bengston *et al.* 2018; Fischer *et al.* 2018). Such an approach would provide useful insights into whether responses to object novelty and spatial novelty involve similar or distinct patterns of neural activity. Characterizing the brain region expression patterns sets the foundation for a higher resolution approach. For example, one could use a region-specific RNA-seq approach alongside gene ontology analyses in order to identify relevant genes implicated in putative exploratory behaviours. This is consequential because in order to understand how a behavioural trait evolved and may respond to selection and/or experience, it is important to understand the underlying genetic pathways (Bubac *et al.* 2020). Identifying how the expression of these genes diverge across individuals and/or species will allow us to gain a better understanding of the architecture of these behavioural traits. Ultimately these can go on to help us develop hypotheses with respect to how plasticity evolved and allow us to better characterize the evolutionary forces and constraints (*e.g.* genetic correlations) operating on behavioural plasticity.

Early versus Late life influences

An organism's environment and upbringing influence how its behavioural traits develop. Even subtle environmental differences during development may have noticeable impacts on the expression of individual exploratory behaviour. When genetically identical female laboratory mice were placed in a shared large environment and their exploratory behaviour tracked over three months, individual differences emerged in exploratory behaviour (Freund *et al.* 2013). Moreover, these differences were amplified as individuals aged. How important are very early life experiences versus late life experiences? There is reason to believe that experiences earlier in life have the potential to more strongly affect future behaviour. In *Heliconius* butterflies for example, freshly eclosed individuals shift away from an unlearned colour preference to a higher degree than adult individuals (Hollenbeck 2006). In **Chapters 2 and 3**, I worked with adult fish, but it may be the case that juveniles may show increased flexibility in behaviour, perhaps for example making training for familiar and blue objects possible.

The role of learning in evolution

One of the controversies concerning plasticity is whether it promotes or constrains genetic evolution (Duckworth 2009). The hypothesis that plasticity is often the first step toward new adaptations is particularly controversial (de Jong & Crozier 2003; Laland *et al.* 2014). Some argue plasticity weakens the strength of directional selection by shielding the genotype from selection. That is, regions of the genome would be under selection were it not for behavioural plasticity being present (Huey *et al.* 2003). Others argue that plasticity can be the initial step in adaptation with plastically induced phenotypes becoming genetically determined. Experimental evolution studies have established that where increased learning performance is selected for, learning has costs. Fruit flies with a higher learning performance devote more of their energy budget to the neural structures underlying learning and memory, with a correlated decrease in reproduction and longevity (Mery & Kawecki 2003; Niven *et al.* 2007; Burger *et al.* 2008). If having to consistently learn that novelty is rewarding is costly, then this may be a mechanism for the genetic accommodation of increased novelty preferences which could then cascade into the species level consequences. Guppies are a species that have been amenable to experimental evolution studies (Cole & Endler 2015; Kotrschal *et al.* 2020) and experiments which provide a selective environment where learned novelty responses are beneficial may provide us with insight into the potential for learning to play an active role in evolution, an area of research that has received rising attention in recent times (Laland *et al.* 2014, 2015; Heyes *et al.* 2020).

Concluding remarks

My thesis aimed to test hypotheses on constraints in behavioural plasticity arising as a result of putative evolutionary relationships between colour and reward as well as those arising from trait correlations as proposed by the field of animal personality using instrumental conditioning paradigms. I have shown that consistency in exploratory behaviour can be induced as a result of prior experience and the level of exploratory behaviour present in individuals can be a reflection of prior experience in the environment, supporting previous research documenting environmental sensitivity of exploratory behaviour (Burns *et al.* 2016). This is not the only study to find that behavioural traits are not constrained in their plasticity as a result of other behavioural traits. Smith & Blumstein 2012 find that a tri-contextual behavioural syndrome between general activity, boldness, and exploration did not prevent domain specific changes in boldness after training guppies to be shy in response to a predator model (although they did not successfully demonstrate associative learning, instead ascribing their results to sensitization). Constraints are often inferred in the personality literature but demonstrations that trait correlations or consistency in behaviour serve as a strong constraint to shifts in behaviour in response to experience are rare. I add to evidence arguing against a constraint hypothesis by showing that object exploration can change independently of spatial exploration. Of note is that both my work and that of Smith & Blumstein studied low predation guppies and a common finding is that behavioural trait correlations are stronger in high predation environments (Bell & Sih 2007; Dingemanse *et al.* 2007). A key weakness of my work is that I was only able to investigate one population. Whether plasticity experiences stronger constraints in environments where the costs of an incorrect response to novelty is stronger would be interesting to establish. Further research determining what factors promote or constrain plasticity remain important for explaining the diversity of behaviour we observe across animals, why we observe the behavioural trait combinations we do, and our understanding of how adaptive behaviour arises.

References

Adriaenssens, B. & Johnsson, J.I. (2013). Natural selection, plasticity and the emergence of a behavioural syndrome in the wild. *Ecol. Lett.*, 16, 47–55.

Audet, J.-N., Kayello, L., Ducatez, S., Perillo, S., Cauchard, L., Howard, J.T., *et al.* (2018). Divergence in problem-solving skills is associated with differential expression of glutamate receptors in wild finches. *Sci. Adv.*, 4, eaao6369.

Baldwin, J.M. (1896). A new factor in evolution. *Am. Nat.*, 30, 441–451.

Balon, E.K. (2004). About the oldest domesticates among fishes. *J. Fish Biol.*, 65, 1–27.

Beekman, M. & Jordan, L.A. (2017). Does the field of animal personality provide any new insights for behavioral ecology? *Behav. Ecol.*, 28, 617–623.

Bell, A.M. & Sih, A. (2007). Exposure to predation generates personality in threespined sticklebacks (*Gasterosteus aculeatus*). *Ecol. Lett.*, 10, 828–834.

Bengston, S.E., Dahan, R.A., Donaldson, Z., Phelps, S.M., van Oers, K., Sih, A., *et al.* (2018). Genomic tools for behavioural ecologists to understand repeatable individual differences in behaviour. *Nat. Ecol. Evol.*, 2, 1–12.

Bevan, P.A., Gosetto, I., Jenkins, E.R., Barnes, I. & Ioannou, C.C. (2018). Regulation between personality traits: individual social tendencies modulate whether boldness and leadership are correlated. *Proc. R. Soc. B.*, 285.

Brown, G.E., Ferrari, M.C.O., Elvidge, C.K., Ramnarine, I. & Chivers, D.P. (2013). Phenotypically plastic neophobia: a response to variable predation risk. *Proc. R. Soc. B.*, 280, 20122712–20122712.

Bubac, C.M., Miller, J.M. & Coltman, D.W. (2020). The genetic basis of animal behavioural diversity in natural populations. *Mol. Ecol.*, 29, 1957–1971.

Buehlmann, C., Wozniak, B., Goulard, R., Webb, B., Graham, P. & Niven, J.E. (2020). Mushroom bodies are required for learned visual navigation, but not for innate visual behavior, in ants. *Curr. Biol.*, 30, 3438-3443.e2.

Burger, J.M.S., Kolss, M., Pont, J. & Kawecki, T.J. (2008). Learning ability and longevity: a symmetrical evolutionary trade-off in *Drosophila*., *Evolution*, 62, 1294–1304.

Burns, J.G., Price, A.C., Thomson, J.D., Hughes, K.A. & Rodd, F.H. (2016). Environmental and genetic effects on exploratory behavior of high- and low-predation guppies (*Poecilia reticulata*). *Behav. Ecol. Sociobiol.*, 70, 1187–1196.

Burns, J.G., Saravanan, A. & Helen Rodd, F. (2009). Rearing environment affects the brain size of guppies: lab-reared guppies have smaller brains than wild-caught guppies. *Ethology*, 115, 122–133.

Collado, M.Á., Menzel, R., Sol, D. & Bartomeus, I. (2021). Innovation in solitary bees is driven by exploration, shyness and activity levels. *J. Exp. Biol.*, 224, jeb232058.

Cole, G. L., & Endler, J. A. (2015). Artificial selection for food colour preferences. *Proceedings of the Royal Society B: Biological Sciences*, *282*, 20143108. doi: 10.1098/rspb.2014.3108

Daniel, M.J., Koffinas, L. & Hughes, K.A. (2020). Mating preference for novel phenotypes can be explained by general neophilia in female guppies. *Am. Nat.*, 196, 414–428.

Deacon, A.E. & Magurran, A.E. (2016). How behaviour contributes to the success of an invasive Poeciliid fish : the Trinidadian guppy (*Poecilia reticulata*) as a model species.

De Serrano, A.R., Daniel, M.J. & Rodd, F.H. (2021a). Experimentally altered male mating behaviour affects offspring exploratory behaviour via nongenetic paternal effects. *Behav. Brain Res.*, 401, 113062.

De Serrano, A.R., Fong, C. & Rodd, F.H. (2016). Effects of methylphenidate on responses to novelty in a teleost fish (*Poecilia reticulata*). *Behav. Brain Res.*, 302, 53–59.

De Serrano, A.R., Hughes, K.A. & Rodd, F.H. (2021b). Paternal exposure to a common pharmaceutical (Ritalin) has transgenerational effects on the behaviour of Trinidadian guppies. *Sci. Rep.*, 11, 1–13.

Dingemanse, N.J., Wright, J., Kazem, A.J.N., Thomas, D.K., Hickling, R. & Dawnay, N. (2007). Behavioural syndromes differ predictably between 12 populations of three-spined stickleback. *J. Anim. Ecol.*, 76, 1128–1138.

Duckworth, R.A. (2009). The role of behavior in evolution: a search for mechanism. *Evol. Ecol.*, 23, 513–531.

Dunlap, A.S. & Stephens, D.W. (2009). Components of change in the evolution of learning and unlearned preference. *Proc. R. Soc. B Biol. Sci.*, 276, 3201–3208.

Dunlap, A.S. & Stephens, D.W. (2014). Experimental evolution of prepared learning. *Proc. Natl. Acad. Sci.*, 111, 11750–11755.

Elvidge, C.K., Chuard, P.J.C. & Brown, G.E. (2016). Local predation risk shapes spatial and foraging neophobia patterns in Trinidadian guppies. *Curr. Zool.*, 62, 457–462.

Firth, J.A. & Sheldon, B.C. (2015). Experimental manipulation of avian social structure reveals segregation is carried over across contexts. *Proc. R. Soc. B.*, 282.

Fischer, E.K., Westrick, S.E., Hartsough, L. & Hoke, K.L. (2018). Differences in neural activity, but not behavior, across social contexts in guppies, *Poecilia reticulata*. *Behav. Ecol. Sociobiol.*, 72, 131.

Forss, S.I.F., Schuppli, C., Haiden, D., Zweifel, N. & van Schaik, C.P. (2015). Contrasting responses to novelty by wild and captive orangutans. *Am. J. Primatol.*, 77, 1109–1121.

Freund, J., Brandmaier, A.M., Lewejohann, L., Kirste, I., Kritzler, M., Kruger, A., *et al.* (2013). Emergence of individuality in genetically identical mice. *Science*, 340, 756–759.

Giurfa, M., Núñez, J., Chittka, L. & Menzel, R. (1995). Colour preferences of flower-naive honeybees. *J. Comp. Physiol. A*, 177, 247–259.

Greenberg, R. & Mettke-hofmann, C. (2001). Ecological aspects of neophobia and neophilia in birds. In: *Current Ornithology, Volume 16*. Springer US, Boston, MA, pp. 119–178.

Greggor, A.L., Masuda, B., Flanagan, A.M. & Swaisgood, R.R. (2020). Age-related patterns of neophobia in an endangered island crow: implications for conservation and natural history. *Anim. Behav.*, 160, 61–68.

Greggor, A.L., McIvor, G.E., Clayton, N.S. & Thornton, A. (2016). Contagious risk taking: social information and context influence wild jackdaws' responses to novelty and risk. *Sci. Rep.*, 6, 1–7.

Grill, J., Blum, C., Wagner, R.H., Mann, M., Urban, D., Schaedelin, F.C., *et al.* (2012). Exploring novelty: a component trait of behavioural syndromes in a colonial fish. *Behaviour*, 149, 215–231.

Heyes, C., Chater, N. & Dwyer, D.M. (2020). Sinking in: the peripheral baldwinisation of human cognition. *Trends Cogn. Sci.*, 24, 884–899.

Hollenbeck, E. (2006). Associative color learning and age in *Heliconius* butterflies. University of Pennsylvania.

Huey, R.B., Hertz, P.E. & Sinervo, B. (2003). Behavioral drive versus behavioral inertia in evolution: a null model approach. *Am. Nat.*, 161, 357–366.

Jolles, J.W., Boogert, N.J., Sridhar, V.H., Couzin, I.D. & Manica, A. (2017). Consistent individual differences drive collective behavior and group functioning of schooling fish.

Curr. Biol., 27, 2862-2868.e7.

de Jong, G. & Crozier, R.H. (2003). A flexible theory of evolution. *Nature*, 424, 16–17.

Jungwirth, A., Wedell, N., Bshary, R. & Milinski, M. (2017). Animal personalities: an empty placeholder feigning understanding: a comment on Beekman and Jordan. *Behav. Ecol.*, 28, 629–630.

Knight, Z.A., Tan, K., Birsoy, K., Schmidt, S., Garrison, J.L., Wysocki, R.W., *et al.* (2012). Molecular profiling of activated neurons by phosphorylated ribosome capture. *Cell*, 151, 1126–1137.

Kotrschal, A., Lievens, E.J., Dahlbom, J., Bundsen, A., Semenova, S., Sundvik, M., *et al.* (2014). Artificial selection on relative brain size reveals a positive genetic correlation between brain size and proactive personality in the guppy. *Evolution*, 68, 1139–1149.

Kotrschal, A., Szorkovszky, A., Herbert-Read, J., Bloch, N.I., Romenskyy, M., Buechel, S.D., *et al.* (2020). Rapid evolution of coordinated and collective movement in response to artificial selection. *Sci. Adv.*, 6, 5–12.

Kraemer, P.J. & Golding, J.M. (1997). Adaptive forgetting in animals. *Psychon. Bull. Rev.*, 4, 480–491.

Laland, K., Uller, T., Feldman, M., Sterelny, K., Müller, G.B., Moczek, A., *et al.* (2014). Does evolutionary theory need a rethink? *Nature*, 514, 161–164.

Laland, K.N., Uller, T., Feldman, M.W., Sterelny, K., Müller, G.B., Moczek, A., *et al.* (2015). The extended evolutionary synthesis: its structure, assumptions and predictions. *Proc. R. Soc. B.*, 282, 20151019.

Lucon-Xiccato, T., Bisazza, A. & Pilastro, A. (2019). Exploratory behaviour covaries with preference for unfamiliar males in female guppies. *Anim. Behav.*, 155, 217–224.

Magory Cohen, T., Kumar, R.S., Nair, M., Hauber, M.E. & Dor, R. (2020). Innovation and decreased neophobia drive invasion success in a widespread avian invader. *Anim. Behav.*, 163, 61–72.

Magurran, A.E. & Seghers, B.H. (1991). Variation in schooling and aggression amongst guppy (*Poecilia reticulata*) populations in Trinidad. *Behaviour*, 214–234.

McCabe, C.M., Reader, S.M. & Nunn, C.L. (2015). Infectious disease, behavioural flexibility and the evolution of culture in primates. *Proc. R. Soc. B.*, 282, 20140862.

Merrill, R.M., Rastas, P., Martin, S.H., Melo, M.C., Barker, S., Davey, J., *et al.* (2019). Genetic dissection of assortative mating behavior. *PLOS Biol.*, 17, e2005902.

Mery, F. & Kawecki, T.J. (2003). A fitness cost of learning ability in *Drosophila melanogaster*. *Proc. R. Soc. B.*, 270, 2465–2469.

Niven, J.E., Anderson, J.C. & Laughlin, S.B. (2007). Fly photoreceptors demonstrate energy-information trade-offs in neural coding. *PLoS Biol.*, 5, 828–840.

Olla, B.L., Davis, M.W. & Ryer, C.H. (1998). Understanding how the hatchery environment represses or promotes the development of behavioral survival skills. *Bull. Mar. Sci.*, 62, 531–550.

Peterson, A.T., Soberón, J. & Sánchez-Cordero, V. (1999). Conservatism of ecological niches in evolutionary time. *Science*, 285, 1265–1267.

Pyron, R.A., Costa, G.C., Patten, M.A. & Burbrink, F.T. (2015). Phylogenetic niche conservatism and the evolutionary basis of ecological speciation. *Biol. Rev.*, 90, 1248–1262.

Raine, N.E. & Chittka, L. (2008). The correlation of learning speed and natural foraging success in bumble-bees. *Proc. R. Soc. B Biol. Sci.*, 275, 803–808.

Royauté, R., Hedrick, A. & Dochtermann, N.A. (2020). Behavioural syndromes shape evolutionary trajectories via conserved genetic architecture. *Proc. R. Soc. B Biol. Sci.*, 287, 20200183.

Rymer, T.L. & Pillay, N. (2012). The development of exploratory behaviour in the african striped mouse rhabdomys reflects a gene × environment compromise. *Behav. Genet.*, 42, 845–856.

Sathyan, R. & Couldridge, V.C.K. (2013). Female preference for blue in Japan blue guppies (*Poecilia reticulata*). *Environ. Biol. Fishes*, 96, 953–959.

Schuett, W., Dall, S.R.X., Wilson, A.J. & Royle, N.J. (2013). Environmental transmission of a personality trait: foster parent exploration behaviour predicts offspring exploration behaviour in zebra finches. *Biol. Lett.*, 9, 20130120.

Seghers, B.H. (1974). Schooling behavior in the guppy (*Poecilia reticulata*): an evolutionary response to predation. *Evolution.*, 28, 486.

Sih, A., Bell, A. & Johnson, J.C. (2004). Behavioral syndromes : an ecological and evolutionary overview. *Trends Ecol. Evol.*, 19.

Sinn, D.L., Cawthen, L., Jones, S.M., Pukk, C. & Jones, M.E. (2014). Boldness towards novelty and translocation success in captive-raised, orphaned Tasmanian devils. *Zoo Biol.*, 33, 36–48.

Smetzer, J.R., Greggor, A.L., Paxton, K.L., Masuda, B. & Paxton, E.H. (2021). Automated telemetry reveals post-reintroduction exploratory behavior and movement patterns of an endangered corvid, ʻAlalā (*Corvus hawaiiensis*) in Hawaiʻi, USA. *Glob. Ecol. Conserv.*, 26, e01522.

Smid, H.M., Wang, G., Bukovinszky, T., Steidle, J.L.M., Bleeker, M.A.K., van Loon, J.J.., *et al.* (2007). Species-specific acquisition and consolidation of long-term memory in parasitic wasps. *Proc. R. Soc. B.*, 274, 1539–1546.

Smith, B.R. & Blumstein, D.T. (2012). Structural consistency of behavioural syndromes: does predator training lead to multi-contextual behavioural change? *Behaviour*, 149, 187–213.

Snijders, L., Kurvers, R.H.J.M., Krause, S., Ramnarine, I.W. & Krause, J. (2018). Individual- and population-level drivers of consistent foraging success across environments. *Nat. Ecol. Evol.*, 2, 1610–1618.

Sol, D., Bacher, S., Reader, S.M. & Lefebvre, L. (2008). Brain size predicts the success of mammal species introduced into novel environments. *Am. Nat*, 172, 63–71.

Sol, D., Duncan, R.P., Blackburn, T.M., Cassey, P. & Lefebvre, L. (2005). Big brains, enhanced cognition, and response of birds to novel environments. *Proc. Natl. Acad. Sci.*, 102, 5460–5465.

Ungerstedt, U. (1971). Adipsia and aphagia after 6-hydroxydopamine induced degeneration of the nigro-striatal dopamine system. *Acta Physiol. Scand.*, 82, 95–122.

Wilson, A.C. (1985). The molecular basis of evolution. *Sci. Am.*, 253, 164–73.

Zweifel, L.S., Parker, J.G., Lobb, C.J., Rainwater, A., Wall, V., Fadok, J.P., *et al.* (2009). Disruption of NMDAR-dependent burst firing by dopamine neurons provides selective assessment of phasic dopamine-dependent behavior. *Proc. Natl. Acad. Sci. U. S. A.*, 106, 7281–7288.