

**THE 'HOW': THE ROLE OF LEARNING AND FLEXIBILITY IN PROBLEM
SOLVING IN GREY AND RED SQUIRRELS.**



A dissertation submitted for the degree of
Doctor of Philosophy

By

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Submitted by Pizza Ka Yee Chow, to the University of Exeter as a thesis for the degree of Doctor of Philosophy in Psychology, November 2015.

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ACKNOWLEDGEMENTS

I would like to thank my supervisors Stephen Lea, Natalie Hempel de Ibarra, and Lisa Leaver. Their patience, support and guidance to improve my research skills and thoughts. They also gave me freedom to explore my ideas and of course, the opportunity to work with squirrels. Thanks for their advice, lots of inspiring discussions, and comments on my work. A special thanks for his effort to build my confidence to write these years (he said he would not push me to be the best writer now because it is a life-long task which could take up to 70 years old).

I also thank Mrs. and Mr. Gilbert for their generous funding for my PhD and support me to go to conferences to share my findings with fellows. Thanks to David Taylor who helped me to build the puzzle box and supported for other technical problems. Thanks to Leila Goss who puts squirrels' welfare as top priority and enriches their living environment in laboratory.

I thank Peter Lurz and the Scottish National Heritage for permission to conduct research in the Brodick Country Park on Isle of Arran. Especially Kate Sampson and all rangers in the Ranger Service Centre provided lots of help and support throughout the red squirrels study.

Special thanks to my family and friends, Theo and Ilianna. Without their love and supports, I could not take up this challenge and follow my dream career. I also thank other PhD buddies who tried to drag me out of the laboratory during the weekends.

Finally, my special thanks to the laboratory squirrels, Arnold, Leonard, Sarah, Simon and Suzy, who accompanied me. The trust relationship that built between the squirrels and me is invaluable; it builds a core platform for me to explore how do they perceive the world. It follows that without them, there will not be me and this thesis.



Arnold



Leonard



Sarah



Simon



Suzy

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THESIS SUMMARY

Recent studies have advanced our knowledge of factors that could affect problem solving performance, and also of the positive effects of problem solving ability on fitness measures (the ‘what’ of problem solving). However, a missing linkage exists between this ‘what’ and the corresponding ‘how’. Such linkage requires the understanding of how these factors contribute to problem solving. Therefore, the central aim of this thesis is to examine this ‘how’. The roles of learning and behavioural flexibility in the context of problem solving are shown across the experiments, primarily with laboratory and free-ranging grey squirrels and to a lesser extent with wild red squirrels.

Under a recurring change, laboratory grey squirrels showed a rapid decrease in the number of errors they made per reversal phase in a serial spatial reversal learning task. Such efficiency is achieved by a gradual tactic change, from sequential to integrative tactics, with increased experience. It also involves support from cognitive mechanisms such as attention and inhibitory control. In a puzzle box task, wild grey squirrels showed that they were better problem solvers than the wild red squirrels. However, red squirrels that solved the puzzle box were more efficient than the grey solvers. Detailed analysis of the results showed that learning and flexibility play independent roles in problem solving. Each process is associated with particular traits that to increase efficiency. For grey squirrels, behavioural selectivity (effective behaviours) and persistence increased with increased experience. Flexibility, however, showed minimal positive effect for them, given that it decreased behavioural selectivity. In contrast, flexibility primarily provided a positive effect for red squirrels’ solving efficiency. These results showed that the two species appear to use both similar and different cognitive processes in solving the task.

The discussion gathers the results and explores how learning and flexibility, along with other behavioural traits, vary in their contributions to problem solving performance. As learning and flexibility are definitely not limited in problem solving, the discussion also addresses how these two processes might be involved a construct of general intelligence (‘g’) in animals, and how they are relevant to wider ecological aspects.

PEER – REVIEWED PUBLICATIONS**CHAPTER 2**

Serial reversal learning in grey squirrels: learning efficiency as a function of learning and change of tactics. Published in *Journal of Experimental Psychology: Animal Learning and Cognition*

CHAPTER 4

How practice makes perfect: the role of persistence, flexibility and learning in problem solving efficiency. Accepted in *Animal Behaviour*

WRITTEN AS MANUSCRIPT AND TARGETED JOURNAL**CHAPTER 3**

Colour reversal learning in grey squirrels: the roles of inhibitory control and attention depend on learning stage. Targeted journal: Brief communication in *Animal Cognition*

CHAPTER 5

Do grey squirrels outperform red squirrels in problem solving?

Targeted journal: Behavioural Ecology

DECLARATION

All parts of this thesis are my work and I developed all experiments and apparatuses. Two out of the four chapters (Chapter 2 and Chapter 4) have been submitted for publications. For Chapter 2, I have changed the US spelling to UK in order to align the whole thesis format. Chapter 3 and Chapter 5 are written in manuscript format for later submissions. However, some of these published materials are reused in the Introduction. I referred 'we' across the thesis, which included the contribution from my supervisors Stephen Lea (SL), Natalie Hempel de Ibarra (NH) and Lisa Leaver (LL) and collaborators Ming Wang (MW) and Peter Lurz (PL). Details of contribution are as follow:

Chapter 2:

SL commented and provided advice to written drafts before submission. LL commented on the initial experimental design and edited the final draft. MW provided R code for the Generalised Estimating Equations (GEE) for adjusted variance for very small sample size.

Chapter 3:

SL provided detailed comments and feedback on all drafts and LL edited the final draft. MW provided the GEE R code for analysis.

Chapter 4:

SL provided detailed feedback, comments and edited on all written drafts and LL edited final draft. MW provided the GEE R code for analysis.

Chapter 5:

SL and NH provided comments and contributed to discussion on all written drafts. LL made establish the contact with PL. PL supported for my field work on Arran Island.

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* $p < 0.005$

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CHAPTER 1 GENERAL INTRODUCTION

Natural selection endows animals with the ability to solve problems: an ability where variation appears to be heritable (e.g. Galsworthy et al., 2005; Roth, LaDage, & Pravosudov, 2010) and exhibits as an independent general cognitive trait (e.g. Cole, Cram, & Quinn, 2011; Shaw, Boogert, Clayton, & Burns, 2015). By definition, such an ability allows animals to overcome obstacles and achieve a goal. To study problem solving ability, Thorndike (1898) sets forth the definition of a ‘problem’ as ‘when an individual cannot simply use the behaviours within their repertoire to directly obtain a goal’ and he also developed quantitative methods for measuring sophisticated behavioural responses of animals using a puzzle box (p.6). Such design requires animals to use alternative ways to overcome obstacles; this is an important method for understanding behavioural and cognitive processes such as learning and flexibility that underlie problem solving (Griffin & Guez, 2014; Roth & Dicke, 2005). Most current evidence focuses on the ‘what’: the factors that contribute to problem solving success from biological (see **1.2**) and psychological aspects (see **1.3**). Some evidence focuses on the ‘why’: the functional or adaptive significance of problem solving, by considering its impact on fitness measures (see **1.1**). This thesis focuses on the ‘how’, the missing linkage between the ‘what’ and problem solving performance. The experiments principally use grey squirrels (*Sciurus carolinensis*) as the study model.

I first provide a brief overview of the rationale for my thesis, using insights gained from an evolutionary perspective: the ‘why’ of problem solving in **Section 1.1**. Then I highlight the ‘what’, variables that are related to problem solving success from biological aspects in **Section 1.2** and psychological (or behavioural traits, behavioural syndromes) in **Section 1.3**. Each section is accompanied by a consideration of corresponded problems in the

research field. In **Section 1.4**, I narrow down my focus on learning and instantaneous behavioural flexibility in problem solving. **Section 1.5** highlights possible new research angles to address the ‘how’, the role of learning and flexibility in the context of problem solving and their contribution to problem solving performance. **Section 1.6** introduces and explains my choice of species to study the potential research questions. **Section 1.7** standardises all the other terminologies use across the thesis and finally, **Section 1.8** provides an overview of the thesis in addressing each new proposed angle.

1.1 THE ‘WHY’: PROBLEM SOLVING ABILITY AND FITNESS MEASURES

This section mainly considers the ‘why’ from evolutionary perspective and adaptation assumptions to explain the rationale behind the questions proposed in **Section 1.5**. Two key assumptions from adaptability perspective are 1) traits or ability that are functional or adaptive are selected; and 2) traits that have higher benefits compared with costs. With this in mind, how do the two assumptions fit the case of problem solving ability?

In the context of solving food-extraction problem tasks, one clear immediate benefit of successful problem solving is that it provides direct, high-value food sources. Both laboratory and field studies use baits and rewards in novel problem solving tasks are generally the species preferred food (e.g. raw meat for keas, *Nestor notabilis*, and spotted hyenas, *Crocuta crocuta*, meal worms for great tits, *Parus major*, and scorpion for meerkats, *Suricata suricatta*). Accordingly, individuals could increase the amount of high valuable food that they consumes, if they solve the food-extraction task more than once (e.g. Benson-Amram & Holekamp, 2012), solve multiple obstacles (e.g. Biondi, Bó, & Vassallo, 2010; Cole & Quinn, 2012) or solve other problem tasks (e.g. Thornton & Samson, 2012) and such increased consumption through solving problem could possibly directly lead to weight gain

(Reader & Laland, 1999).

Another possible benefit of successful problem solving is that it is positively related to fitness measures. For example, at the individual level, problem solvers show increased mating success in males Satin bowerbirds, *Ptilonorhynchus violaceus* (Keagy, Savard, & Borgia, 2009) and lay larger clutches eggs and fledge more offspring in females great tits (Cole, Morand-Ferron, Hinks, & Quinn, 2012). Other evidence shows that if either parent is an efficient problem solvers, great tits also lay larger clutch size, have higher hatching success and higher fledgling numbers of young than when both parents that are non-problem solvers (Cauchard, Boogert, Lefebvre, Dubois, & Doligez, 2013).

Although there is little evidence of the costs to mating success that are associated with problem solvers, one study by Cole and colleagues (2012) found that problem solvers abandoned their nest more often than non-problem solvers. Overall, however, evidence from the ultimate consequences of problem solving ability suggests that it tends to be positive and seems to be favoured by natural selection as well as sexual selection (reviewed by Boogert, Fawcett, & Lefebvre, 2013; but also see empirical study by Isden, Panayi, Dingle, & Madden, 2013). If problem solving ability provides higher benefits than costs, then this cognitive ability and its associated traits are assumed to co-evolve due to the relatedness of their characteristics (Price & Langen, 1992). It follows that it is necessary to understand the factors associated with this ability.

1.2 THE ‘WHAT’ I: BIOLOGICAL VARIABLES AND PROBLEM SOLVING

Biological variables vary from an individual’s physiological state to its physical characteristics. Here I highlight five aspects of biological characteristics that have been shown to be related to problem solving performance:

Brain size: Amongst the biological variables, brain size has received the most attention. The ‘brain size – environmental change’ hypothesis states that species that have a bigger brain size relative to their body mass have enhanced cognitive ability to deal with environmental change compared to other similar species that have smaller brain size. For example, bigger brain species use more innovative foraging techniques than relative small brain species (Sol, Duncan, Blackburn, Cassey, & Lefebvre, 2005) at both within- and between- species level in birds (Lefebvre, et al., 1998) and mammals (Lefebvre, Reader, & Sol, 2004). Further investigation highlights the contribution of enhanced cognitive ability is related to large forebrain size (e.g. Lefebvre, Whittle, Lascaris, & Finkelstein, 1997; Nicolakakis & Lefebvre, 2000) or involves particular areas such as large telencephalon size (Shultz, Bradbury, Evans, Gregory, & Blackburn, 2005) in avian species and large neocortex in non-human primates (Reader & Laland, 2002).

Age: possibly related to increased experience in life, some studies show that adults tend to be problem solvers (e.g. Boogert, Reader & Laland, 2006; Botero et al., 2009) whereas in others juveniles are more successful (e.g. Biondi, Bó, & Vassallo, 2010; Morand-Ferron, Cole, Rawles, & Quinn, 2011; Thornton & Samson, 2012). Other evidence shows no difference between age groups in problem solving performance (e.g. male satin bowerbirds: Keagy, Savard, & Borgia, 2009).

Sex: Males and females in a species typically have distinct roles in reproduction, and this in turn, may affect problem solving performance or its adaptive value. Males are better problem solvers than females in canaries, *Serinus canaria* (Cadieu, Fruchard, & Cadieu, 2010) but females are better problem solvers than males in guppies, *Poecilia reticulata* (Laland & Reader, 1999a; Reader & Laland, 2000) and in common marmosets, *Callithrix jacchus* (Yamamoto, Domeniconi, & Box, 2004). In other species, there are no differences between males and females e.g. in great tits (Cole, Morand-Ferron, Hinks, & Quinn, 2012), or in common mynas, *Sturnus tristis* (Sol, Griffin, & Bartomeus, 2012).

Physiological states: Physiological states such as hunger level are related to the ‘necessity drive hypothesis’. This hypothesis states that individuals in a certain state or condition have higher needs than others to seek alternative foraging strategies. For example, guppies that have been food-deprived solved the task quicker than those that have not been food-deprived (Laland & Reader, 1999a; 1999b, Reader & Laland, 2000) regardless sex or which social rank an individual belongs to. However, other researchers (e.g. Cole, Morand-Ferron, Hinks & Quinn, 2012; Keagy, Savard, & Borgia, 2009) find that motivation does not account for problem solving performance, and it has been argued that in some species such as rhesus monkeys solving problem that does not lead to any rewards may be related to motivation to manipulate objects (e.g. Harlow, Harlow, & Meyer, 1950).

Morphology: little evidence shows any relationship between physical characteristics and problem solving performance. Nevertheless, physical characteristics may indicate good health condition, which may be related to problem solving ability. For example, the length of the carotenoid-based yellow wing stripe of siskin, *Carduelis spinus* (Mateos-Gonzalez, Quesada, & Senar, 2011) is positively related to problem solving ability. In other species,

however, no correlation has been found between any morphological characteristics and problem solving performance, e.g. carib grackles, *Quiscalus lugubris* (Overington, Cauchard, Côté, & Lefebvre, 2011).

1.2.1 PROBLEMS OF BIOLOGICAL VARIABLES

Two major problems so far make biological variables an unreliable predictor. Firstly, the role of brain size in cognitive ability is still under debate (e.g. Healy & Rowe, 2008; Jönsson, Fabre, & Irestedt, 2012; Mery, 2012). One of the arguments is related to unstandardised measurements of brain size. For example, other than brain-to-body ratio, studies found that residual brain size (e.g. Overington, Morand-Ferron, Boogert, & Lefebvre, 2009), overall brain size (e.g. Deaner, Isler, Burkart, & van Schaik, 2007), and particular brain areas such as telencephalon in birds (Shultz, Bradbury, Evans, Gregory, & Blackburn, 2005) are related to cognitive ability.

Another difficulty with the brain size hypothesis is that there can be huge variation in brain size that a particular area may be unrelated to problem solving ability. For example, how can the tiny brains of insects perform complex form of learning (Chittka & Niven, 2009)? Or why do big brained species like parrots fail to solve complex problem such as the trap-tube task, which is expected to be showed by large brain animals (Liedtke et al., 2011)? Hence, suggests further investigations should narrow down to neural connections or neuronal density (Chittka & Niven, 2009) to identify which neural network is responsible for a function or ability or beyond brain size as in other ecological or behavioural traits in explaining problem solving performance.

The second problem with using biological variables as predictors is their interaction

with social variables or psychological variables such as behavioural traits (or behavioural syndromes), which creates difficulties in disentangling their contribution to problem solving performance. For example, social rank defines the position of an individual in a group, rank or social status is related to an individual's competitive ability and possibly, physiological states. This may create a 'necessity drive' (Reader & Laland, 2003) which may explain why subdominants tend to be the problem solvers, as in chimangos, *Milvago chimango* (Biondi, Bó, & Vassallo, 2010), in great tits, *Parus major* (Cole & Quinn, 2012), in guppies, *Poecilia reticulata* (Laland & Reader, 1999a) and in meerkats (Thornton & Samson, 2012).

1.2.2 BRIEF SUMMARY OF THE FINDINGS OF BIOLOGICAL VARIABLES

Studies that use biological variables as predictors show inconsistent results at the *between*-species level. Nevertheless, the results seem to be robust in explaining variations in problem solving performance at the *within*-species level (e.g. Benson-Amram & Holekamp, 2012; Cole & Quinn, 2012; Hopper et al., 2013; Thornton & Samson, 2012; Yamamoto, Domeniconi, & Box, 2004). This evidence suggests that the scope of investigations should either go beyond biological variables or take an inter-disciplinary approach when studying inherent problem solving performance. For example, recent studies have attempted to widen the analysis by controlling biological variables and analysing them along with behavioural traits such as neophobia, exploration, persistence and behavioural variety. Such studies show that biological and behavioural traits such as openness or exploration either interact with each other (e.g. Hopper et al., 2013), or that biological variables are not as reliable as behavioural traits (e.g. Overington, Cauchard, Côté, & Lefebvre, 2011) in predicting problem solving performance. Accordingly, we now turn to consider behavioural traits.

1.3 THE 'WHAT' II: PSYCHOLOGICAL VARIABLES AND PROBLEM SOLVING

Emerging evidence shows that behavioural traits may be of higher explanatory values for variations in problem solving performance than biological variables. Traits that have received attention include:

Neophobia: Neophobia is an aversive reaction towards novel objects (Greenberg, 2003). A number of studies show that problem solvers tend to be less object or device neophobic (e.g. Heinrich, 1995; Overington, Cauchard, Côté, & Lefebvre, 2011; Sol, Griffin, & Bartomeus, 2012). On the other hand, other studies show that neophobia does not determine problem solving success or failure (e.g. Cole, Morand-Ferron, Hinks & Quinn, 2012). Instead, neophobia is an intervening variable for problem solving performance (e.g. Overington, Cauchard, Cote, & Lefebvre, 2011; Webster & Levebvre, 2001). For example, an individual with high device neophobia might be less likely to approach an apparatus initially and hence, less likely to succeed.

Exploration: Exploration refers to the behavioural reaction towards a novel environment or towards a problem task. Some studies show that exploration is one of the determinants of problem solving performance (e.g. Benson-Amram & Holekamp, 2012; 2013); individuals that explore more in a problem task are more successful in solving the task than those that explore less. However, others do not (Cole, Cram & Quinn, 2011; Cole & Quinn, 2012; Cole, Morand-Ferron, Hinks & Quinn, 2012); individuals that explore more a novel environment is not related to problem solving success than those explore less.

Persistence: Persistence reflects the motivation of an individual in problem solving. It can be measured as the duration that individuals spend interacting with the task before they

obtain a reward. Individuals who persist longer during problem-solving have been shown to be more likely to solve a problem, for example among hyenas (Benson-Amram & Holekamp, 2012), carib grackles, *Quiscalus lugubris* (Overington, Cauchard, Côté, & Lefebvre 2011), great tits (Cauchard, Boogert, Lefebvre, Dubois, & Doligez 2013), and meerkats (Thornton & Samson 2012). Persistence has been measured either as the numbers of attempts or the rate of attempts in solving a task (e.g. Biondi, Bó, & Vassallo, 2008; Griffin, Diquelou and Perea, 2014). In either case, results show that persistence is particularly important for problem solving success (Sol, Griffin, & Bartomeus, 2012), especially for the first problem of a series of problem solving tasks (Griffin, Diquelou & Perea, 2014).

Behavioural variety: this trait is indicated by the number of different ways an individual approaches a problem solving task. Behavioural variety has been suggested is a core factor for problem solving success (Griffin & Guez, 2014). Individuals that exhibit more behavioural types during problem solving tend to be problem solvers rather than non-problem solvers (e.g. Benson-Amram & Holekamp, 2012, 2013; Griffin, Diquelou & Perea, 2014; Griffin & Diquelou, 2015).

1.3.1 PROBLEMS OF PSYCHOLOGICAL VARIABLES

Although behavioural traits provide higher explanatory value than biological variables, measurement variations create limitations. Contradictory results may be due to unstandardised measurements across studies: researchers apply the same label to different behaviours under different contexts. For example, Cole and Quinn (2012) and Benson-Amram and Holekamp (2012) both examined exploration in relation to problem solving performance. Cole and Quinn (2012) measured exploration in great tits in a novel environment whereas Benson-Amram and Holekamp (2012) measured exploration towards

the problem solving device. With different measures of the same trait, Cole & Quinn (2011) found that exploration was not related to problem solving success, while Benson-Amram and Holecamp (2012) found that it was. Different measurements inevitably pose a problem for obtaining reliable results and drawing any valid conclusions. This suggests that it is necessary to standardise definitions and consistently apply the same label for targeted behavioural trait. Ideally, we should measure direct responses to the task, which are more relevant than other indirect measures that shares the same concept.

1.3.2 BRIEF SUMMARY OF FINDINGS ON PSYCHOLOGICAL VARIABLES

This section has reviewed the psychological variables that are associated with problem solving performance. Neophobia was once thought to be a core factor to predict problem solving success. But current evidence indicates that neophobia is better thought of as a ‘barrier’ that may interfere with success on the task, and in turn, mask the measure of actual cognitive ability (e.g. Overington, Cauchard, Cote, & Lefebvre, 2011; Webster & Lefebvre, 2001). This suggests that experimental paradigms should include a habituation period for individuals so as to measure actual cognitive ability. When neophobia is controlled, traits such as persistence and behavioural variety become important for problem solving performance. Problem solvers are more persistent and they exhibit more behavioural types than non-problem solvers. It has also been shown that the relative importance of each trait depends on the stage of problem solving. For example, persistence is more important at the beginning of problem solving and behavioural variety is more important in the latter stages when solving a series of problem task (Griffin, Diquelou, & Perea, 2014). Investigators should standardise their measures and use direct responses towards the problem solving task in order to be able to draw conclusion across studies. But how do these traits make their contributions to the problem solving process?

1.4 THE ‘HOW’: A MISSING LINKAGE BETWEEN THE ‘WHAT’ AND THE ‘WHY’

The question that was posed at the end of last section reflects the fact that current investigations have overlooked the ‘how’, the actual process of problem solving. The examination of this process requires the understanding of learning and behavioural flexibility. In this section, I give an overview for each mechanism separately. For each mechanism, I first define the terminology, then provide evidence to justify the importance of its role in problem solving (the ‘why’).

1.4.1 LEARNING: WHAT DOES IT MEAN IN PROBLEM SOLVING?

Learning is a core feature in the problem solving process, as has been mentioned by Reader and Laland (2003). Learning could be defined broadly as the ability to acquire, store, and retrieve information (Shettleworth, 2010), but it could be also defined narrowly as the neural representation of information processing (Dukas, 1999). Most working definitions are specific to the context being studied, and here, I focus on learning in the context of problem solving, which is a process of acquiring information or knowledge or skills through practice or experience in the task.

1.4.1.1 WHY LEARNING?

Some forms of learning exist in all animals where it has been looked for. It manifests broadly in different contexts such as learning potential food sources, habitats, mate choice and predators. The evidence is clear that learning has positive direct effects on fitness such as improved growth rate by learning potential food locations (Dukas, 2002), consuming novel foods and repeatedly returning to a problem task for feeding (Sol, Griffin, & Bartomeus, 2012) or increased mating success by avoiding mating with heterospecifics (Verzijden et al.,

2012). Enhanced learning ability is also shown in harsh environments facilitating individuals in novel foraging or problem solving relative to counterparts that are living in a stable environment (Roth, LaDage & Pravosudov, 2010) or in innovation (Dukas, 2013).

In the context of problem solving, learning is observed as the change in behaviour due to experience with familiarisation to a context or an object. When a naïve animal is first presented with a problem, the time costs in solving a problem are high because most animals have to learn through trial-and-error. But with increased experience at solving the same problem, individuals develop better motor skills in coordinating and manipulating the same task and thus, becoming more efficient at solving a problem task (e.g. Biondi, Bó, & Vassallo, 2010; Sol, Griffin, & Bartomeus, 2012; Taylor et al., 2010; Thorndike, 1898; Thornton & Samson, 2012). They also make fewer errors or increase efficiency in solving, perhaps by finding new tactics to address the problem.

Learning also maximises energy gain. With increased experience, problem solvers can increase their consumption of highly valuable food if they repeatedly solve the same task (e.g. Benson-Amram & Holekamp, 2012; Biondi, Bó, & Vassallo, 2010; Cole & Quinn, 2012) or solve other problem tasks (e.g. Thornton & Samson, 2012). This highlights that learning provides direct benefits for individuals faced with problem solving tasks.

1.4.1.2 PRIOR EXPERIENCE OR KNOWLEDGE IN PROBLEM SOLVING

Prior experience of a task has been shown to facilitate learning a similar task, and thus increase efficiency to obtain a goal (food reward). Such facilitation on the next task performance is called positive transfer or generalisation. Positive transfer has been demonstrated either through exhibiting a similar motor solution in solving a novel problem

(e.g. Seibt & Wickler, 2006), applying similar tactics, in the serial reversal learning task (e.g. Bond, Kamil, & Balda, 2007) or prior training to use a tool (e.g. Bird & Emery, 2009; Taylor, Elliffe, Hunt, & Gray, 2010). This evidence highlights that learning allows an individual to increase efficiency by acquiring and applying the same information in another novel situation. As well as such positive transfer, however, there is also evidence for negative transfer in which prior experience could hamper learning another task (negative transfers) as in the discrimination reversal learning task (e.g. Riopelle, 1955). But with repeatedly experiencing a recurring task, animals would also show a decrease in the errors to reach the learning criterion in a serial reversal learning task, and this suggests that experience shapes animals to *'learning to learn efficiently'* (Harlow, 1949).

1.4.1.3 SOCIAL LEARNING AND PROBLEM SOLVING

Evidence on the relationship between social contexts and problem solving performance is inconsistent. Studies showed that observers successfully learn from demonstrators how to solve a problem (e.g. Bouchard, Goodyer, & Lefebvre, 2011; Seibt & Wickler, 2006) and the presence of conspecific can increase efficiency in problem solving (e.g. Boogert, Monceau, & Lefebvre, 2010). But there are also situation where the presence of conspecifics does not affect individual problem solving performance (Benson-Amram & Holekamp, 2012; Morand-Ferron, Cole, Rawles, & Quinn, 2011) or where observers learn unsuccessfully from demonstrators (Seibt & Wickler, 2006; Gajdon, Fijn, & Huber, 2006). This evidence suggests that the presence of conspecifics could be a confounding variable when measuring individuals' performance. Therefore, measuring individual problem solving performance ideally should be in an asocial condition.

1.4.1.4 LEARNING, BEHAVIOURAL TRAITS AND PROBLEM SOLVING

As discussed in **Section 1.2**, certain behavioural traits have been found to be important for problem solving success. Although very few studies have explicitly highlighted the relationship between behavioural traits and learning, studies using novel food extraction tasks consistently show that learning and behavioural traits co-vary during the problem solving process. For example, animals that exhibited more effective behaviour with increased experience to solve the same task (e.g. Overington, Cauchard, Côté, & Lefebvre, 2011), spend more time on manipulating the functional part of a problem (Thornton & Samson, 2012). Although the number of attempts (a measure of persistence) may decrease across trials in some situations, the rate of attempts increases with successive trials (e.g. Biondi, Bó, & Vassallo, 2008; Griffin, Diquelou and Perea, 2014). These examples suggest that if we could identify the behavioural factors that vary across trials, then we could build a more complete picture of how these contribute to the problem solving process. Accordingly, I will consider evidence on a key factor that has been found to correlate with problem solving success, behavioural flexibility.

1.4.2 BEHAVIOURAL FLEXIBILITY AND PROBLEM SOLVING

1.4.2.1 BEHAVIOURAL FLEXIBILITY: WHAT IS IT IN PROBLEM SOLVING?

Behavioural flexibility is core feature in problem solving (Reader & Laland, 2003) and is a type of phenotypic plasticity (West-Eberhard, 2003, pp. 34-55). Similar to learning, behavioural flexibility is loosely defined as ‘the ability to modify a behaviour’ in the context of problem solving (Reader & Laland, 2003, pp.20). However, the conceptual framework of behavioural flexibility mainly surrounds the word ‘change’ in behaviours (West-Eberhard, 2003, pp.34) to tackle a problem, challenge or demand. It differs from learning, which involves the refinement of behaviour as a result of experience; rather, it manifests as an

instant change of behaviour that allows an individual to use or seek alternative ways of solving a problem. This instant adjustment is different from basic reflexive response (see detailed discussion in West-Eberhard, 2003) but may co-vary or associate with other cognitive mechanisms such as decision making, or memory, with behavioural traits (discussed in **Section 1.3**).

1.4.2.2 WHY BEHAVIOURAL FLEXIBILITY?

The linkage between behavioural flexibility and fitness measures has been directly illustrated across many studies. Considering first its ultimate consequences, behavioural flexibility is positively related to invasion success in reptiles (Amiel, Tingley, & Shine, 2011), in birds (Sol, Timmermans, & Lefebvre, 2002; Sol, Duncan, Blackburn, Cassey, & Lefebvre, 2005; Sol, Székely, Liker, & Lefebvre, 2007), and in mammals (Sol, Bacher, Reader, & Lefebvre, 2008). This shows that flexibility allows individuals to use alternative food sources in novel or unstable environments to maximise survival and thereby, is a functional trait that natural selection favours. While this evidence has established the long-term significance of behavioural flexibility, investigation of its role in the process of problem solving is still in its infancy.

1.4.2.3 BEHAVIOURAL FLEXIBILITY IN PROBLEM SOLVING

As mentioned above, behavioural flexibility provides an instant form of change in behaviour. Such change is manifested as different forms in different studies, for example, scatter-hoarding birds such as ravens, *Corvus corax*, and western scrub-jays, *Aphelocoma californica*, employ various caching strategies to minimise the pilferage risk (e.g. Bugnyar & Kotrschal, 2002; Clayton, Dally & Emery, 2007); tree squirrels such as thirteen-lined ground squirrel, *Spermophilus tridecemlineatus*, and grey squirrels, *Sciurus carolinensis*, adjust their

body position to increase the visibility of the surroundings when visual access to the environment is obstructed (e.g. Arenz & Leger, 1997; Makowska & Kramer, 2007); high competitive ability individuals may dominate food sources in foraging, such that individuals with low competitive ability in their population such as seen in great tits (*Parus major*) and northern pike (*Esox lucius*) have to use alternative strategies such as solving problem tasks to obtain food (Cole & Quinn, 2012; Pintor, McGhee, Roche & Bell, 2014). Behavioural flexibility could be seen as the frequency of using innovative foraging techniques or consumption of novel food in invasive species as in reptiles (Amiel, Tingley, & Shine, 2011), birds (Sol, Timmermans, & Lefebvre, 2002; Sol, Duncan, Blackburn, Cassey, & Lefebvre, 2005; Sol, Székely, Liker, & Lefebvre, 2007) and mammals (Sol, Bacher, Reader, & Lefebvre, 2008). However, recent studies have shown that the frequency of consuming novel foods is a different form of behavioural flexibility than that seen in technical problem solving (Sol, Griffin, & Bartomeus, 2012; Logan, 2015). Both forms of behavioural flexibility may share similar correlations with behavioural traits such as neophobia, exploration, or motivation, but unlike the consumption of novel food which relies on an individual's motivation, technical problem solving depends on overcoming neophobia (Sol, Griffin, & Bartomeus, 2012).

In terms of technical problem solving, behavioural flexibility could be shown in various ways depending on the design of the task. The first form is switching to a new solution when the old solution to the same task is not available. This requires individuals to abandon a previously successful response. To record such a change in behaviour, studies in birds (e.g. Auersperg, Gajdon, & von Bayern, 2012) and primates (e.g. Manrique, Volter, & Call, 2012; Huebner & Fichtel, 2015) have given animals a problem that could be solved in several ways and once the individual mastered a solution type, the researcher would block

that solution and hence, force the individual to abandon the old solution and seek an alternative for the same task. Such a design, on the one hand, could illustrate how a change in behaviour is related to problem solving performance. On the other hand, the procedures of ‘blocking’ the old solution may inhibit spontaneous flexibility in using alternative solutions. For example, when various solution types become available for chimpanzees (*Pan troglodytes*), they showed that chimpanzees were reluctant to abandon old solutions that could decrease efficiency in obtaining a food reward (Hrubesch, Preuschoft, & van Schaik, 2009).

Another form of behavioural flexibility requires individuals to inhibit a learned behaviour and adjust their behaviour to new reward patterns. Examples of the paradigms include the serial reversal learning task (Pavlov, 1927) that is frequently examined with animals both in the laboratory (e.g. Warren, 1965), in temporary captive testing enclosures (e.g. Bond, Kamil, & Balda, 2007) or at least with a single reversal learning in the wild (e.g. Boogert, Monceau, & Lefebvre, 2010; Isden, Panayi, Dingle, & Madden, 2013). Briefly, the single reversal task requires individuals first to go through a discrimination training phase and then a phase in which the contingency of reinforcement are reversed (details in **Chapter 2**). Individuals have to reach a pre-set learning criterion in both learning phases. In serial reversal learning task, the cycle is repeated across a number of phases. Flexibility is measured by the number of trials that an individual takes to reach a learning criterion following reversal or the number of errors that an individual makes before reaching the learning criterion following reversal.

The final form of behavioural flexibility introduced here is switching between tactics while attempting to solve a problem. This form of flexibility is suggested by Roth & Dicke

(2005) as a measure of intelligence. Once again, this measure records a behavioural ‘change’, specifically the individual’s tendency to employ alternative means to solve a problem as a result of failure. In the experimental work to be described in this thesis, I refine this measurement in terms of the number of changes between defined behaviours as a species shown during problem solving. The behavioural change could be new as well as old behaviours to solve a task. This measurement is unlike the approach taken by Ramsey et al. (2007) that require entirely novel solution to solve a problem. Instead, such alternative means are not required to be entirely novel during problem solving. It could be also any strategies that an animal has tried on a previous trial, or a previous problem. Nor did I consider whether the behaviours concerned were potentially effective or not. The number of switches between tactics does not depend on an individual’s behavioural repertoire size, as an individual with a limited repertoire could make numerous switches between its few available behaviours, whereas an individual with a large repertoire might make very few switches between its many available behaviours (for details see **Chapter 3**).

1.4.2.4 BEHAVIOURAL FLEXIBILITY, INFLEXIBILITY AND BEHAVIOURAL TRAITS IN PROBLEM SOLVING

Research on the relationship between behavioural flexibility and the behavioural traits considered in **Section 1.3** is scant. In a design that could be solved in multiple ways, exploratory behaviours were positively correlated with high behavioural flexibility in keas, *Nestor notabilis* (Auersperg, Gajdon, & von Bayern, 2012). Indirect evidence using similar methods has reflected that a possible positive correlation between high persistence (or motivation) and behavioural flexibility in falconiformes, *Milvago chimango* (Biondi, Bó, and Vassallo, 2008); birds increased their persistence as well as obtained more success using various solution types with increased experience.

Consideration of animals that fail to solve a novel problem may shed useful light on the relationship between behavioural traits and behavioural flexibility. Cole, Cram, and Quinn (2011) reported that only 44% (out of 570 great-tits) could solve a novel lever-pulling task to obtain four waxworms (*Galleria mellonella*), Benson-Amram & Holekamp (2012) showed that 85% (53 out of 62) hyenas could not open a puzzle box to gain access to raw meat, and Seibt and Wickler (2006) showed that only 23% (12 out of 52) of goldfinches and 62% (18 out of 29) siskins could solve the string-pulling task. This evidence highlights that individuals vary in their ability to solve problem, but why do these individual fail? are 'their cognitive abilities simply inferior' (Thornton & Lukas, 2012)?

In a number of studies, non-problem solvers have shown some characteristics that are different from their successful counterparts. While a lack of persistence has been considered as a key factor that relates to unsuccessful problem solving (e.g. Overington et al., 2011; Thornton & Samson, 2012), in some experiments unsuccessful individuals spent similar amount of time on manipulating the task as successful individuals, but still failed to solve the problem (Benson-Amram & Holekamp, 2012). With this in mind, here, I do not rule out the importance of persistence in problem solving. The evidence, however, suggests that persistence is not the only factor in the relationship between behavioural flexibility and behavioural traits. With persistence held constant between individuals that come to the problem solving task, studies have mentioned non-problem solvers consistently using ineffective behaviours that lead to unsuccessful problem solving in a reversal learning task (Leal & Powell, 2011), or to less efficient way in food-extraction task (Hrubesch, Preuschoft, & van Schaik, 2009). This evidence suggests that, in these cases, unsuccessful problem solving is due to behavioural inflexibility; non-solvers do not show the 'change' from

ineffective to effective behaviours.

1.4.2.5 DEVELOPMENTAL CONSTRAINTS, INFLEXIBILITY AND PROBLEM SOLVING

The above section mainly addresses the relation between inflexibility and behavioural traits. However, there are other explanations for behavioural inflexibility in problem solving which investigators should be aware of. Inflexibility could also be due to developmental constraints (e.g. Holekamp, Swanson, & van Meter, 2013). For example, Holekamp and colleagues (2013) mentioned that morphological constraints prohibit carnivores and primates solving a given task. Motor dexterity may also depend on age and body size, and can affect problem solving performance (e.g. Thornton & Samson, 2012; Thornton & Lukas, 2012). Colour bias is seen in some species when it provides ecologically important information, for example, male spotted bowerbirds prefer green colour (Isden, Panayi, Dingle, & Madden, 2013), pikes attack red colour more often than blue colour (Pintor, McGhee, Roche, & Bell, 2014), tropical arboreal lizards chose more black colour than white colour (Leal & Powell, 2011).

1.4.2.6 LEARNING AND FLEXIBILITY: INDEPENDENT MECHANISMS OR ALONG AN AXIS IN PROBLEM SOLVING?

One thing that has not been discussed so far is whether learning and flexibility are linked cognitive processes/behavioural traits or they are independently supporting individuals in problem solving. Some researchers have proposed that learning and flexibility are related to each other, for example, that learning provides additional flexibility (Mery, 2012), is a minor form of flexibility (van Schaik, 2013) or that flexibility is the end product of learning (Dukas, 2013). On the one hand, strictly speaking, learning and behavioural flexibility serve

different purposes in problem solving; learning is slow and gradual process whereas behavioural flexibility is an instant adjustment to demand. On the other hand, learning and behavioural flexibility could lie on a continuum of the need for experience to elicit plasticity. The relationship between learning and flexibility in the context of problem solving can be further illustrated by considering solution types. A solution type can be categorised along the learning-flexibility axis *and* also along an old vs. new behaviours axis. Hence, four types of solutions are generated along these two axes when approaching a problem and the involvement of learning and flexibility varies according to the type of solution: solving an old problem with learned behaviours; solving an old problem with novel behaviours (Kummer & Goodall, 1985); solving a novel problem with learned behaviours (Kummer & Goodall, 1985) and solving a novel problem with novel behaviours (Ramsey, Bastian, & van Schaik, 2007).

1.5 PROPOSED AREAS TO INVESTIGATE THE ‘HOW’

To study the ‘*how*’ of problem solving, I take the variables that have been identified in the ‘*what*’ part and examine *how* flexibility varies in relation to problem solving performance. My core focus of flexibility includes two forms, learning and immediate flexibility. Proposed studies include:

1.5.1 TACTICS USE IN THE SERIAL REVERSAL TASK

We know that the most efficient way to solve a two stimuli reversal learning task is to use the ‘win-stay, lose-shift’ strategy (Shettleworth, 2010, pp.210-212). In WSLS, individuals follow the same stimulus if it is immediately rewarded (win-stay) and shift to the alternative stimulus following non-reinforcement (lose-shift). Although learning a tactic such as WSLS should apparently lead to efficiency, this does not happen immediately. Typically, the formation of a task efficient tactic over the course of learning is progressive, as it gradually

replaces the trial-and-error tactics employed at the start. The advantage of learning an appropriate tactic for a task is that it enables rapid solution of the same problem on future occasions even if individuals no longer remember the specific task information (Bonney & Wynne, 2002), or if specific task information becomes misleading, as is does in reversal tasks. This evidence suggests that learning a tactic provides a route for individuals to *'learn how to learn efficiently'* (Harlow, 1949) and should not be affected by memory. However, this suggestion has yet to be tested.

1.5.2 WHAT ARE THE MECHANISMS UNDERLYING LEARNING THAT CAN AFFECT PROBLEM SOLVING PERFORMANCE?

In order to understand how learning contributes to problem solving, we need to consider underlying mechanisms that support learning. Factors that have been shown to have a substantial effect include:

Attention. Across different study designs, one consistent finding in relation to problem solving performance and the importance of learning is attention to cues. For example, in a serial discrimination-reversal task, individuals need to attend to the cue that is associated with reward. In the novel food extraction task, paying attention to the functional cues (Werdenich & Huber, 2006; Seed, Call, Emery, & Clayton, 2009), properties (e.g. Thornton & Samson, 2012) or using the motor-perceptual feedback (Taylor, Medina, Holzhaider, Hearne, Hunt, & Gray, 2010; Taylor et al., 2010) facilitates goal achievement. Examples including kea paying attention to the end of a string in retrieving the food reward (Werdenich & Huber, 2006), and chimpanzees paying attention to the trap could avoid the food fall into the trap (Seed, Call, Emery, & Clayton, 2009).

Memory, or proactive interference. Previous memories are certainly not completely erased by new experience. However, the influence of previous memories on learning efficiency is not necessarily positive. For example, in a reversal task, on the one hand, improved retention of information from the current phase is implied if individuals learn the reversal faster than the initial acquisition phase (Calhorn & Handley, 1973; Chittka, 1998); on the other hand, memories from the previous phase can proactively interfere with individuals' performance on the current task (e.g. Chittka, 1998; Mackintosh et al., 1968; Strang & Sherry, 2014, but also see Raine & Chittka, 2012).

Tactics: To achieve efficiency, animals usually develop some kind of tactic in a task, depending on the task design. For example, in a serial reversal task with two stimuli with different colours, shapes or sizes that are simultaneously presented to animals, animals would develop a win-stay, lose-shift strategy (WSLS) with successive exposures to the same problem to achieve efficiency. However, some tactic could impair efficiency, for example the position habits ubiquitous in discrimination learning tasks (Mahut, 1954) or colour stimuli that are highly ecologically-related for a species, as described in **Section 1.4.2.5**

Inhibitory control. Inhibitory control is another mechanism that may increase efficiency. For example, in a discrimination-reversal task, an individual needs to inhibit their proponent responses in order to obtain their goal quickly in the reversal phase and hence, it is inhibitory control promotes learning efficiency. When facing any problem that requires individuals to use an alternative solution other than the previous solution, inhibitory control is possibly involved in facilitating the process of learning (e.g. Manrique, Völter, & Call, 2013). Like attention, inhibitory control may not be easily seen, however, researchers can capture individuals' inhibitory control as the increased latency or reduced tendency to respond to the

incorrect cues (Jenkins & Harrison, 1962) and correlated it with learning performance by using advanced technology such as automatic touch screen.

1.5.3 VARIATION OF BEHAVIOURAL TRAITS ACROSS LEARNING AND PROBLEM SOLVING PERFORMANCE

The review above has shown that learning and behavioural flexibility are related to other behavioural traits that play a part in the problem solving process. These varied contributions would expect to affect problem solving efficiency, which is an overlooked measure of performance in many studies. For example, experience can lead to an increase in persistence and in effective behaviours (e.g. Bondi, Bó, & Vassallo, 2008) or to a decrease in behavioural variety (e.g. Benson-Amram & Holekamp, 2012). These changes of behavioural traits would be expected to increase efficiency, but the actual contributions remain to be examined. In order to tease apart their contributions to efficiency, we need to look at tasks that are solvable but are sufficiently complex that efficiency could increase over a substantial number of trials.

1.5.4 A COMPARATIVE STUDY OF PROBLEM SOLVING ABILITY

In **Section 1.4.2.5**, I mentioned that developmental constraints could pose a challenge in task design. It follows that designing a task that is appropriate across a range of different species is difficult. Accordingly, a more probable approach may be to conduct comparative studies of species that belong to the same family using a standardised problem task. The evidence is that this approach is possible, and can reveal inter-species level differences. For example, Webster & Lefebvre (2001) have compared three species in the order Passeriformes included the Carib grackle, *Quiscalus lugubris*, the Lesser Antillean bullfinch, *Loxigilla noctis*, and the shiny cowbird, *Molothrus bonariensis*, and they showed that these

Passeriformes species were more successful than the two Columbiformes species including the zenaida dove, *Zenaida aurita*, and the common ground dove, *Columbina passerina*, in a puzzle box task in which individuals obtained a reward by solving it in one of four available ways. Similarly, tests of flexibility have been carried out by comparing the learning ability of species that belong to the same family using a standardised task. For example, Bond, Kamil, and Balda (2007) used a serial discrimination-reversal task to compare the flexibility of three caching species of corvid, pinyon jays, *Gymnorhinus cyanocephalus*, Clark's nutcrackers, *Nucifraga columbiana*, and western scrub jays, *Aphelocoma californica*, which vary in the complexity of their social dynamics.

As mentioned in the review that behavioural flexibility is crucial for invasive species when establishing new habitats, further work should conduct a comparative study involving an invasive and a non-invasive species that belong to the same genus and between closely related species such as grey and red squirrels that have very different population trends. Experiments could be carried out to try to understand whether the two species are different in their problem solving ability and the behavioural traits that are associated with this ability. By controlling or matching other possible confounding variables from social and biological contexts, the results from this comparison can be strengthened so as to yield a new way of understanding whether invasive species have better problem solving performance than non-invasive species, and if so, why.

1.6 A STANDARDISED TERMINOLOGY FOR BEHAVIOURAL TRAITS

This section aims to standardise the measurements that will be used throughout the thesis. Measurements are defined and are recorded as direct behavioural reaction towards the problem task.

Persistence: Griffin, Diquelou and Perea, 2014 (2014) measured persistence as the actual number of attempts to solve a problem on each trial. However this is confounded with the solution time. Accordingly, we followed Biondi, Bó, and Vassallo (2008), who measured persistence as the *rate* of attempts to solve the problem, i.e. the number of attempts at solution in a given trial divided by the solution time on that trial.

Behavioural variety: The usual definition of behavioural variety is the total number of different behaviours emitted in a given trial (e.g. Benson-Amram & Holekamp, 2012; Griffin, Diquelou, & Perea, 2014), and we recorded this quantity.

Behavioural selectivity: In the light of the literature cited above, we were also interested in the proportion of the behaviours emitted that were potentially effective. In addition to the three variables discussed above, therefore, we measured the proportion of effective behaviours in a given trial or successful problem solving.

Flexibility: As mentioned in **Section 1.4.2.3**, we measured flexibility in terms of the number of changes between defined behaviours the squirrel made in a given trial. However, it is clear that, as with persistence, the number of switches between tactics on a trial will almost inevitably be confounded with the solution time for the trial – the longer it takes the animal to solve the problem, the more chance it has to switch tactics. Accordingly, we measured flexibility by the rate of switching, calculated by dividing the number of switches between tactics on a trial by the solution time for that trial.

1.7 THE ‘WHO’: GREY SQUIRRELS AS A STUDY SPECIES

Throughout the thesis, grey squirrels are my primary study species, although red squirrels are included in a comparative experiment. Both grey squirrels and red squirrels belong to the same family, *Sciuridae*, and same genus, *Sciurus*, and have a higher brain-to-body size than other rodents (Mace, Harvey, and Clutton-Brock, 1981). The two species are mostly solitary and share many ecological characteristics, see Koprowski (1994) for grey squirrels and Lurz, Gurnell, & Magris (2005) for red squirrels. Despite these similarities, grey squirrels expanded their population quickly and have replaced red squirrels in their habitats since the 19th century in UK (Grunell, 1987; Gurnell, Wauters, Lurz, & Tosi, 2004) and more recently elsewhere in Europe (Huxley, 2013). Grey squirrels are regarded as one of the ‘100 World’s Worst Invasive Alien Species’ (Global invasive species database, 2015) and especially in U.K, the population of grey squirrels is still increasing and is predicted to expand to other European countries in the next 50 years (Huxley, 2003).

Evidence reviewed above indicates invasive species possess high behavioural flexibility. Accordingly, grey squirrels are assumed to have high flexibility in adapting to novel environment and this implies that they have good problem solving ability.

Other reasons include grey squirrels are scatter-hoarders that flexibly adjust their caching managements when they encounter socio-ecological challenges. For example, field studies have shown that grey squirrels flexibly adjust their food protection strategies when conspecifics (Hopewell & Leaver, 2008; Hopewell, Leaver, & Lea, 2008; Leaver, Hopewell, Caldwell, & Mallarky, 2007, Steele et al., 2008) and heterospecifics (Schmidt & Ostfeld, 2008) are present. This evidence shows that grey squirrels respond flexibly to the social context, but to what extent it is a result of general cognitive flexibility is less clear.

Additionally, food extraction problem solving tasks should not be a constraint for them, as grey squirrels have flexible motor skills, and use their front paws and mouth to manipulate objects such as twigs in the natural environment and bird feeders in the anthropogenic environment.

Finally, given that grey squirrels are active around all the year and in urban parkland are well habituated to human, it is easy to observe them. So they are a reliable subject in which we can address multiple different problem solving questions.

1.7.1 SQUIRREL COGNITION

To date, most of studies on sciurid cognitive ability have focused on caching behaviours, cache decision and cache management, in fox squirrels, *Sciurus niger*, (e.g. Delgado, Nichols, Petrie, & Jacobs, 2014) and in grey squirrels, *S. carolinensis* (e.g. Spritzer & Brazeau, 2003; Steele et al., 2006; Steele et al., 2011); intraspecific cache pilferage and pilfering behaviours in American red squirrels, *Tamiasciurus hudsonicus* (e.g. Gerhardt, 2005), interspecific pilfering behaviours least chipmunks, *Tamias minimus*, and eastern chipmunks, *Tamias striatus* (Penner & Devenport, 2011), and interspecific food competition in grey and red squirrels, *S. vulgaris* (e.g. Wauters, Grunell, Martinoli, & Tosi, 2001; Wauters, Tosi, & Grunell, 2002).

There is also evidence of social cognition in squirrels, specifically the effect of social contexts on caching strategies and management in grey squirrels (Hopewell & Leaver, 2008; Hopewell, Leaver, & Lea, 2008; Leaver, Hopewell, Caldwell, & Mallarky, 2007, Schmidt & Ostfeld, 2008; Steele et al., 2008), social learning in choosing food reward in grey squirrels

(Hopewell, Leaver, Lea, & Wills, 2009) and food handling in red squirrels (Weigl & Hanson, 1980).

Although there is currently limited evidence indicating how sciurids remember cache locations and status, it is clear that they do have an accurate memory of the locations of their caches. This has been shown in yellow pine chipmunks, *Tamias amoenus* (Vander Wall, 1991; 2000), in thirteen-lined ground squirrels, *Spermophilus tridecemlineatus* (Devenport, Luna, Devenport, 2000), in grey squirrels (Jacobs & Liman, 1991; Macdonald, 1997), though less accurate memory is held about caches in red squirrels (Macdonald, 1997). Their cache recovery has been shown to mainly rely on spatial memory and to some extent, olfactory cues, in fox squirrels, (Jacobs & Liman, 1991) and in grey squirrels (Macdonald, 1997; McQuade, Williams, & Eichenbaum, 1986). This is also true for yellow pine chipmunks pilfering caches (Vander Wall, 2000).

There is some evidence about learning and flexibility. Previous studies have used successive object discrimination learning tasks (e.g. using small toys or jewellery as discriminanda) in which the same reward contingency is applied to a new pair of stimuli in each discrimination phase (e.g. Harlow, 1949). Fox squirrels and round-tailed ground squirrels, *Citellus tereticaudus*, failed to learn any task after the first discrimination phase in such experiments (Flaningam, 1969; Rees, 1968). Flexibility has been shown in using spatial cues. Beacon, global and relative array cues are interchangeable in southern flying squirrels, *Glaucomys volans* recovery hidden food (Gibbs, Lea, & Jacobs, 2007), in fox squirrels (Waisman & Jacobs, 2008) and in Columbian ground squirrels, *Spermophilus columbianus* (Vlasak, 2006).

In summary, although the literature on squirrel cognition is limited, enough is known to provide an overview for the experiments to be reported here.

1.8 THESIS OVERVIEW

This thesis primarily uses grey squirrels to address the research areas that have been mentioned in **Section 1.5**. **Chapter 2** mainly examines under a recurring change whether squirrels are '*learning to learn efficiently*' in a serial spatial reversal learning task? If so, how do they do it? **Chapter 3** aims to examine the learning mechanisms, attention and inhibition control, that support squirrels to achieve learning efficiency using a colour reversal task on touch screen. **Chapter 4** examines learning and other three behavioural traits in the context of a novel food extraction problem solving task using a puzzle box. The design of the puzzle box intended to have the properties mentioned in **Section 1.5.3**. **Chapter 5** conducted a comparative study in grey and red squirrels. I aimed to gain ecological validity for the results in the laboratory as well as to examine whether there are differences between the problem solving ability and its related behavioural traits among the squirrels from two species.

**CHAPTER 2: SERIAL REVERSAL LEARNING IN GREY SQUIRRELS:
LEARNING EFFICIENCY AS A FUNCTION OF LEARNING AND CHANGE OF
TACTICS.¹**

ABSTRACT

Learning allows individuals to adapt their behaviours flexibly to a changing environment. When the same change recurs repeatedly, acquiring relevant tactics may increase learning efficiency. We examined this relationship, along with the effects of proactive interference and other interference information, in a serial spatial reversal task with five grey squirrels (*Sciurus carolinensis*). Squirrels completed an acquisition and 11 reversal phases with a poke box in which two out of four possible reward locations were baited diagonally in a square array. In this situation, an efficient tactic is to locate the diagonally related locations consecutively (integrative search tactic) instead of searching rewards in a clockwise or anti-clockwise direction (sequential search tactic). All the squirrels formed a learning set acquiring successive reversals in fewer trials. Although four individuals gradually employed more integrative tactics in locating the rewards both within and between phases, sequential tactics were used in the first trial of each phase. This suggests the integrative tactic did not depend on an association between the rewarded locations but was learned as a spatial pattern and/or by use of extra-apparatus cues to locate individual rewards. Generalized Estimating Equation (GEE) models showed that learning efficiency increased with experience and tactic change. Although tactic change partially mediated the effect of learning on learning efficiency, learning retained an independent contribution to improved efficiency. Squirrels that used more integrative tactics made fewer total errors than squirrels that used less integrative tactics; suggesting learning a task relevant tactic using spatial cues can provide direct benefits in maximising rewards and minimising time costs.

¹The content of this chapter is published: Chow, P. K. Y., Lea, S. E. G., & Leaver, L. A. (2015). Serial reversal learning in grey squirrels: learning efficiency as a function of learning and change of tactics. *Journal of Experimental Psychology: Animal Learning and Cognition*, 41, 343-353.

2.1 INTRODUCTION

Learning provides a mild form of flexibility by which individuals can adapt their behaviours according to environmental demands or changes (van Schaik, 2013). The occurrence of learning, as argued by Dukas (2013), confers advantages on a variety of fitness measures. Such ultimate gain for fitness presumably outweighs the inevitable time and effort costs of the learning process, especially when the demand or change recurs. Hence, it is important to understand the learning process, in particular how animals learn and how they learn '*how to learn efficiently*' (Harlow 1949, p.51) under such recurring changes.

To assay flexibility in the learning process under recurring change, investigators have often used discrimination reversal learning (Shettleworth 2010, p. 210-211). Pavlov (1927) introduced the reversal learning paradigm, in which the reinforcement contingency switches between two stimuli. In the acquisition phase, individuals need to associate one of two stimuli with a reward (A- B+). Once they reach a predetermined learning criterion, the reinforcement contingency is switched, for a reversal phase in which the previously unrewarded stimulus becomes rewarded while the previously rewarded stimulus becomes unrewarded (A+ B-). In *serial* reversal learning, the reinforcement contingency repeatedly switches between the two stimuli (e.g. Mackintosh & Cauty, 1971). Under such recurring change, a wide range of species (Warren, 1965, 1974) have been shown to reduce the number of errors across successive reversals. Such a trend indicates that individuals have increased their learning efficiency, thus increasing the reward gain and reducing the time cost, with cumulative experience (Flaningam, 1969). Harlow (1949) called such gains in learning efficiency across repeated tasks of the same type 'learning set' or 'learning to learn'.

So, how do animals improve their efficiency under recurring change? Previous research has consistently shown that attention and memory are the key factors (Shettleworth, 2010).

Attention. Selective attention to the rewarded cue (e.g. Mackintosh, Brendan, & Valerie, 1968; Mackintosh & Little, 1969) or to local feedback (e.g. Rayburn-Reeves, Stagner, Kirk, & Zentall, 2013) is an important factor in completing the reversal learning task more efficiently. Appropriately directed attention allows animals to achieve efficiency by making associations between the relevant cues and the rewards, but this is more likely to happen if the experimental design is ecologically relevant to the study species. For example, bumblebees associate olfactory cues with food rewards in an olfactory reversal paradigm (e.g. Mota & Giurfa, 2010) and rats associate extra- or intra- apparatus distance cues with the goal in a spatial learning task (e.g. Kraemer, Gilbert, & Innis, 1983).

Memory. Previous memories are certainly not completely erased by new experience. However, the influence of previous memories on learning efficiency is not necessarily positive. On the one hand, improved retention of information within the current phase is implied if individuals learn the reversal faster than the initial acquisition phase (e.g. Calhoun & Handley, 1973; Chittka, 1998). On the other hand, memories from the previous phase can proactively interfere with individuals' performance on the current task (e.g. Chittka, 1998; Mackintosh et al., 1968; Strang & Sherry, 2014, but also see Raine & Chittka, 2012).

These factors, however, concern the mechanisms involved in learning each reversal task; they do not as such allow for the formation of learning set, or any other form of increased flexibility in the learning process. Increased flexibility during the course of learning

could, however, result from a change in the response strategies or tactics that an individual uses in solving a task.

Tactic change. A tactic can be considered as a specific behavioural pattern that an individual shows in responses to a task. The best illustration of how changing of tactics could increase efficiency comes from the typical two stimulus serial reversal task; the most efficient tactic for maximising reward gain under this design is the ‘win-stay, lose-shift’ (WSLS) strategy (Shettleworth, 2010). In WSLS, individuals follow the same stimulus if it is immediately rewarded (win-stay) and shift to the alternative stimulus following non-reinforcement (lose-shift). Depending on the reversal paradigm, the manifestation of such behavioural pattern may reflect several possible cognitive processes: the individual has learned an associative rule between a stimulus and a reward as in a two stimulus serial reversal task, the individual has formed a spatial relationship between the rewarded locations as in a spatial pattern learning (e.g. Brown & Terrinoni, 1996; Brown, Zeiler, & John, 2001), the individual is using intra- and/or extra- apparatus cues to remember rewards individually, or the individual is using any combination these of mechanisms. Although learning a tactic such as WSLS should lead to improved efficiency, it does not happen immediately. The formation of a task efficient tactic over the course of learning is progressive, as it gradually replaces the trial-and-error tactics employed at the start. In learning paradigms other than two-stimulus reversal, individuals may employ more than one tactic in a given learning phase, which allows us to measure change of tactics by observing the proportions in which given tactics are exhibited during the course of learning. Learning an appropriate tactic for a task has been shown to be advantageous for solving the same problem in future occasions even if individuals no longer remember the specific task information (Bonney & Wynne, 2002), or if specific task information becomes misleading, as it does in reversal tasks. This evidence

suggests that learning a tactic is one way in which individuals can '*learn how to learn efficiently*' (Harlow, 1949).

In the present study, we first examined whether Eastern grey squirrels (*Sciurus carolinensis*) would show a learning set in the serial reversal task. Previous studies of learning set in the *Sciuridae* family have used successive discrimination learning tasks, in which the same reward contingency is applied to a new pair of stimuli in each discrimination phase (e.g. Harlow, 1949). In this task, fox squirrels, *Sciurus niger*, and round-tailed ground squirrels, *Citellus tereticaudus*, failed to learn any task after the first discrimination phase (Flanigan, 1969; Rees, 1968). However, the response strategies that are readily learned are likely to be those that are ecologically relevant to the species in question (e.g. Day, Crews, & Wilczynski, 1999; Liedtke & Schneider, 2014; Mota & Giurfa, 2010), and these studies all used discrimination of objects (e.g. small toys or jewellery), which is not an obviously ecologically relevant ability for sciurids. To accommodate squirrels' natural learning style, we utilized spatial learning, which is certainly ecologically relevant for Eastern grey squirrels since they are scatter hoarders. Squirrels were required to remember which two of four locations contained food (see *Methods*). The four locations were arranged in a square and the two rewarded locations were always at opposite ends of a diagonal; reward contingency was only switched between the two diagonal pairs of wells across phases. We recorded the sequence in which the squirrels visited them, so that we were able to categorise the sequences as resulting from two different types of tactics, sequential search tactics (Fig. 2.1a) and integrative search tactics (Fig. 2.1b). Under this set up, the efficient way to maximise the gain and minimise the time costs was to use integrative search tactics. If squirrels formed a learning set in this situation, we would then be able to examine how they achieved the improved efficiency across the reversal phases and the possible cognitive processes

underlying the factor(s), in particular whether changing to integrative tactics made a contribution. Figure 2.2 illustrates the predictors that would potentially affect the learning efficiency in this serial reversal task. We examined how each predictor varied across the learning process and its relationship to learning efficiency.

We chose grey squirrels as a study species because field studies have shown that they adjust their food protection tactics flexibly under intra-conspecific food competition (Hopewell & Leaver, 2008; Hopewell, Leaver, & Lea, 2008; Leaver, Hopewell, Caldwell, & Mallarky, 2007; Schmidt & Ostfeld, 2008; Steele, et al., 2008), so it is reasonable to suppose that this species would also show flexibility in spatial learning. Also, grey squirrels are scatter-hoarders and cache thousands of nuts every year (Thompson & Thompson, 1980), so the number of locations they were required to remember in this task should not pose a problem for them. Moreover, although there is currently limited evidence indicating how squirrels remember cache locations and status, it is clear that they have an accurate memory of the locations of their caches (Jacobs & Liman, 1991; Macdonald, 1997) and they can update this memory to reflect the current state of each cache (unused, used, or pilfered). Finally, given that the food preferences of grey squirrels imply that they tend to maximise energy gain (Smith & Follmer, 1972), the use of a highly preferred food reward should lead to rapid learning.

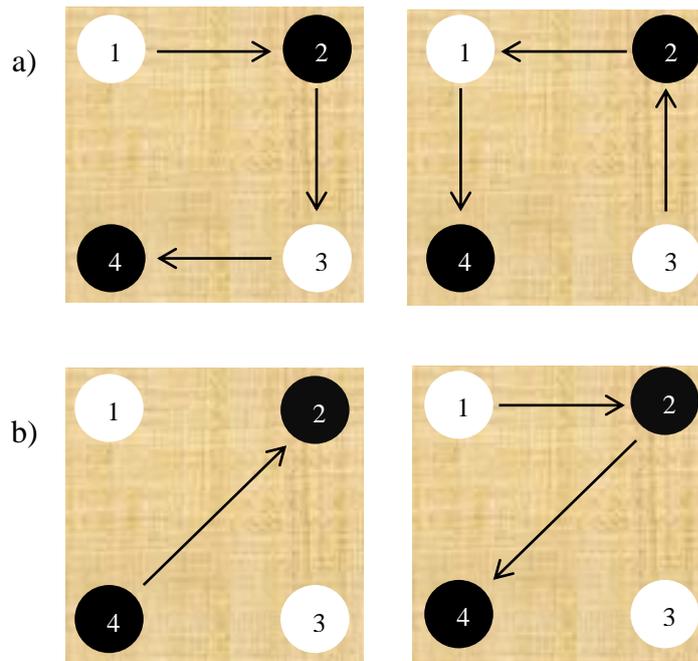


Figure 2.1. Examples of response strategies. Circles filled with black colours are rewarded wells while white colour circles are non-rewarded wells. a) two examples show the use of sequential strategy in this task. Choice directions are made from either clockwise (left) or counter-clockwise (right). Note that both responses are *incorrect*; b) two examples show the use of integrative strategy in this task. Squirrels may show *correct* response in choosing the diagonal pair (left) in the consecutive choices or using integrative strategy after making the *incorrect* choice (right).

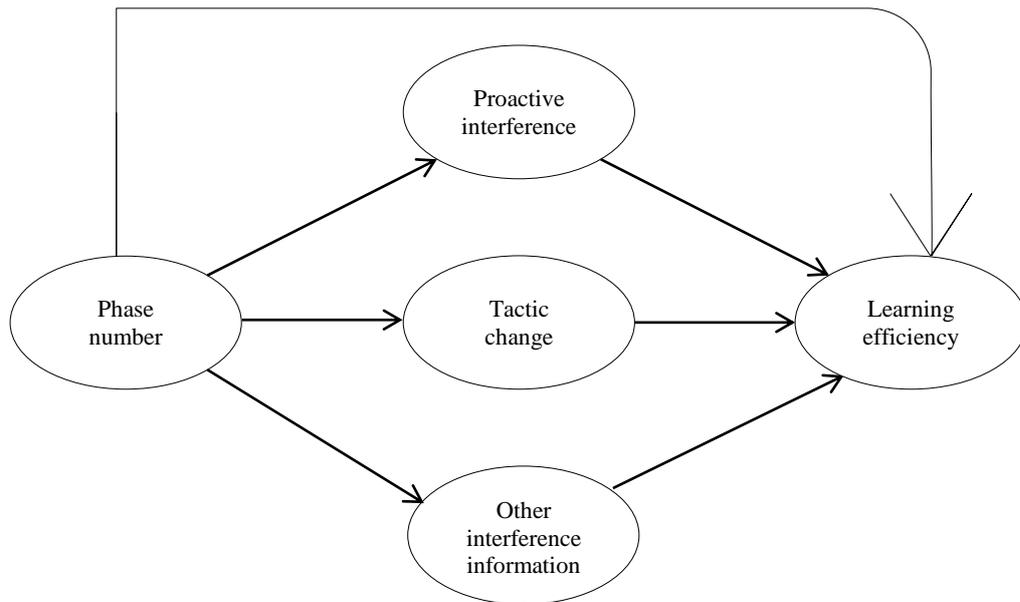


Figure 2.2. This figure shows the hypothetical model between all the variables. Covariates are phase number, tactic change, proactive interference, and irrelevant interference behaviours. Dependent variable is learning efficiency.

2.2 METHODS

Subjects

Five captive squirrels (three males and two females) housed at the University of Exeter were used in this study. They were housed in large cages, from which they could be given access to the test room via an overhead mesh tunnels controlled by sliding doors (for full details, see Hopewell, Leaver, Lea, & Wills, 2010). Accordingly, the squirrels were not handled directly in the experimental procedures or normal husbandry. They were not food deprived during the experiment. Water was provided *ad libitum* and their daily diet included fresh fruit, pumpkin seeds, sunflower seeds, dried vegetables and tiger nuts. Data collection was from Mar-July, 2014. Experiments were conducted when squirrels were most active, usually during 0700-0900 and 1400-1700. This study was approved by the Ethical Review Group at the University of Exeter. Squirrels were treated in accordance with Association for the Study of Animal Behaviour guidelines on animal welfare and UK law.

Apparatus

Fig. 2.3a and 2.3d show the apparatus (hereafter, the poke box). It was a square wooden box (length x width x depth dimension: 21cm x 21cm x 4.5cm) composed of four layers. Layers (from top to bottom) consisted of an aluminium plate (21cm x 21cm x 0.1cm), a wooden *upper* container (21cm x 21cm x 1.8cm), a piece of metal mesh (21cm x 21cm x 0.1cm), and a wooden *base* container (21cm x 21cm x 2.7cm). The entire assembly was secured with wing-nuts. As Fig 2.3b shows, the upper and base containers had 16 food wells (each was 4.5cm in diameter and 0.6cm gap between wells), with four wells in a row and divided by the metal mesh. As Fig. 2.3c shows, the metal plate had 12 holes of the same diameter, corresponding with the food wells.

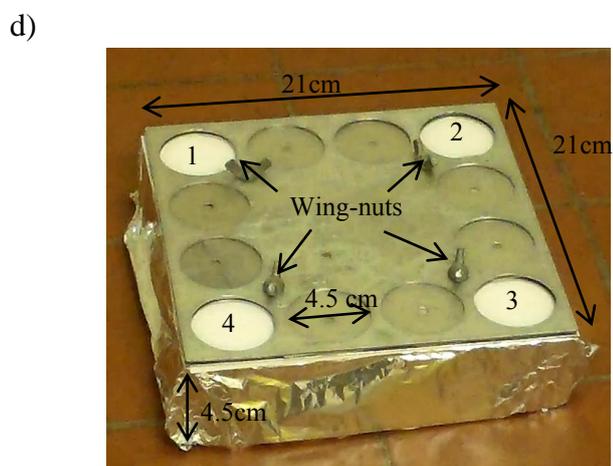
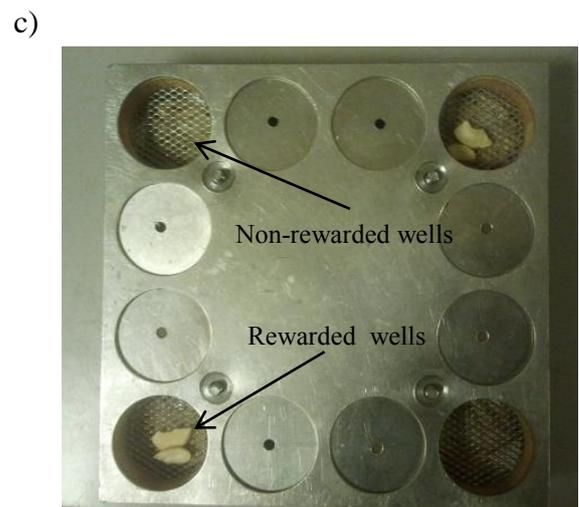
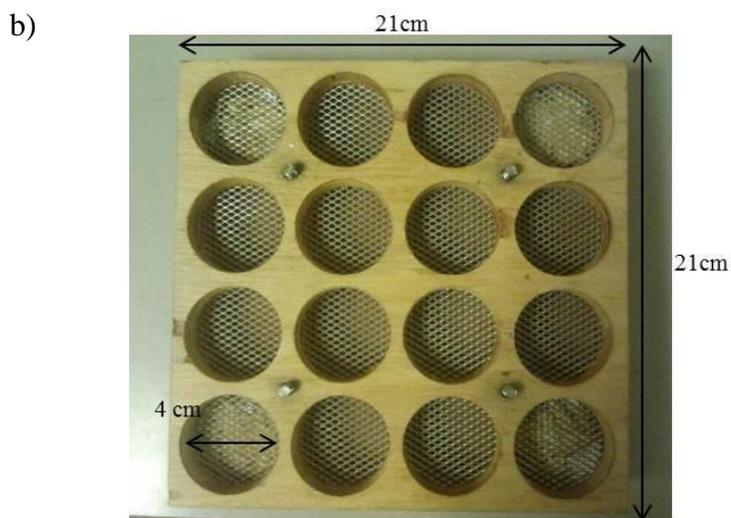
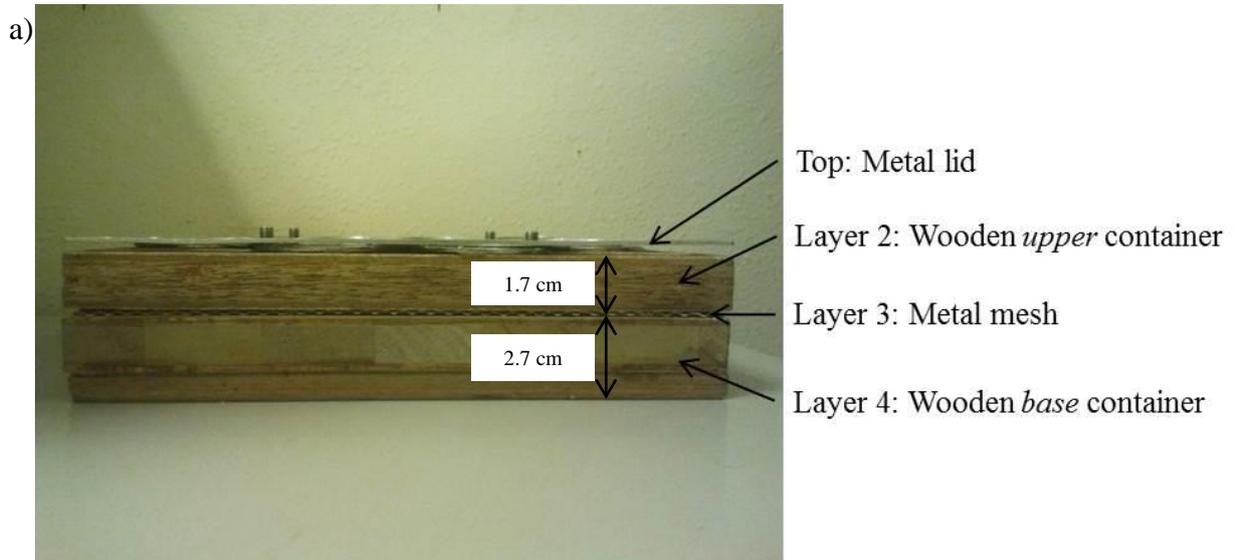


Figure 2.3. a) this figure shows the poke box is composed by four layers. The top part is a metal plate, followed by a wooden *upper* container, then a metal mesh, and finally a wooden *base* container. b) this figure shows that all the layers have 16 compartments, except the metal mesh which is used to separate the upper and the base container. c) only four wells that are located at the corner are used during the experiment. Reward locations and non-rewarded (control) locations are in diagonal direction; d) the finally prepared poke box used in the main experiment, a white sheet and two foil sheets (21cm x 21cm). Four wing-nuts are used to secure the whole apparatus. The number of the white paper indicates each well-corresponded number.

Procedures

Pre-training

Squirrels went through standardised pre-training before the main experiment. In the pre-training, we used all 12 food wells. Cheerio pieces (Nestlé® Cheerios Cereal) or pine nuts (according to the known food preference of each squirrel) were placed in the base container of each well as a control for olfactory cues. We covered these baits with the metal mesh so that squirrels could smell but could not eat the food. We then baited the upper container with accessible food rewards, either one-third of a Cheerio or one pine nut per food well. A sheet of aluminium foil (21cm x 21cm) and a sheet of white paper (21cm x 21cm) were placed between the upper container and the metal plate. This aimed to further minimise the chance of squirrels using olfactory cues in locating the hidden food.

Pre-training was divided into four stages: opened-well stage (habituation), crossed-stage, diagonal-cut stage, and closed-well stage. The first stage aimed to encourage squirrels to come close to the apparatus and to obtain food from the wells. No foil was used and the

paper had holes corresponding to the food wells. In the next two stages, the crossed-stage and diagonal-cut stage, we aimed to allow squirrels to gain experience of peeling off the paper or using their front paws to scratch to open the wells by themselves. No foil was used in these stages. In the crossed-stage, there were two perpendicular diagonal cuts in the paper above each food well. In the diagonal-cut stage, a single cut was used and the diameter of this cut was gradually reduced. In the final stage, the closed-well stage, foil was introduced and the paper was not cut, so that the squirrels had to open the wells either by their claws or teeth. Pre-training was conducted on alternate days with three trials per pre-training day, and each trial lasted for a maximum of 10 minutes (30 minutes in total per training day). The first trial of each day was a repeat of the previous training stage. Individuals advanced to the next training stage after they had successfully obtained all baits across three trials. At the end of each trial, we slowly approached the testing squirrel, removed the poke box, and re-baited the food wells outside the test room.

Training

The same poke box and a similar procedure to that in the pre-training stage were used in the training phase. The training phase used only the four wells at the corners of the poke box (the other wells were capped) and we changed the food reward to hazelnuts or cashews, depending on each squirrel's preference, to increase their motivation. Two half hazelnuts were used for four squirrels and two pieces of cashews were used for one squirrel. As in the pre-training phase, we controlled for olfactory cues by first baiting all four base wells with the corresponding food rewards, either hazelnuts or cashews, under the mesh. As shown in Fig. 2.3c, we minimised any side preference by placing baits in diagonally opposite wells while the wells on the opposite diagonal were empty. We further minimised olfactory cues by placing two sheets of foil, instead of a single sheet, between the metal plate and the upper

container and rotating the box randomly between trials. Fig. 2.3d shows the poke box as finally prepared for the training phase. The poke box was then put in the centre of the test room so as to equalise the distance between the box and the corners of the testing room. The sides of the box were always parallel to the walls of the testing room, and well numbers were defined in terms of their location relative to the testing room, e.g. well 3 was the one nearest to the corner between the side wall and the door. Squirrels could therefore use structures in the test room as extra-apparatus cues to identify the rewarded wells.

There were 12 phases in total (an acquisition phase and 11 reversals). We tested one squirrel at a time and pseudo-randomized which diagonal pair of wells (either wells 1 and 3 or wells 2 and 4, Fig. 2.1a and 2.1b) was positive for a squirrel in the acquisition phase. The learning criterion was three consecutive correct trials. Correct trials were those in which squirrels obtained food from both of the rewarded wells as their first and second choices, without choosing any non-rewarded wells before or between choices of the rewarded wells. At each reversal, both wells that had previously been rewarded became non-rewarded and vice versa.

A trial started when a squirrel approached the poke box. A well selection was indicated by the squirrel tearing the corresponding paper and the foil sheet. The trial ended when the squirrel moved 25 cm away from the poke box or had not obtained a reward for 10 minutes. Squirrels received a maximum of four trials each day, depending on their motivation. All the behavioural responses were captured by a video camera (Panasonic SHD-90) that was set adjacent to the cage.

To minimise the possibility of squirrels learning the location of rewards from direct observation, the experimenter (the first author) approached the box quietly and removed it for re-baiting outside the test room after each trial. As squirrels can use odour cues to locate caches (Jacobs & Liman, 1991; Macdonald, 1997), we randomised the orientation of the poke box for the next trial to avoid any odour cues being left on the poke box which might aid in locating rewards. We then applied disinfectant on the poke box using wipes after we re-baited the wells so as to minimise any odours left by the experimenter. The whole set up procedure did not last longer than two minutes. The next trial began after the experimenter quietly approached the test cage and placed the poke box in the centre of the test cage. We re-applied the disinfectant procedure before the next squirrel was tested. This aimed to minimise the scent that the previous squirrel left on the poke box, which might affect the decision making of the next individual tested.

Measurement

Learning efficiency. We measured learning efficiency as the number of errors (trials in which a squirrel opened either unbaited well before or between opening any baited wells) that a squirrel made in each phase.

Proactive interference. To examine whether squirrels' performance was affected by proactive interference from the previous reward contingency, we counted the number of non-rewarded first choices across trials and divided this number by the total number of trials.

Learning tactics. To examine the tactics that a squirrel employed in the task, we recorded the sequence of wells that the squirrel chose in each trial for each phase. Each trial was categorised as using either sequential or integrative tactic. Trials were counted as errors if the squirrels visited either non-rewarded well before it had visited both rewarded wells. Fig. 2.1a shows examples of a sequential tactic, in which squirrels made choices in clockwise or anti-

clockwise directions with no diagonal transitions. Fig. 2.1b shows examples of an integrative tactic, in which the squirrels followed a diagonal direction between two choices. Amongst the tactics, only the left-panel of integrative tactic of Fig. 2.1b shows the most efficient tactic for this task, and this was accordingly considered as the correct response. Incorrect responses could be made while using either of the tactics: Fig. 2.1a shows how the incorrect responses could be made by a sequential tactic while the right-panel of Fig. 2.1b shows how the incorrect responses could be made by using an integrative tactic. We further calculated the proportion of integrative tactics used in each phase by dividing the total number of integrative tactics (both correct and incorrect) by the total number of trials taken in each phase. This calculation included the last three (criterion) trials, in order to include the data from one squirrel that showed no errors in two phases.

Other interference information. We included a measure of possible interference information that might affect learning efficiency. In each correct trial we counted the number of wells that the squirrels opened after opening the rewarded wells. We then divided the total number of extra wells opened by the number of correct trials in each phase to obtain the rate of irrelevant behaviours induced by interference for each squirrel.

Data analysis

Page's trend test (Page, 1963) was used to test the change in learning efficiency across phases. Wilcoxon signed rank tests were used to examine whether squirrels took more trials to learn one of the diagonal pairs rather than the other and to compare the number of errors in the first reversal phase with the acquisition phase.

To assay proactive interference, binomial tests were applied to each individual. We tested whether the proportion of first choice preferences for a rewarded well was different from the 50% expected by chance in the acquisition phase and the first reversal phase. We then pooled the significance levels across the five individuals using Fisher's formula ($-2 \sum \ln(p)$; Sokal & Rohlf, 1995, p. 794) to obtain a χ^2_{10} value to test the null hypothesis that all the squirrels behaved in accordance with chance. To minimise any possible bias, the first trial and the last three criterion trials of each phase were excluded, as the first trial in the acquisition phase was a random choice and the first trial of the first reversal phase was immediately affected by the previous contingency.

For each squirrel in each phase, we obtained the proportion of trials in which integrative tactics were used, and we used Page's test to examine the trend in the proportion of integrative search tactics employed across phases. We used a Spearman's rank correlation coefficient to examine whether the squirrels' tendency to use integrative tactics was correlated with its overall number of errors in completing the reversal task.

To assay the hypothetical model in Fig. 2.2, a Generalized Estimating Equations (GEE: Hardin & Hilbe, 2003; Liang & Zeger, 1986) analysis with exchangeable "working" correlations was used. GEE is a marginal model for population-averaged parameter estimation based on the quasi-likelihood using robust "sandwich" variance for hypothesis testing as well as considering the individual correlations under repeated measures. Under mild regularity conditions, the parameter estimates are consistent and asymptotically normal even under the misspecified "working" correlation structure of the responses. As GEE modelling with small samples can underestimate the true variance of the sample, we applied Wang and Long's (2011) adjusted variance estimator, which has been proven to provide

robust inference for datasets with extreme small-sample size and comprised entirely of repeated measures, as in our case (Wang & Long, 2011). Table 2.1 shows the covariates and the corresponding measurement used for the GEE models. To compare the effect size of the predictors, we standardised the covariates (phase number, tactic change, proactive interference and other irrelevant interference behaviours) in each phase, but not the dependent variable, learning efficiency. We used the Poisson distribution for count dependent variable, learning efficiency, and the Gaussian distribution for other continuous dependent variables (e.g. tactic change). We did not test any interactions so as not to exhaust the degrees of freedom. R version 2.15.2 (R Development Core Team, 2012) was used to analyse the data; the ‘gee’ package was used to apply GEE (Carey, 2015), and ‘geesmv’ package was used to get adjusted variance estimator (Wang, 2015) and the ‘crank’ package was used to apply Page’s trend test (Lemon, 2014). All the tests were two-tailed with significance level as $\alpha = .05$.

Standardised covariates	Measurements
Phase number	Total 12 phases, includes an acquisition phase and 11 reversal phases.
Proactive interference	The average number of non-rewarded first choice in each trial of each phase. This variable also excludes the first trial in each phase.
Tactic change	The average of sequential search tactics across trials of each phase; this recorded the change of sequential search tactic in proportion across phases. This measurement includes the last three criterion trials in order to maximise squirrels that show zero error in a phase.
Irrelevant interference behaviour	The average of extra choices made after an individual made all the correct choices.

Table 2.1. *Standardised covariates and its corresponded measurement for the first GEE model to examine the predictors for learning efficiency.*

2.3 RESULTS

Learning efficiency

All the squirrels completed 11 reversals. Fig. 2.4 shows that individual squirrels made fewer errors as the 12 phases progressed, and this trend was significant (Page trend test: $\chi^2(1)=18.31, p<0.001$). Squirrels did not require more trials to learn one diagonal pair of wells than the other (Wilcoxon signed rank test: $p=0.313$), nor did they require more trials to learn the acquisition phase than the first reversal phase (Wilcoxon signed rank test: $p=0.625$).

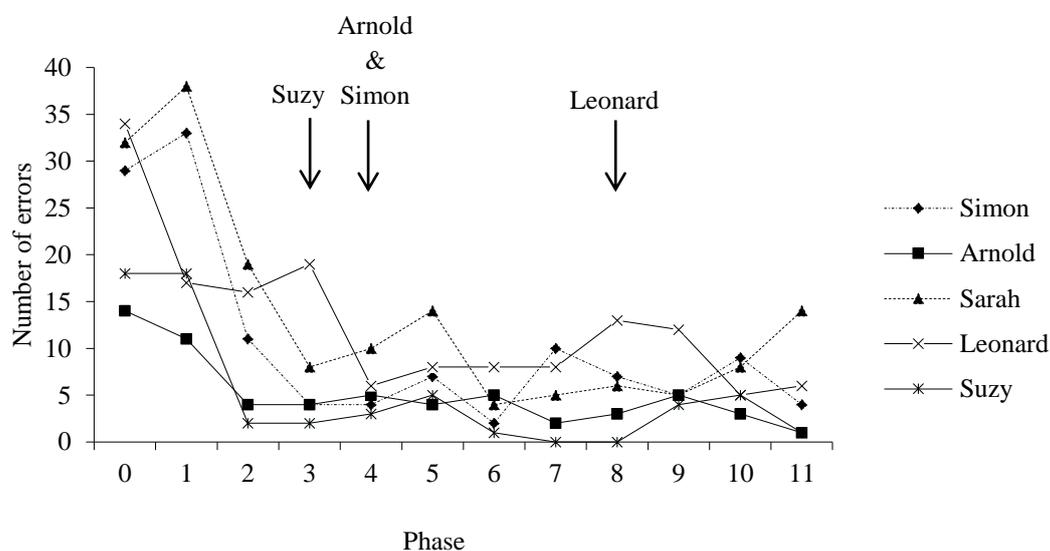


Figure 2.4. This figures show the number of errors across the acquisition phase (phase 0) and the 11 reversal phases; each arrow indicates the phase that a squirrel significantly starts to use more integrative strategy over sequential strategy. *** $p<0.001$.

Proactive interference

Fig. 2.5a shows that when the first trial and the last three criterion trials of each phase were excluded, the proportion of squirrels' first choices in each trial for one of the rewarded wells was greater than chance in the acquisition phase, and this trend was significant (77%; Fisher's pooled: $\chi^2(10)=65.43, p<0.001$). Fig. 2.5b shows that when the first trial and the last three criterion trials were excluded, the proportion of trials on which squirrels' first choice for

any of the rewarded wells was also significantly greater than chance in the first reversal phase (69%; Fisher's pooled: $\chi^2(10)=20.56, p<0.02$).

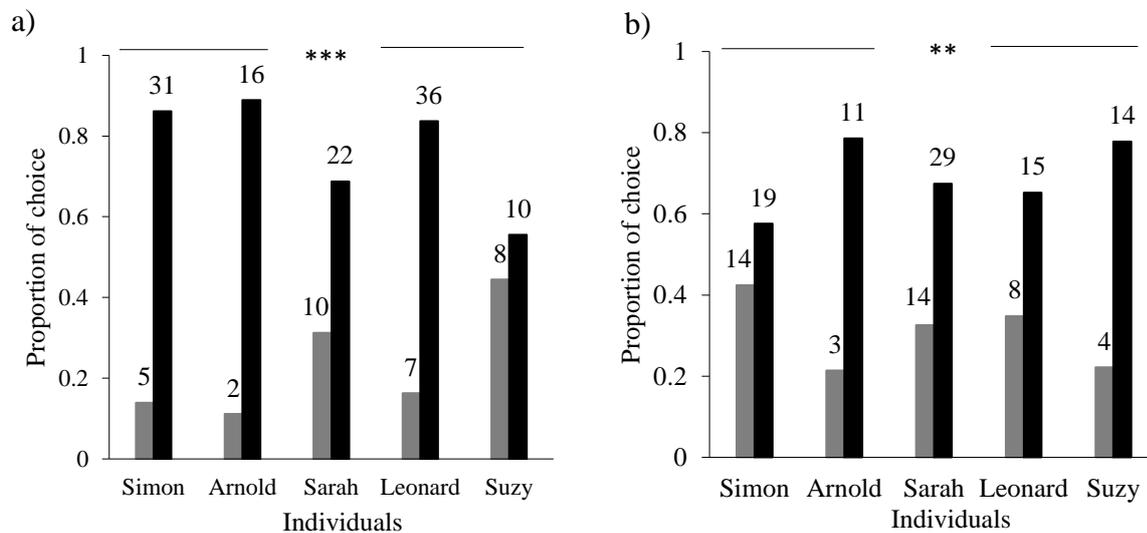


Figure 2.5. a) proportion of choices between the non-rewarded wells (■) and the rewarded wells (■) in the *acquisition* phase; b) proportion of choices between the non-rewarded wells (■) and the rewarded wells (■) in the first *reversal* phase. Note that the number above each bar indicates the trial numbers that excluded the three criterion trials in *acquisition* phase and a further exclusion of the first trial in the first *reversal* phase. *** $p<0.001$; ** $p<0.02$.

Tactic change

Fig. 2.6a shows that the proportion of integrative tactics used increased across phases, and this trend was significant (Page trend test: $\chi^2(1)=8.11, p<0.005$). The mean proportion of integrative tactics used by a squirrel across all phases was positively correlated with the overall errors it made to complete all the reversals ($r_s=0.7$), but this result was not significant. Fig. 2.6b shows the proportion of first trials across 12 phases on which the squirrels used sequential and integrative tactics. Overall, squirrels tended to use the sequential tactic rather than the integrative tactic on the first trial of each new reversal (Fisher's pooled: χ^2

(10)=23.88, $p<0.01$); however, one squirrel, Suzy, used the integrative tactic more often than the sequential tactic, although this trend was not significant (binominal test: $p=0.388$).

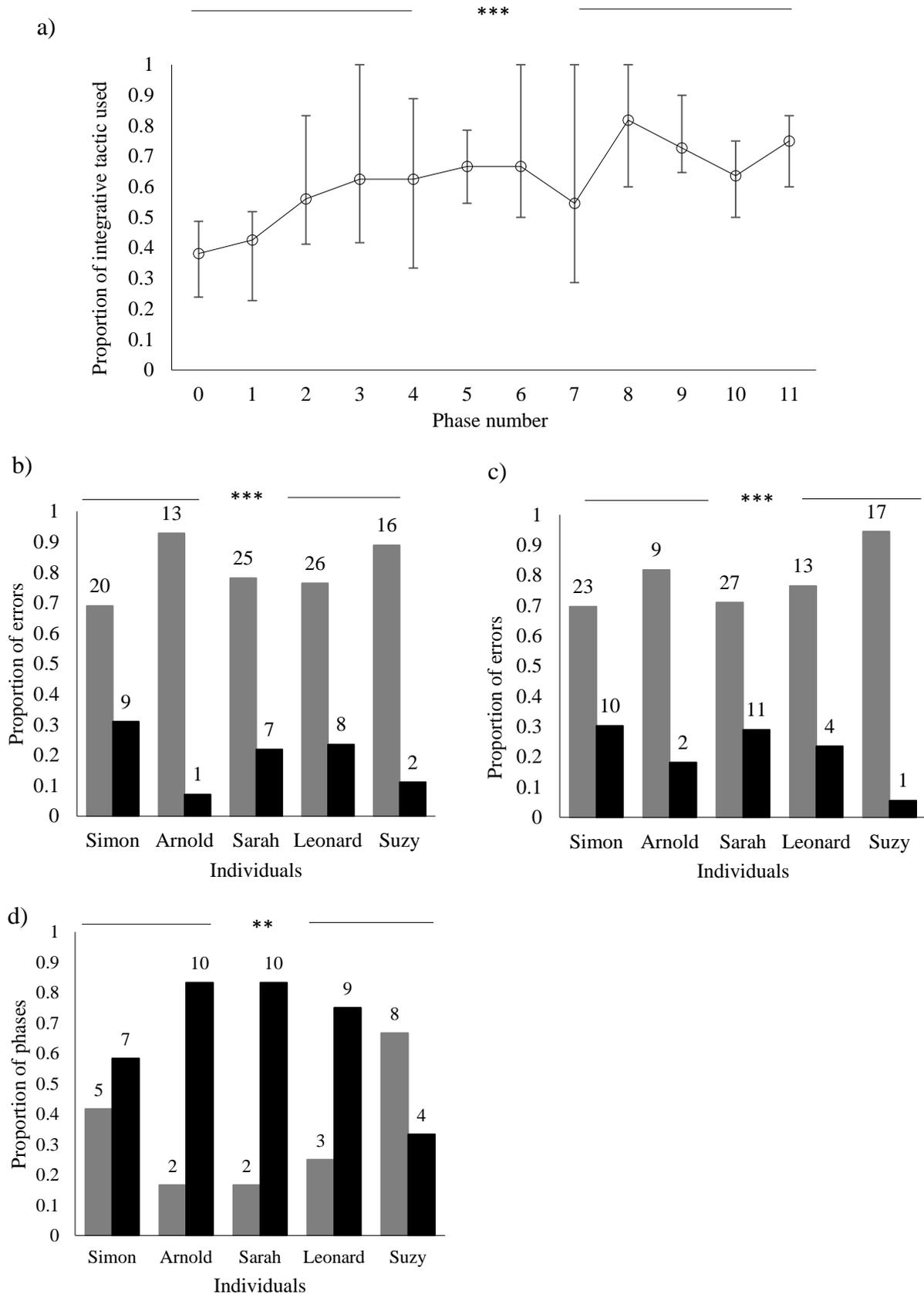


Figure 2.6. a) this figure shows the median (maximum and minimum) proportion of integrative strategy across all the phases; b) proportion of errors that are using sequential strategy (■) or integrative strategy (■) for each individual in the *acquisition* phase; c) proportion of errors that are using sequential strategy (■) or integrative strategy (■) for each individual in the *reversal* phase; Note that the number above each bar indicates the total trial numbers d) proportion of the first trial across phases that squirrels used sequential strategy (■) and integrative strategy (■). Note that the number above each bar indicates the total numbers of tactics. *** $p < 0.001$, ** $p < 0.01$.

Predictors of learning efficiency

Table 2.2 shows the results of GEE modelling. The number of errors made in a phase decreased across reversals, decreased with proportion of integrative tactics, increased with the amount of proactive interference, and decreased with the amount of other irrelevant interference. Only phase number ($p < 0.001$) and tactic change ($p = 0.025$) showed significant effects. The effect of other interference also approached significance ($p = 0.057$) but the effect of proactive interference did not ($p = 0.197$).

Table 2.2 The first GEE model using Poisson distribution to examine the predictors for learning efficiency. Learning efficiency measures as the number of error in each phase and is not standardised for analysis. Standardised covariates include phase numbers, an acquisition phase and 11 reversal phases; proactive interference, measured as the average number of first choosing non-rewarded wells in each phase; tactic change, measured as the proportion of using integrative search tactic in each phase; other interference information, measured as the average number of extra wells chosen after an individual made correct responses in each

phase. This table shows the coefficient estimates, Z and p -values. Covariates are standardised prior to the analysis for effect comparison purpose.

Predictors	Estimates	df	Z	p
Phase numbers	-0.49	1	-5.86	<0.001
Proactive interference	0.07	1	1.29	0.197
Tactic change	-0.28	1	-2.24	0.025
Irrelevant interference behaviour	-0.15	1	-1.91	0.056

Effect of learning on proactive interference, tactic change, and other interference information

Following the hypothetical model in Figure 2.2, we tested whether phase number had an effect on tactic change, proactive interference and other interference information, and therefore whether any of these variables could be mediating the effect of phase number on efficiency. Table 2.3 shows the result of the GEE model: phase number significantly had an effect on tactic change ($p<0.001$) but not proactive interference ($p=0.357$) and irrelevant interference behaviour ($p=0.091$). Hence, the proportional use of integrative tactics increased across phases. Taken together, the two GEE analyses show that even with tactic change included in the model, phase number still has a significant effect on efficiency (Table 2.2), tactic change therefore partially mediated the effect of phase numbers.

Table 2.3. A GEE model using Gaussian distribution to examine the predictor for tactic change, proactive interference, and other interference information. Phase number is the only independent variable which is standardised for the analysis whereas each dependent variable is unstandardised. This table shows the coefficient estimates, Z and *p*-values. Only tactic is a significant predictor for memory.

Dependent Variable	Estimates	<i>df</i>	Z	<i>p</i>
Proactive interference	-0.02	1	-0.92	0.357
Tactic change	0.09	1	7.91	<0.001
Irrelevant interference behaviour	-0.19	1	-1.69	0.091

2.4 DISCUSSION

We examined whether squirrels would form a learning set in a spatial reversal learning task and if so, how squirrels achieved this improved efficiency, by examining the variation of four potential predictors, learning (phase number), proactive interference, tactic change, and other irrelevant interference information across phases. The discussion here focuses on how the squirrels appear to be ‘*learning how to learn efficiently*’ (Harlow, 1949), particular attention is paid to the role of tactic change in the learning process in relation to improved learning efficiency. We also discuss the possible cognitive processes that are involved in the tactic change under this specific design and the response strategies in respect to squirrels’ ecological behaviours. Our results showed that both accumulated experience and tactic change led to increased efficiency. Although the effect of learning on efficiency was partially mediated by tactic change, its significance was not completely negated when tactics were included in the model.

Firstly, our results showed that the squirrels did form a learning set (Harlow, 1949), since they showed clear improvement over successive reversals, becoming more efficient in adjusting their behaviour to the recurring change in reward contingencies (Fig. 2.4). Our result is apparently contrary to previous studies in which other species in *Sciuridae* family have failed to improve over a successive object-discrimination task (eastern fox squirrels, Flaningam, 1969; round-tailed ground squirrels, Rees, 1968). It is possible that the difference of results may be due to the different methodology used in the serial reversal *versus* the successive discrimination tasks, but it is also important to consider that our use of a spatial task, which is certainly ecologically relevant to squirrels, as scatter hoarders, plays an important part in the squirrels' ability to engage with the task.

Secondly, we examined the process by which squirrels were '*learning how to learn efficiently*' (Harlow, 1949). Our results showed that the simple accumulation of experience has the greatest effect amongst the variables. Apparently, experience may allow squirrels to become familiar with the recurring change. Individuals did not use more trials or make more errors in the first reversal phase than in their acquisition phase. It appears that squirrels may be predisposed not to rely on previous information, given that our model shows that neither proactive interference nor other irrelevant information is a significant predictor of learning efficiency. It is also notable that squirrels visited one of the two rewarded locations as their first choice significantly more often than the non-rewarded locations in the first reversal phase (Fig. 2.5b). These results suggest that squirrels quickly learn to adapt to the change of contingency, allowing current reward information to override memories of past contingencies. Altogether, the evidence supports the idea that learning to be flexible can have adaptive significance in fitness measures (Dukas, 2013), here, we show that learning provides direct

advantages in maximising reward gain as well as minimising time cost to achieve learning efficiency.

As we predicted, another significant predictor of increased learning efficiency was tactic change. The tactics that animals use in a task may reflect the formation of an abstract rule about the alternating pattern of reward contingency across the phases. In our case, squirrels would form a diagonal rule between the two paired rewards (i.e. pick the diagonally opposite well if a rewarded well is found) and apply it in each phase to increase efficiency. Our results seem to support this explanation, given that the squirrels changed the tactics they used *within* phases and by the end of the experiment, some squirrels were making zero, one or two errors before reaching criterion (Fig. 2.4). However, detailed analysis of the tactics used in the first trials after a contingency switch makes us question whether this explanation is complete. If squirrels have learned the rule, they should make the integrative tactic errors that are similar to the right panel of Fig. 2.1b in the new reward contingency. But this did not happen: as shown in Fig. 2.6b, squirrels reverted to sequential tactics at the beginning of each new phase, even for the individuals that reached the criterion with one or two trial errors. These results suggest that squirrels do not become efficient by forming the diagonal rule. Instead, results suggest that this integrative tactic is implemented by learning the spatial pattern of the reward locations, perhaps through the use of extra-apparatus cues. Although we have no evidence for the formation of spatial pattern in our case, the use of extra-apparatus cues is possible for two reasons: firstly, the apparatus was always parallel to the walls of the test room, which then provide unique information for squirrels to locate the reward. Secondly and more importantly, if squirrels could only use spatial pattern for this task, then first choice between rewarded and non-rewarded wells should be at chance level, as happened with rats in the pole box experiment (e.g. Brown & Wintersteen, 2004). However, squirrels located one

of the rewarded wells significantly more than the non-rewarded wells as their first choice both in the acquisition phase (Fig. 2.5a) and the reversal phase (Fig. 2.5b), and this clearly shows that they relied on more than internal spatial representation to locate the reward. The use of extra-apparatus use have also been shown in other members of the *Sciuridae* family such as northern flying squirrels (Gibbs, Lea, & Jacobs, 2007) and fox squirrels (Waisman & Jacobs, 2008), and these species were found to be flexible in cue use and to use more than one frame of reference in remembering the locations of rewards.

Tactic change may be related to increased efficiency because it is associated with attention. Attention to cues and local feedback have been suggested to be important for reversal tasks (e.g. Mackintosh, Brendan, & Valerie, 1968; Mackintosh & Little, 1969; Rayburn-Reeves, Stagner, Kirk, & Zentall, 2013). Although a serial reversal task puts a premium on attention to the recent rewards received, here we also suggest that attention to extra-apparatus cues is useful with stable reinforcement contingencies.

Our results highlight the advantages of changing tactics in response to the task demands so as to increase learning efficiency, maximise energy gain and minimise time cost. Although the correlation result was not significant, individuals that used integrative tactics made fewer total numbers of errors across phases than individuals that used this tactic less, and they thus secured the same number of rewards at a lower cost of time and effort. This trend confirms our expectation that changing tactics in the learning process brings advantage. The apparent variation of our squirrels in how soon they switched to use integrative tactics may suggest that there is variation in intrinsic learning ability, with some squirrels requiring more trials to memorise the reward value of each well, whereas others reached the criterion with no or only a single error trial. However, given that all locations had contained rewards

some of the time, depending on the reinforcement contingency in force in a particular phase, squirrels that preferred to use the sequential search behaviour might not be making ‘errors’ but instead using an alternative strategy in foraging (Cakmak et al., 2009; Evans & Raine, 2014) or involving a different speed/accuracy trade-off (Chittka, Dyer, Bock, & Dornhaus, 2003), even if the time cost of sequential tactics is higher than that of integrative tactics. A quick but inaccurate foraging style has been shown to be adaptive in some foraging situations (Burns, 2005).

Although the cost of making an ‘error’ is small in this design, the fact that squirrels significantly increased their proportional use of integrative tactic *within* each phase shows that they were motivated to increase efficiency in obtaining the hidden rewards (Fig. 2.6a). Grey squirrels have the capacity to re-locate their caches within 5 cm accuracy (Macdonald, 1997), and in field conditions searching at random could be less efficient than relying on memory and using appropriate search tactics. Our squirrels’ preference for using sequential search tactics in the first trial of a new reward contingency (Fig. 2.6d) – that is, in response to a failure to obtain expected reward may be an example of an ecologically driven tendency in response tactics towards change, as in lizards (Day et al., 1999), honeybees (Mota & Giurfa, 2010) and jumping spider (Liedtke & Schneider, 2014). Squirrels may consider the distance between food locations during foraging or cache retrieval, and they will initiate a search in adjacent locations that are around the remembered cache location when search in the expected place for a cache fails. Hence, if well 1 is unexpectedly empty, the likelihood of a squirrel to search in well 2 and 4 is higher than well 3, as both well 2 and 4 are closer to well 1 (10.8cm) than well 3 (17.2cm). Such a search in an adjacent location may be a more natural response, and a more efficient one under natural conditions, than moving to the diagonally opposite well. Hence, both the search tactics we considered are ecologically

relevant and may have adaptive advantages. Future research could look at these possibilities by using a larger poke box which then allows squirrels to reveal their response strategy with rewards that are hidden further apart.

In conclusion, we provide the first evidence that squirrels increase learning efficiency with repeated exposure to changing reward contingencies in an ecologically relevant task, and furthermore we have been able to show how they achieve this. Squirrels rapidly form a learning set after experiencing successive reversals. This rapid decrement in errors is predicted by increased experience but is accelerated if they are flexible enough to change tactics under the recurring change of contingencies. This is the kind of cognitive capacity that should be useful to a scatter-hoarding animal, which needs to return efficiently to cache sites to empty them, but thereafter to avoid wasting time on revisit to sites that have been emptied or found to be pilfered.

CHAPTER 3: THE ROLES OF INHIBITORY CONTROL AND ATTENTION DEPEND ON LEARNING STAGE.

ABSTRACT

Learning mechanisms such as inhibitory control and attention are known to support animals' adaptation to changes. But it remains unclear at what stage of learning, for example before or after reaching learning criteria, they contribute most to achieving learning efficiency. We investigated this question in five grey squirrels (*Sciurus carolinensis*) with a colour reversal learning task on a touch screen. Squirrels increased efficiency through greater inhibitory control *before* reaching the learning criteria. However, increased inhibitory control in combination with heightened attention is the key to achieving efficiency *after* reaching the learning criteria. These results show that the positive effect of each mechanism on learning efficiency depends on the learning stage and the interactions between mechanisms. While greater inhibitory control provides a direct positive effect on achieving efficiency, heightened attention does not always have a positive effect on reversal learning.

3.1 INTRODUCTION

Learning is one of the primary forms of flexibility which allows animals to adjust to change in their environment (van Schaik, 2013). The ability to learn is facilitated by mechanisms such as inhibitory control and attention (e.g. Mackintosh, 1963, Mackintosh & Mackintosh, 1967, Mackintosh & Little, 1969, Rudebeck & Murray, 2011). Early theorists (e.g. Sutherland & Mackintosh, 1971, Mackintosh, 1975) propose that attention allows animals to focus on relevant information, and learn selectively about it. Theorists (e.g. Sutherland & Mackintosh, 1971, Hulbert & Anderson, 2008) have also proposed a role for inhibitory control as well as attention, with interaction between the two mechanisms;

inhibitory control suppresses attention to irrelevant information and in turn, allows individuals to focus on relevant information. Although these theories emphasise the role of multiple cognitive mechanisms in the learning process, it is not easy to disentangle the two mechanisms when seeking to account for poor learning performance or an increased number of error responses in a task (e.g. Tait & Brown, 2007).

To overcome this difficulty, many studies have incorporated invasive methods such as injecting neuro-chemicals to induce lesions or depletion in targeted brain areas that are thought to be responsible for either mechanism (e.g. Boulougouris, Glennon, & Robbins, 2008; Clark et al., 2005; Clark et al., 2009; Voytko, Richardson, Gorman, Tobin, & Price, 1994; Tait & Brown, 2007), then assessing the impaired learning performance by comparing with a control group that did not have the lesion. Such assessments have used a wide range of cognitive tasks, but they frequently involve a reversal learning task (Shettleworth, 2010, pp. 210). The reversal learning task involves two stimuli, and requires animals first to learn to associate one stimulus with reward while the other is not. Once the animal reaches a stringent criterion, the reward contingency is reversed and the previously non-rewarded stimulus becomes rewarded in the reversal phase. The change of contingency requires a series of adjustments in which individuals have to notice the change, inhibit their previously learned response, overcome the learned but now irrelevant association with the non-reward stimulus, and pay attention to learn the new association (Boulougouris, Glennon, & Robbins, 2008). Accordingly, the two mechanisms, attention and inhibitory control, may vary in their contributions in this learning process and the final performance both in the initial acquisition and in the reversal learning phase. Such effects on performance may be indicated by the speed of learning, represented either by the number of errors that an individual makes before

reaching the learning criterion, or by the number of trials that the individual takes to reach the criterion.

Instead of using invasive methods, recent studies have increasingly shown the possibility of using behavioural indices to understand the learning process or capture learning mechanisms such as attention and inhibitory control separately. For example, the reaction time to the first incorrect choice and correct choice to understand learning process (e.g. D'Cruz et al., 2011) and learning mechanisms such as attention can be measured as the rate of vicarious trial-and-error (e.g. the frequency of head-switching) between two stimuli (e.g. Tolman, 1938; Kemble & Beckman, 1970). Such head-switching is seen in discrimination task as Tolman (1938) described. His rats showed head-switching in front of two doors under a Y-maze set up; such head turning appeared to be a conflict-like behaviour between two stimuli before the individuals made a choice. If each behaviour is identified correctly as corresponding to a specific mechanism, we should be able to understand their contributions at different learning stages. In particular, their contribution could be broken down by errors made in 'early learning stage' and 'late learning stage' that are assumed to call upon different mechanisms to operate (see review Nilsson, Alsiö, Somerville, Clifton, 2015). Accordingly, in the present study, behaviours were recorded separately at two stages of learning, as detailed below. But *how* do inhibitory control and attention contribute to efficiency, and at what stage of learning are they the most important?

In this study, we examined this question using a colour reversal learning task on a touch screen in grey squirrels (*Sciurus carolinensis*). We disentangled inhibitory control and attention by their corresponded behavioural reactions at different learning stages, and related each mechanism to efficiency, defined as the number of errors made in the course of learning.

To our knowledge, there is little evidence examining the cognitive mechanisms of grey squirrels, but recent studies have shown that squirrels are highly flexible under recurring changes (e.g. Chow, Leaver & Wang, & Lea, 2015) and evidence from their caching behaviours suggests they exhibit attention and inhibitory control: they show inhibitory control by stopping digging and increasing the latency to start caching when conspecifics are present (Hopewell & Leaver, 2008) and they are attentive to the presence of conspecifics (Hopewell, Leaver & Lea, 2008) and heterospecifics (Schmidt & Ostfeld, 2008) during caching. Hence, grey squirrels should be a good candidate to address the questions for this study. We predicted that in the case of reversal learning, increased inhibitory control would play an important role in increasing efficiency (fewer numbers of errors) before than after reaching the learning criteria, as individuals have to inhibit their learned responses. Prediction for head-switching could be two-folded: on the one hand, Gellermann (1933) suggested that increased head-switching is shown with increased experience or correct responses in the task. Such increment in head-switching may reflect individuals are learning or comparing the characteristics between stimuli. If this is the case in this reversal learning task, then we predicted that head-switching would be higher *after* a squirrel reached the learning criteria than before. On the other hand, Tolman (1939) has pointed out this vicarious trial-and-error (conflict-like behaviours) reflects an animal is 'hesitating' between choices, especially at the initial stage of discrimination. However, head-switching rate would decrease with increased experience to the task and thus, better learning performance is expected. If this is the case with our study design, then we predicted that higher head switching rate would be seen *before* the squirrel reached the learning criteria than after.

3.2 METHODS

Five captive squirrels at the University of Exeter went through a standardised pre-training protocol (supplementary materials Table S1) to use a touch screen (Fig. 3.1). Squirrels then went through a five-trial colour preference test before the training task. The colour preference test consisted of a pair of triangles (width x height: 3cm x 3.2cm), one pure red (RGB: 255,0,0) and the other pure green (RGB: 0,255,0) randomly presented on the touch screen at the eye level of the squirrels, 9cm apart. Both colours were equally rewarded (one hemp seed); squirrels had to respond to both colours to minimise any colour-reward associations acquired prior to the training. Colour preference was calculated as the colour that a squirrel chose first for three or more consecutive trials.

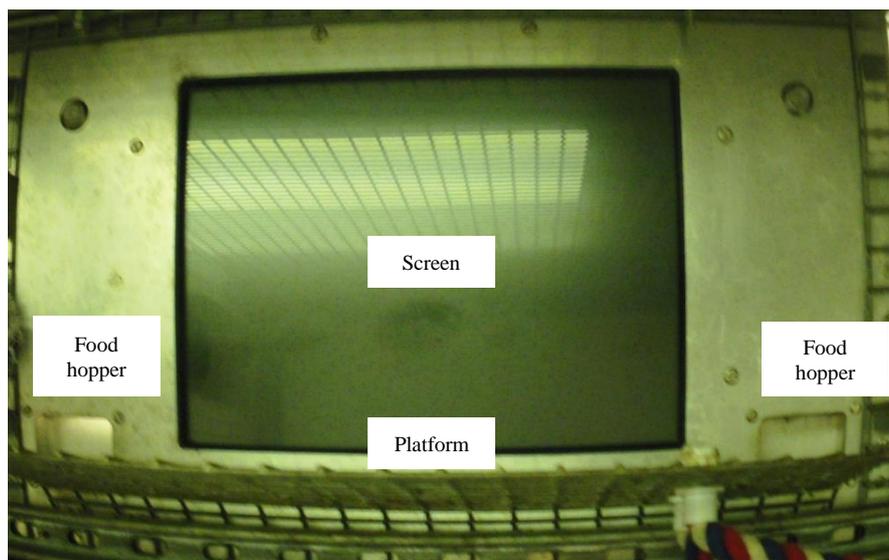


Figure 3.1. Touch screen set up for squirrels. The screen is at the centre with one food hopper on each side. Correct responses lead to food delivery on the corresponding side.

Training involved two phases, an acquisition phase and a reversal phase. Squirrels received a block (60 trials) that lasted one hour daily. Squirrels started each trial by nose-pressing a central key before the same pair of stimuli as in the colour preference test was

presented. To avoid side biases, each colour was presented on each side of the screen 30 times and no more than three consecutive times. Response to the correct colour in each trial led to immediate food delivery (a hemp seed and a honey Cheerios® or ¼ cashew) in the corresponding side hopper, whereas an incorrect response led to a 2-second time out before squirrels were allowed to respond to the correct colour (correction trials). In the acquisition phase, we reinforced squirrels' non-preferred colour (i.e. non-preferred colour +, original preferred colour -). As one individual, Sarah, did not exhibit a preference for either colour, we randomly assigned the reward colour for her. When a squirrel reached both of two learning criteria: 1) 41/60 trials correct (68%, two-tailed binominal test $p=0.006$) and 2) at least 10 or more consecutive correct trials, we switched the reward contingency (i.e. non-preferred colour -, original preferred colour +). In the reversal phase, squirrels learned the new association until they reached the first criterion and had at least 9 or more consecutive correct trials. Training ended each day when squirrels either completed the 60-trial block or did not respond for 20 minutes.

Measurements

Behavioural responses of a phase are recorded in two stages: *before* and *after* reaching the learning criteria.

Learning efficiency. Learning efficiency was the total number of errors for each learning stage of a phase.

Attention. Attention was indexed as head-switching. A head-switch was counted when a squirrel turned its head between the two stimuli before making a choice. We then calculated

the number of head switches for each learning stage by averaging the number of head switches across trials and across blocks.

Inhibitory control. Given that inhibitory control requires individuals to suppress their prepotent response, we could measure inhibitory control as the average of the first response latency where the incorrect stimulus was selected. We first averaged latencies across trials in each block and then averaged across blocks for each learning stage. Lower response latencies to the incorrect stimulus indicated as less inhibitory control, whereas higher latencies reflected greater inhibition.

Data analysis

We examined learning performance, and each mechanism before and after reaching learning criteria (see supplementary materials for details) using paired t-test with effect size >0.8 (Winter, 2013) and used Cohen's d (Cohen, 1988) to confirm the effect sizes of significant results. We used Generalised Estimating Equations (GEE) with adjusted variance and exchangeable 'working' correlation (Wang & Long, 2011) to examine the contribution of inhibitory control and attention for each learning stage on learning efficiency. Gaussian distributions were used when analysing continuous dependent variables including inhibitory control and attention. A Poisson distribution was used for the count variable, the total number of errors at each learning stage. All the tests are reported two-tailed with significance level set at $\alpha < 0.05$.

3.3 RESULTS

Accuracy of performance before and after reaching the learning criteria

Figure 3.2a shows that in both learning phases, squirrels made more errors, on average, before reaching the learning criteria than after. These differences were significant in the acquisition phase ($t(4)=5.31, p=0.006, d=0.94$) and in the reversal phase ($t(4)=4.38, p=0.012, d=0.91$).

Inhibitory control before and after reaching the learning criteria

Figure 3.2b shows that in the acquisition phase, the average response latency towards the incorrect colour (original preferred colour) was lower than towards the correct colour (non-preferred colour). This difference was significant ($t(4)=3.14, p=0.035, d=0.84$). Similarly, in the reversal phase, response latency towards the incorrect colour (original non-preferred colour) was lower than towards the correct colour (original preferred colour) and this difference was also significant ($t(4)=2.9, p=0.044, d=0.82$). Despite this, Figure 3.2c shows that the difference in response latency towards the incorrect colour before and after reaching the learning criteria did not reach significance in the acquisition phase ($t(4)=2.43, p=0.072$) or in the reversal phase ($t(4)=0.48, p=0.658$).

Attention before and after reaching learning criteria

Figure 3.2d shows that on average, squirrels showed higher head-switching rates (vicarious trial-and-error) after than before reaching the learning criteria in both phases. However, the difference was significant only in the reversal phase ($t(4)=-3.27, p=0.031, d=0.85$), not in the acquisition phase ($t(4)=-2.47, p=0.069$).

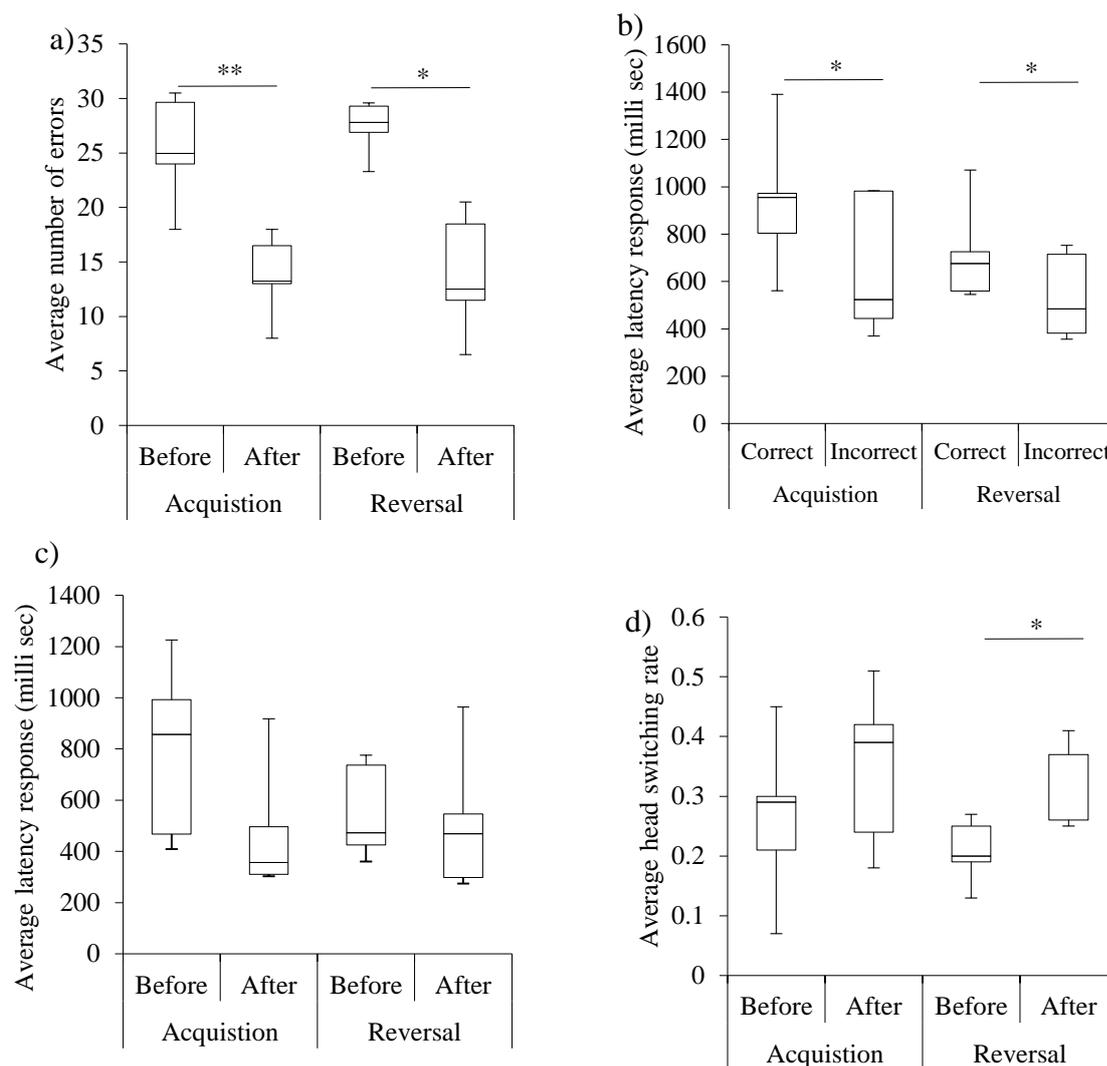


Figure 3.2. The values of the traits for the five squirrels tested in each trial are shown by the line in the box, the top and bottom of the box and the upper and lower vertical lines. a) average number of errors that squirrels made before and after reaching the learning criteria in each learning phase. b) average latency response towards the correct and incorrect colour for each learning phase. c) average latency of the first response to the incorrect stimulus (an index of inhibitory control) before and after reaching the learning criteria for each learning phase. d) average head-switching rate (an index of attention) before and after reaching the learning criteria for each learning phase. All scores are shown as medians; error bars indicate ranges. * <0.05 , ** <0.01 .

Learning efficiency, learning stages, inhibitory control and attention

First model used covariates, learning stage, attention, and inhibitory control to predict learning efficiency. Figure 3.3 shows that all the covariates were significantly related to learning efficiency: learning stages ($\chi^2_1=42.91$, $p<0.001$), inhibitory control ($\chi^2_1=9.11$, $p=0.003$) and attention ($\chi^2_1=26.53$, $p<0.001$). With the other variables held constant, squirrels made fewer errors after reaching the learning criteria, greater inhibitory control was associated with higher learning efficiency, and higher attention was associated with lower learning efficiency.

Learning stages, inhibitory control and attention

We then ran the second model using covariates learning stage and attention to predict inhibitory control. Figure 3.3 shows that learning stages and attention were significantly related to inhibitory control. Greater inhibitory control (i.e. higher latency of responding to the incorrect stimulus) was observed before reaching the learning criteria ($\chi^2_1=6.36$, $p=0.012$) and was associated with higher attention (i.e. higher head-switching rate) ($\chi^2_1=5.19$, $p=0.023$). Finally, we ran the last model using covariates learning stage to predict attention. Figure 3.3 shows that learning stage was also significantly related to attention ($\chi^2_1=10.32$, $p=0.001$); squirrels increased attention after reaching the learning criteria.

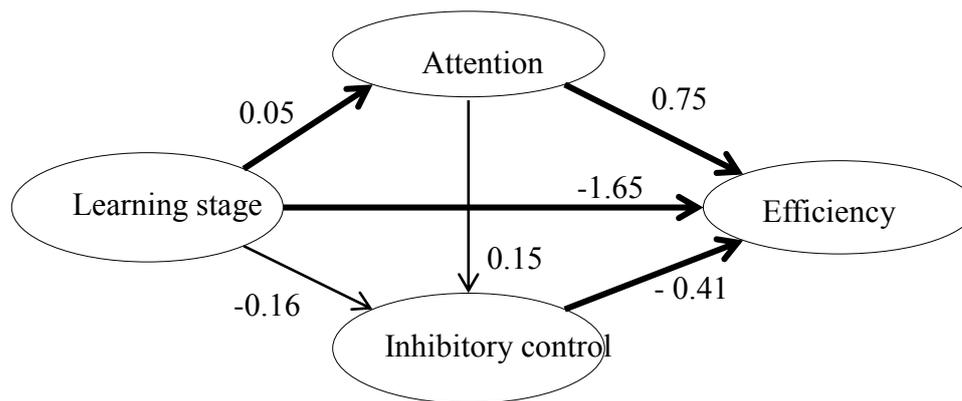


Figure 3.3. GEE models show the estimates for relationships between learning stages, attention, inhibitory control and efficiency. First model include three predictors: learning stages (before or after reaching the learning criteria); inhibitory control (the average reaction time responding to the incorrect stimulus for each learning stage of each phase); attention (the rate of head switching for each learning stage of each phase). The dependent variable (DV) is learning efficiency, the total number of errors for each learning stage of each phase. Second model includes two predictors, learning stages and attention. The DV is inhibitory control. Third model includes only learning stages. The DV is attention. All predictors are standardised for comparison but not dependent variables. Solid lines indicate significance level thick solid lines equal to $p < 0.005$ whereas thin solid lines indicate $p < 0.05$.

3.4 DISCUSSION

Our results provide evidence for *how* inhibitory control and attention vary their contribution to achieve efficiency at different learning stages in a colour reversal learning task: 1) *before* reaching the learning criteria, squirrels increased inhibitory control to increase efficiency and 2) *after* reaching the learning criteria, squirrels used two mechanisms, greater inhibitory control along with increased attention, to achieve continued efficiency.

By analysing behaviours within each learning stage, we found that inhibitory control is a key mechanism for achieving efficiency (Path 1) in this reversal learning task: greater inhibitory control has a direct advantage in minimising the total time cost to obtain a preferred food. Inhibitory control appears to be particularly important *before* reaching the criteria (Path 2): squirrels took longer to choose the correct stimuli than the incorrect stimuli in both learning phases (Fig. 3.2b). This result implies that they are learning that the incorrect colour is their preferred colour in the acquisition phase or a previous reinforced colour in the reversal phase.

Along with previous evidence (Mackintosh, 1963; Mackintosh & Mackintosh, 1967; Mackintosh & Little, 1969) we show that attention is important for this task. But our model shows that heightened attention decreases efficiency (Path 1). Heightened attention only increases efficiency *after* squirrels reached the learning criteria (Path 3), and it has to work with greater inhibitory control (Path 2). These results suggest that attention supports inhibition in learning and matches with the theory (Sutherland & Mackintosh, 1971; Hulbert & Anderson, 2008) that increased attention along with greater inhibitory control could allow individuals to focus on relevant information by suppressing attention on irrelevant information. It also implies that squirrels learn the characteristics of correct/relevant stimuli at this stage.

In summary, we show the significant role of inhibitory control and attention under change in squirrels. These mechanisms may have adaptive significance for grey squirrels, an exceptionally successful invasive species (Global Invasive Species Database, 2005), and are, hence expected to show high flexibility in their behaviour, as has been shown in invasive birds (Sol, Timmermans & Lefebvre, 2002). Future studies could compare the learning

performance between an invasive group of grey squirrels and the native red squirrels to highlight whether these learning mechanisms provide advantages for their success. Overall, our findings emphasise that mechanisms involve at different stages of learning provide advantages on achieving efficiency.

**CHAPTER 4: HOW PRACTICE MAKES PERFECT: THE ROLE OF
PERSISTENCE, FLEXIBILITY AND LEARNING IN PROBLEM SOLVING
EFFICIENCY.²**

ABSTRACT

To fully understand how problem solving ability provides adaptive advantages for animals, we should understand the mechanisms that support this ability. Recent studies have highlighted several behavioural traits including persistence, behavioural variety and behavioural/cognitive flexibility that contribute to problem solving success. However, any increment in these traits will increase time and energy costs in natural conditions, so they are not necessarily advantageous. To examine how behavioural traits vary during learning to solve a problem efficiently, we gave grey squirrels (*Sciurus carolinensis*) a problem solving task that required squirrels to obtain out-of-reach but visible hazelnuts by making a lever drop in the laboratory. We recorded persistence, measured as attempt rate, flexibility, measured as the rate of switching between tactics, and behavioural selectivity, measured as the proportion of effective behaviours, in relation to problem solving efficiency on a trial-by-trial basis. Persistence and behavioural selectivity were found to be directly associated with problem solving efficiency. These two factors also mediated the effects of flexibility and increased experience. We also found two routes that led to more efficient problem solving across learning trials: increasing persistence or increasing behavioural selectivity. Flexibility was independent from learning. Flexibility could increase problem solving efficiency, but it also has a time cost; furthermore it seemed to involve a trade-off with behavioural selectivity, with high flexibility being associated with a higher frequency of some disadvantageous ineffective behaviours. These results suggest that flexibility is an independent cognitive process or behavioural trait that may not always bring advantages to animals.

²The content of this chapter has been accepted for publication: Chow, P. K. Y., Lea, S. E. G., & Leaver, L. A. (in press). How practice makes perfect: the role of persistence, flexibility and learning in problem solving efficiency. *Animal Behaviour*.

4.1 INTRODUCTION

Direct demonstrations of correlations, within species, between general cognitive abilities and fitness measures have shown the functional significance of problem solving success (e.g. Cole, Morand-Ferron, Hinks, & Quinn, 2012, Keagy, Savard, & Borgia, 2009; but also see Isden, Panayi, Dingle, & Madden, 2013). The recent focus on individual or species differences in specific behavioural traits that may underlie animals' success or failure in innovative problem solving has helped us to understand some traits that contribute to problem solving success. Examples of behavioural traits that have been implicated in this way include persistence, behavioural variety, behavioural and cognitive flexibility, with each of the traits providing different advantages for an individual during the problem solving process:

Persistence: Since complex problems are unlikely to be solved immediately. It is necessary to be persistent in order to solve them. Individuals who persist longer in their problem-solving attempts have been shown to be more likely to solve a problem, for example among hyenas (Benson-Amram & Holekamp, 2012), carib grackles (Overington, Cauchard, Côté, & Lefebvre, 2011), great tits (Cauchard, Boogert, Lefebvre, Dubois, & Doligez, 2013), and meerkats (Thornton & Samson, 2012).

Behavioural variety: In their studies of hyena problem-solving, Benson-Amram & Holekamp (2012) and Benson-Amram, Weldele, & Holekamp (2013) showed that behavioural variety, the number of *types* of contact that an individual employs to manipulate an apparatus, was a good predictor of whether an animal would solve a problem; Griffin, Diquelou, & Perea (2014) obtained a similar result in Indian hill mynas, *Sturnus tristis*.

Behavioural and cognitive flexibility: Animals may vary in their capacity to change their behaviour as a function of success or failure at solving a problem, or the speed with which they do so. Ramsey, Bastian, & van Schaik (2007) set the capacity to find novel behaviours in response to novel problems at the heart of their analyses of innovation. Kummer and Goodall (1985) argue that flexibility may also involve the capacity to mobilise old behaviours in new situations. Reader & Laland (2003) consider that both of these conditions could indicate flexibility in problem solving.

Two theoretical difficulties are posed by this list of factors. Firstly, increasing any of them is likely to increase the time and energy spent on finding a solution to a problem. In a natural context, spending extra time or energy solving a problem has a cost, especially when the individual first encounters the novel problem: it decreases the net worth of whatever resource a solution makes available, and it increases exposure to risks such as predation. Secondly, they are to some extent opposed to one another. In particular, persistence could be the opposite of either flexibility or behavioural variety, though it need not be, as we discuss later.

Both these difficulties can potentially be resolved by considering what happens when animals are faced with a problem that allows access to high-value food, and the same problem recurs. Committing time and energy to solving a problem is more worthwhile if the net worth is high and the same problem is likely to recur. Similarly, the apparent contradictions between the needs for behavioural variety and flexibility on the one hand, and persistence on the other, may perhaps be broken down by looking at how they each vary across trials. For example, persistence might be important in the earliest trials with a problem, when the animal has had little experience of obtaining the ultimate reward; flexibility might

become more important later, in helping the animal adjust its behaviour to reach the most efficient solution. Griffin and colleagues' (2014) results on Indian hill mynas support this idea by showing persistence was important in solving the first problem of a series whereas behavioural variety was important for solving further problems.

The primary goal of the present experiment, using Eastern grey squirrels (*Sciurus carolinensis*) as subjects, was to disentangle these different factors by studying how flexibility, behavioural variety and persistence vary between individuals and across successive trials on a problem, and then examine how these factors contribute to the efficiency of problem solving (See Methods). To do this, we designed a problem task that afforded specific ineffective and effective contact types for obtaining rewards, although squirrels were allowed to employ any techniques to make a lever drop to obtain visible rewards (see Methods), and we focused on the variations in the time each squirrel took to solve the problem on each trial.

The factors of persistence, variety and flexibility have all been defined in varying and sometimes confused ways in the past and variables may have confounded with the solution time. For example, Griffin et al. (2014) measured persistence, or motivation, as the actual number of attempts to solve a problem on each trial. To examine these variables' separate impacts on the time it takes an animal to solve a problem, it is necessary to define them so that they are logically independent of each other and of solution time. To achieve this, we adopted definition of each factor of interest based on previous studies (details see Methods). In summary, we followed the method of Biondi, Bó, & Vassallo (2008) and Griffin & Diquelou (2015), measuring persistence as the rate at which the squirrels used behaviours directed at the apparatus regardless of what kind of behaviours they were, behavioural variety

as the number of different behaviours employed, flexibility as the frequency with which the squirrels changed the behaviour they directed at it, and behavioural selectivity as the proportion of effective behaviours. All these measures were taken trial by trial, so that we could observe how they changed in the course of learning. But how would we expect them each to impact on the efficiency of problem solving?

Since we were measuring persistence in the same way as Biondi et al. (2008) and Griffin & Diquelou (2015), we predicted that persistence would emerge as one of the contributors to problem solving efficiency, as what the authors found in their studies. Specifically, we predicted that persistence would increase across trials, and in turn, reduce solution time, since perfect performance would entail a rapid rate of (successful) attempts. The prediction for behavioural selectivity is also straightforward; as the squirrels learn to solve the problem more efficiently, the proportion of effective behaviours should increase across trials, and hence, lead to lower solution time. At least at the beginning of training, behavioural variety and flexibility should also facilitate learning, as having a wide range of contact types available, and switching between them frequently, should assist individuals in identifying the successful behaviours for a task; however, later in training, we might expect to see success associated with lower levels of these variables.

If we have correctly identified these four factors as accounting for problem solving performance and its improvement with experience, we can then investigate which, if any, of the factors we were measuring in fact mediate the effect of experience (operationalised by trial number) on solution time, and how. That is to say, some or all of these factors should be correlated with both trial number and solution time; if the variables of persistence, behavioural variety, flexibility and behavioural selectivity are included as covariates in a

model along with trial number, then there should be no remaining correlation of solution time with trial number. Figure 4.1 illustrates one possible explanatory model for problem solving efficiency. In this model, the four factors introduced above mediate the effect of experience. However, it is not the only possible model; at least some of the four component skills such as persistence and behavioural variety could be personality traits (or behavioural syndromes), and flexibility and behavioural selectivity could be cognitive processes and their contributions to individual differences in problem solving performance are not easily modified by experience.

Grey squirrels are well suited for studies of problem solving ability for several reasons. They have excellent motor skills which they use in natural conditions such as manipulating twigs and leaves to build dreys, and in anthropogenic situations, for example for extracting food from even the best protected bird feeders. Accordingly, the manipulatory skills of grey squirrels should not be a limiting factor in a problem solving task. Grey squirrels also belong to the family *Sciuridae*, whose members have a comparatively larger brain to body size ratio than other rodents (Mace, Harvey, & Clutton-Brock, 1981; Roth & Dicke, 2005). Species with relatively larger brains are more successful than those with relatively smaller brains in invading new environments (avian species: Sol, Duncan, Blackburn, Cassey, Lefebvre 2005; amphibians and reptiles: Amiel, Tingley, & Shine, 2011). Birds with larger brains relative to body size are also more flexible than those with a smaller brain relative to body size, and more successful in establishing themselves in a new environment (Sol, Timmermans, & Lefebvre, 2002; Sol, et al., 2005; Sol, Bacher, Reader, & Lefebvre, 2008), surviving in nature (Sol, Székely, Liker, & Lefebvre, 2007) and adapting to city life (Sol, Lapedra, & González-Lagos, 2013). In line with this evidence, the relatively large brain to body size of grey squirrels may have facilitated their spread around most of the

UK since the 19th century and in Italy since the mid- 20th century. This spread of population has been predicted to continue into other European countries (Huxley 2003). Field studies have shown that grey squirrels are flexible in a social context, employing various food protection strategies to minimise food loss during caching (Hopewell & Leaver, 2008; Hopewell, Leaver, & Lea, 2008; Leaver, Hopewell, Caldwell, & Mallarky, 2007; Steele, et al., 2008). Although it is not clear whether such flexibility is also shown in other cognitive domains such as problem solving, the evidence suggests that squirrels are able to adapt to new environments and can therefore be expected to be good at problem solving.

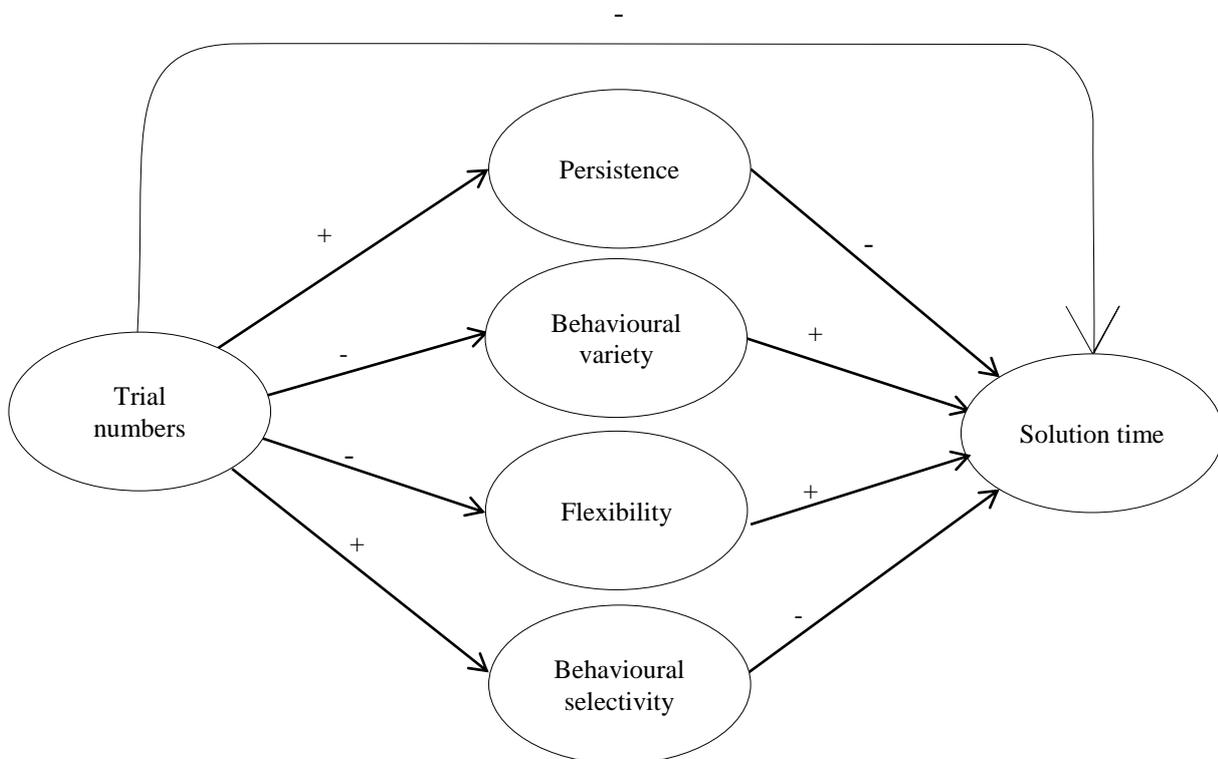


Figure 4.1. This figure shows all the predicted directions and the correlations between all the behavioural traits that vary across time and solution time. Traits include persistence, behavioural variety, flexibility and behavioural selectivity. Persistence is measured as the rate of attempts, behavioural variety is measured as the number of types of contact, flexibility is measured as the rate of switching between contact types and behavioural selectivity is measured as the proportion of effective behaviours.

4.2 METHODS

Ethical Note

The study was approved by the Ethical Review Group at the University of Exeter and in accordance with the Association for the Study of Animal Behaviour guidelines on animal welfare and UK law. Five squirrels (three hand-reared and two recused) living in the laboratory at the University of Exeter participated in this study. Squirrels were two females and three males, aged from 2.5-9 years. For details of the housing and test room, see Hopewell, Leaver, Lea, Wills (2010). Squirrels were not food deprived; daily diet included sunflower seeds, pumpkin seeds, tiger nuts, dried vegetables and fresh fruits and water was provided *ad libitum* throughout the experiment. All squirrels were trained to go voluntarily into the test room through an overhead tunnel that connected their home cage with the test room. The laboratory daylight cycle was 12:12 hrs (0700-1900).

Study information

In the experiment, we used hazelnuts as reward, because these are a preferred food for squirrels and were not included in their normal daily diet. Data were collected from 7th July - 27th Sep, 2013. Testing time was within the period 0900-1500 but depended on the active time of each squirrel. A high-resolution digital camera (Panasonic HD) was set up 15 cm away from the testing cage to capture all the behaviours throughout the experiment. Another mini-camera (Samsung HD brand HMX-W190) was also mounted on the mesh of the adjacent cage, 60 cm away from the centre to capture specific behaviours.

Problem solving apparatus

The problem solving apparatus used was a Plexiglas box, which had ten holes randomly located on each side, and a pyramid-shaped base (Fig. 4.2a). The dimensions of the box were

25 cm x 25 cm x 19 cm, while the base measured 25 cm x 25 cm x 3 cm (Length x Width x Height). The ten holes (2 cm x 0.9 cm, W x H) on each side of the box were horizontally but not vertically aligned with holes on the opposite side (Fig. 4.2b-d), so that levers could be inserted across the box through holes roughly opposite to each other. The box was secured above the base by four wooden legs, and this created a 4.5 cm gap between them where a squirrel could obtain hazelnut rewards when it had solved the problem. The shape of the base allowed the hazelnuts to roll down to the squirrel during the testing phase. In the habituation phase, only the transparent box without any levers was presented to each squirrel. During this phase, the base was made of plastic, but this was replaced by a wooden base for the testing phase. The thickness of the Plexiglas box was also changed from 2 mm to 5 mm in the testing phase. These changes were made to steady the apparatus and prevent squirrels knocking it over during the experiment. During the test phase, ten plastic levers were inserted through holes across the box, protruding from the box by 2.5 cm at each end (Fig. 4.2c). Each lever (1.5 cm x 29.8 cm x 0.5 cm; Length x Width x Thickness) had a 3-sided Plexiglas nut container (back: 2 cm x 1.5 cm; side: 1.5 cm x 1.5 cm) at one end; this was positioned just inside the box. The thickness of each lever was less than the size of the hole, allowing squirrels to smell the hazelnuts when the lever was inserted into a hole. The back of the nut container was transparent and its sides were white, so the squirrel could view the nut from two opposite sides of the box. The design of the apparatus meant that the squirrel could cause the lever to drop, and thereby obtain a nut if there was a nut in the nut container, by pushing the lever end that was near to the nut (henceforth, 'near end'), or by pulling it from the opposite end (hereafter, 'far end'), but not by pulling at the near end or pushing the far end.

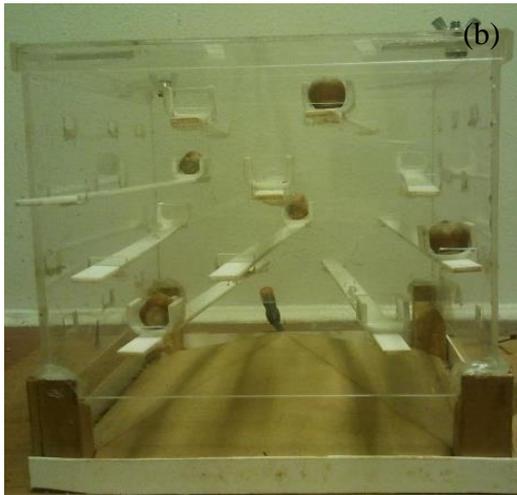
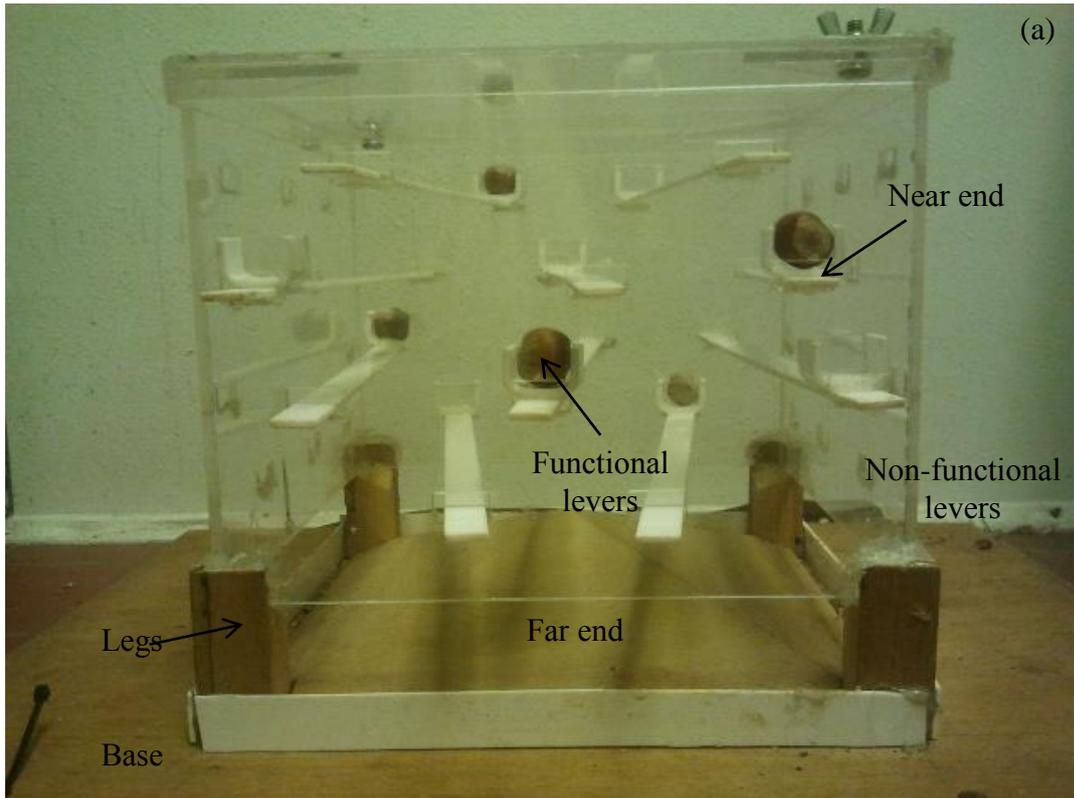


Figure 4.2. (a) figure shows the front side of the puzzle box. This box is constructed as a transparent box (25cm x 19 cm x 25 cm) with ten holes (2 cm x 0.9 cm) located randomly on each side. The holes are horizontally but not vertically aligned to the holes in the opposite side. The pyramidal shape base (25 cm x 3 cm x 25 cm) is to facilitate hazelnuts to roll down the apparatus. Each lever (1.5 cm x 29.8 cm each) has a nut container (back dimension: 2 cm x 1.5 cm; side dimension: 1.5 cm x 1.5 cm). The sides of the container are a solid colour while the back of the container is transparent; (b) the back side of the puzzle box; (c) side view of the puzzle box, levers are positioned horizontally; (d) top view of the puzzle box.

Procedures

The whole experiment lasted for 17 days for each squirrel. The experiment included a standardised habituation phase followed by a testing phase. The habituation phase lasted for three consecutive days and the testing phase for 14 days. Squirrels were habituated and tested individually.

Habituation phase. Each squirrel was given a 30-min habituation phase each day for three consecutive days before the testing phase. The habituation phase aimed to minimise the effect of neophobia on performance and so increase the chance of measuring the intrinsic problem solving ability of each individual. During the habituation phase, we placed the puzzle box without levers in the centre of the test room. To motivate squirrels to get close to the apparatus, we placed eight half hazelnuts around the apparatus.

Testing phase. After the habituation phase, each squirrel went through three blocks of four learning trials of a problem solving task, with one learning trial each day and each trial lasting for a maximum of 45 minutes. Each block lasted for four consecutive days and there was a one-day break between blocks. The apparatus was placed at the same location as in the

habituation phase, but ten levers (five functional and five non-functional) were inserted into the box. Each functional lever contained a hazelnut in its shell in the nut container while each non-functional lever was empty (Fig. 4.2a). We performed the following additional randomisations so as to minimise the probability that an individual would use the positions or direction of the apparatus and levers as a cue to solve the task. In each learning trial, the side of the box presented to the front of the test room was chosen randomly, except that each side of the box was presented once in a block. We also pseudo-randomised the functionality of each lever (with or without a hazelnut) and the direction it faced. Each lever and nut container combination was used as functional twice and non-functional twice within each block of trials. Therefore, no lever could be reliably predicted to be functional or non-functional.

During a learning trial, the squirrels were free to interact with the apparatus without disturbance. The trial began when a squirrel first interacted with the apparatus with any of its body parts. If the squirrel did not interact with the puzzle box for 15 minutes, the trial was terminated and repeated the following day (this only occurred in one trial with one squirrel). The trial ended when the individual obtained all five nuts, when the squirrel had stopped interacting with the apparatus for 15 minutes, or when 45 minutes had elapsed, whichever happened first. Successful problem solving was defined as the squirrel obtaining a nut or causing a lever to drop, whether it was functional or non-functional (see Table 4.1 for full operational definitions).

Table 4.1. Operational definitions for coding the behaviours in the problem solving task.

Behaviours	Definitions
Identifying a nut	A squirrel orients its head towards a lever and sniffs within 0.5 cm of a lever more than 1 seconds.
An attempt	A squirrel uses any of its body part including nose, mouth, teeth, tongue, paw or chin to contact a lever.
Pull	Effective* A squirrel uses its teeth to make an <u>outward</u> movement and a lever subsequently moves outside the box. This pulling behaviour must be performed on the <u>near</u> end of the nut container.
	Ineffective# A squirrel uses its teeth to make an <u>outward</u> movement and a lever does not move out of the box. This pulling behaviour must be performed on the <i>far</i> end of the nut container.
Push	Effective* A squirrel uses any of its body part, including nose (usually), teeth, paw or chin to make an <u>inward</u> movement of a lever and the lever subsequently moves inside the box. This pushing behaviour must be performed on the <i>near</i> end of the nut container.
	Ineffective# A squirrel uses any of its body part, including nose (usually), mouth, teeth, paw or chin to make an <u>inward</u> movement of a lever and the lever would not moves. This pushing behaviour must be performed on the <u>far</u> end of the nut container.
Push up	A squirrel uses its nose to make a push under an end of a lever.
Push down	A squirrel puts force on a lever end with its paws or teeth. This behaviour makes the lever appears in a curve shape.
Tilted up	A squirrel uses its nose to level up a lever end. This behaviour makes a lever turns 45 degrees.
Claw	A squirrel uses it front paws to scratch a lever end.
Lick	A squirrel uses its tongue to touch a lever end.
Shake	A squirrel uses its teeth to bite a lever end and makes an up-and-down movement.
Combined behaviours	At least two of the behavioural types that mentioned above appear.

* indicated as direct effective contact types.

indicated as non-effective contact types.

Measurements

Problem solving efficiency

Problem solving efficiency was recorded in three ways. All these solution times included only the time when a squirrel was in contact with the levers. The first measure was the total time spent on solving the entire task in each trial (hereafter ‘solution time for the entire task’). This included the start time that a squirrel manipulated any functional (with hazelnuts) or non-functional levers (without hazelnuts) until the squirrel obtained all the five nuts or stopped working for 15 minutes. The second measure recorded the total time spent interacting only with functional levers in each trial (hereafter ‘solution time for functional levers’). The final measure was the mean time spent on solving each lever per trial for each squirrel (hereafter ‘solution time per lever’). We divided the solution time for the entire task by the total number of levers that an individual caused to drop in that trial.

Persistence

To measure persistence, we followed the method of Biondi and colleagues (2008) and Griffin & Diquelou (2015), measuring the rate of attempts during problem solving to minimise confounding between the number of attempts and overall problem solving efficiency (measured by solution time). This rate of attempts could reflect squirrels were either showing high frequent of attempts within a short period of time or spending a longer amount of time for each attempt. We first measured the total number of attempts in each learning trial. An attempt was defined as a squirrel starting to use any of its body parts to contact a lever and continued until the squirrel stopped contacting the same lever. If squirrels switched contact from one lever to another that was counted as a new attempt. Then we divided the total number of attempts by the solution time for the entire task.

Behavioural variety

This was measured by the number of different *types* of contact with the apparatus that a squirrel exhibited during problem solving (as listed in Table 4.1). The observed total scores of contact types for a trial ranged from 0 to 9. Behaviours included pull, push in, push up, push down, shake, claw, tilt up, lick and any combination of these such as “tilt up and push in” or “pull and push down” on a lever. Higher scores indicated that an individual exhibited more types of contact during problem solving. We also calculated the rate of behavioural variety, dividing the total number of exhibited types of behaviours by the solution time for the entire task.

Flexibility

Roth & Dicke (2005) suggested that switching between contact types is a measure of intelligence, and this measure is likely to be useful in relation to learning, since it addresses the individual’s tendency to employ alternative means to solve the problem. However, Mery & Burns (2010) have argued that one criterion for adapting to environmental demands is being able to directly observe the consequence of one’s action. It follows that changing the means used to solve a problem should be due to the observed failure of current strategy. Accordingly, we measured flexibility in terms of the number of changes, as a result of failure, between defined contact types the squirrel made in a given trial. Unlike Ramsey et al., (2007), we do not completely insist that such alternative means should be entirely novel: they could include novel strategies as well as strategies that an animal has tried on a previous trial, or a previous problem (Kummer & Goodall, 1985; Reader & Laland, 2003). Nor did we consider whether the behaviours concerned were potentially effective or not. The number of switches between contact types does not depend on an individual’s behavioural repertoire size, as an individual with a limited repertoire could make numerous switches between its

few available behaviours, whereas an individual with a large repertoire might make very few switches between its many available behaviours. However, it is clear that, as with persistence, the number of switches between contact types on a trial will almost inevitably be confounded with the solution time for the trial – the longer it takes the animal to solve the problem, the more chance it has to switch contact types. Accordingly, we measured flexibility by the rate of switching, calculated by dividing the number of switches between contact types on a trial by the solution time for the entire task. This measure examined switches *between* the types of contact used to measure behavioural variety during problem solving. A switch was recorded whenever the current contact type was different from the previous contact type. Only switches as a result of failure were counted, so no switch was recorded if a squirrel had obtained a nut through the immediate previous contact.

Behavioural selectivity

With experience at a task, individuals should learn to narrow down the behavioural types employed to task-relevant behaviours. Benson-Amram & Holekamp, (2012) showed that in successive trials on a problem, hyenas showed reduced behavioural variety across trials on a problem, as they learned to employ fewer ineffective behaviours (as was also found by Thornton & Samson, 2012, in meerkats). They also showed more effective behaviours for the task (as was also found by Manrod, Hartdegen, & Burghardt, 2008, in monitor lizards, and Millot et al., 2014, in cod). In light of this literature, we were interested in the proportion of the behaviours used that were effective. We defined behavioural selectivity as the proportion of the contacts that were effective type. We categorised behaviours as ineffective or effective based on the way that the apparatus design specified ineffective and effective behaviours for solving the problem. We focused on the pull and push contact types which, when correctly applied, led to the most efficient problem solving.

These two behaviours were classified as correct or incorrect, based on which end of the lever a squirrel manipulated (Table 4.1). Ineffective behaviours consisted of contact types that were incompatible with the task requirements, including pulling levers on the near end and pushing them at the far end. These actions could not result in problem solving success. Effective behaviours were contact types that were compatible with the task requirements and were the most efficient behaviours for solving the task in one action. These effective behaviours including correctly pushing the near end or pulling the far end of a lever.

Data analysis

To examine learning, we used non-parametric tests including Page's trend test (Page, 1963) and exact binomial tests. Page's test was applied to examine changes in behaviour across 12 trials (three blocks of four trials each) by examining: solution time for the entire task, solution time for functional levers, solution time per lever, persistence (rate of attempts), flexibility (switch rate), behavioural variety and behavioural selectivity (the proportion of effective behaviours). Exact binomial tests were used to assess whether the distribution of solving attempts between functional and non-functional levers differed from chance for each individual in the first trial. All tests were carried out on individuals, and then we pooled the P -values using Fisher's formula $\chi^2 = -2 \sum \ln(P)$ (Sokal & Rohlf 1995 p. 794).

To examine the variables that were related to problem solving efficiency (Fig. 4.1), we applied a Generalised Estimating Equation (GEE) with exchangeable 'working' correlation (Hardin & Hilbe, 2003; Liang & Zeger, 1986). GEE is a quasi-parametric statistical test that takes individual correlations under repeated measurements into account and has been proven to yield robust results as long as there are no missing data, even if the sample size is small, as in our case (Wang & Long 2011). The dependent variable was

solution time for the entire task in each trial (i.e. including functional and non-functional levers, until squirrels obtained all the five hazelnuts or stopped working for 15 minutes). Covariates included trial number, persistence, flexibility and behavioural selectivity. Behavioural variety was found to be highly correlated with behavioural selectivity ($r=0.68$) and persistence ($r=-0.64$). This correlation held true even after we expressed behavioural variety as rate with selectivity ($r=-0.56$) and persistence ($r=0.67$), and so behavioural variety was dropped from the model to avoid multicollinearity. As small sample size can lead to underestimating the variance in calculation, an adjusted robust variance (Wang & Long, 2011) was applied to calculate the P-values. All the results reported are two-tailed and results were considered as significant when alpha was <0.05 . Statistical analyses were performed in R (version 2.15.2, R Development Core Team, 2012); the ‘gee’ package was used to apply GEE (Carey, 2012) and the ‘crank’ package was used to apply Page’s trend test (Lemon, 2014).

4.3 RESULTS

Trends across trial blocks

All the squirrels solved the entire task and obtained all five nuts on their first trial, with solution time ranging from 38.1 seconds to 69.6 seconds (see Supplementary material for video S1a shows an example for one squirrel, Leonard, solving the puzzle box in his first trial). Variations in solution time also persisted to the last trial, ranging of solution times from 8.4 seconds to 68.8 seconds (see Supplementary material for video S1b shows Leonard solving the puzzle box in his last trial). Fig. 4.3a shows that in the first trial, squirrels approached the functional levers (with hazelnuts) more often than the non-functional levers (without hazelnuts); 84.1% of all approaches were to functional levers, and this proportion is significantly different from 50% (pooled $\chi^2_{10}=35.04$; $P<0.001$). This indicates that squirrels were motivated by the food reward since the beginning. Figs. 4.3b, 4.3c and 4.3d shows that

solution time for the entire task, solution time for functional levers, and solution time per lever decreased across trials (Page's trend tests: $\chi^2_{1}=8.8$, 14.1 and 13.8 respectively, $P<0.005$ in all cases). Figs. 4.4b and 4.4c show that the change of behavioural variety and flexibility across the 12 trials. Neither behavioural variety nor flexibility showed significant changes across trials (Page's trend tests: $\chi^2_{1}=0.5$ and 0 respectively, $P>0.05$ in both cases). Figs. 4.4a and 4d show the change of persistence and behavioural selectivity across trials. Both persistence and behavioural selectivity showed a significant increase across trials (Page's trend tests: $\chi^2_{1}=9.8$ and 20.83, respectively, $P<0.005$ in both cases), indicating that squirrels gradually increased their attempts and exhibited more effective behaviours. Fig. 4.5 shows scatterplots, across all trials and subjects, of the relationship between flexibility and selectivity (Fig. 4.5a) and persistence (Fig. 4.5b), and of the relationships between those two variables and solution time (Figs. 4.5cd). These plots show the first-order relationships between variables, as well as the directions in which flexibility was related to other behavioural traits.

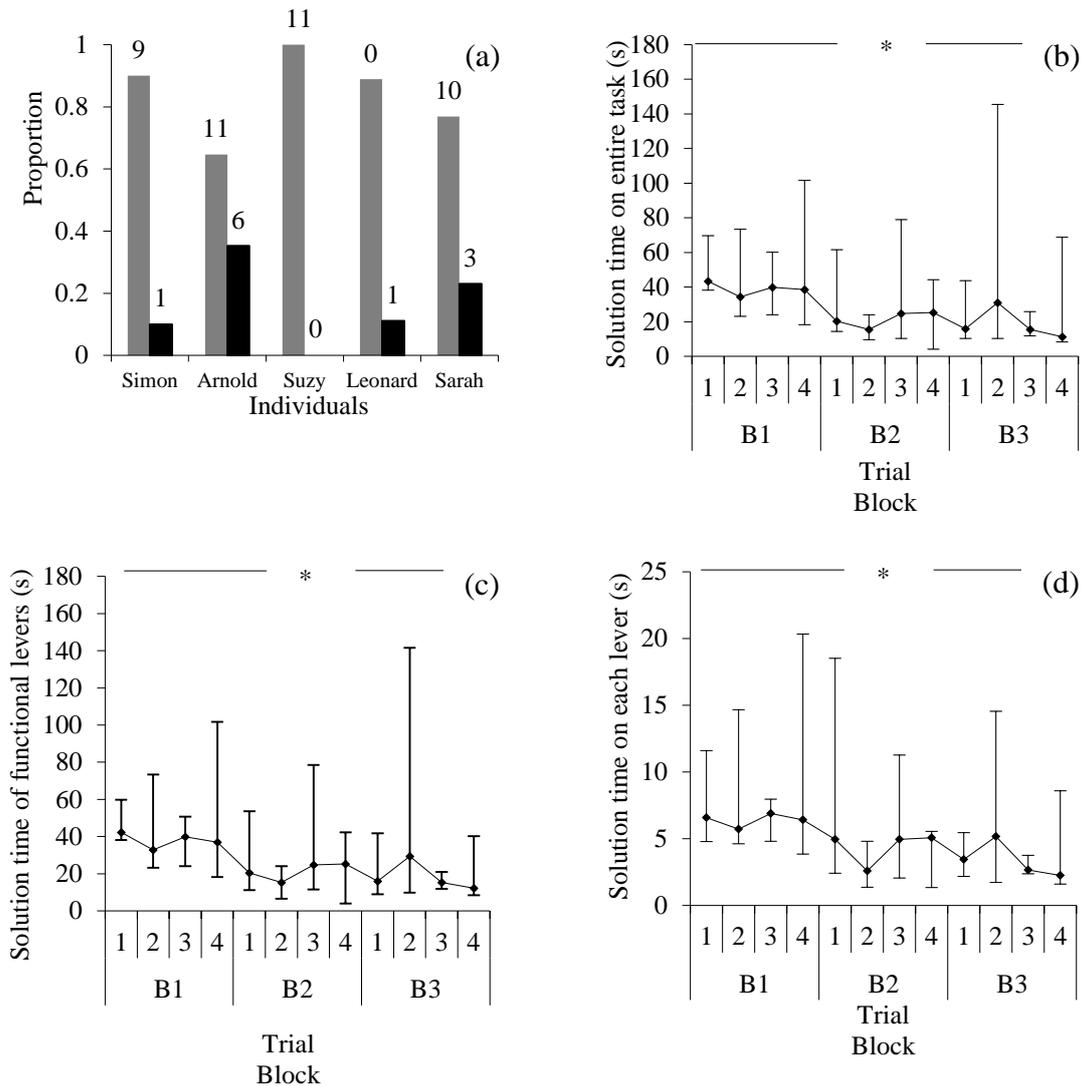


Figure 4.3. (a) Proportion of choices of functional levels (■) and non-functional levels (■) in the first trial. Numbers above bars indicate the actual number of times that each squirrel approached functional and non-functional levels. (b) median, maximum and minimum of total solution time in seconds to solve the entire task, including functional and non-functional levels across trials. (c) median, maximum and minimum of total solution time in seconds on solving the *functional* levels across trials. (d) median, maximum and minimum of average solution time in second in solving *any* lever across blocks. $N=5$. $*p<0.05$

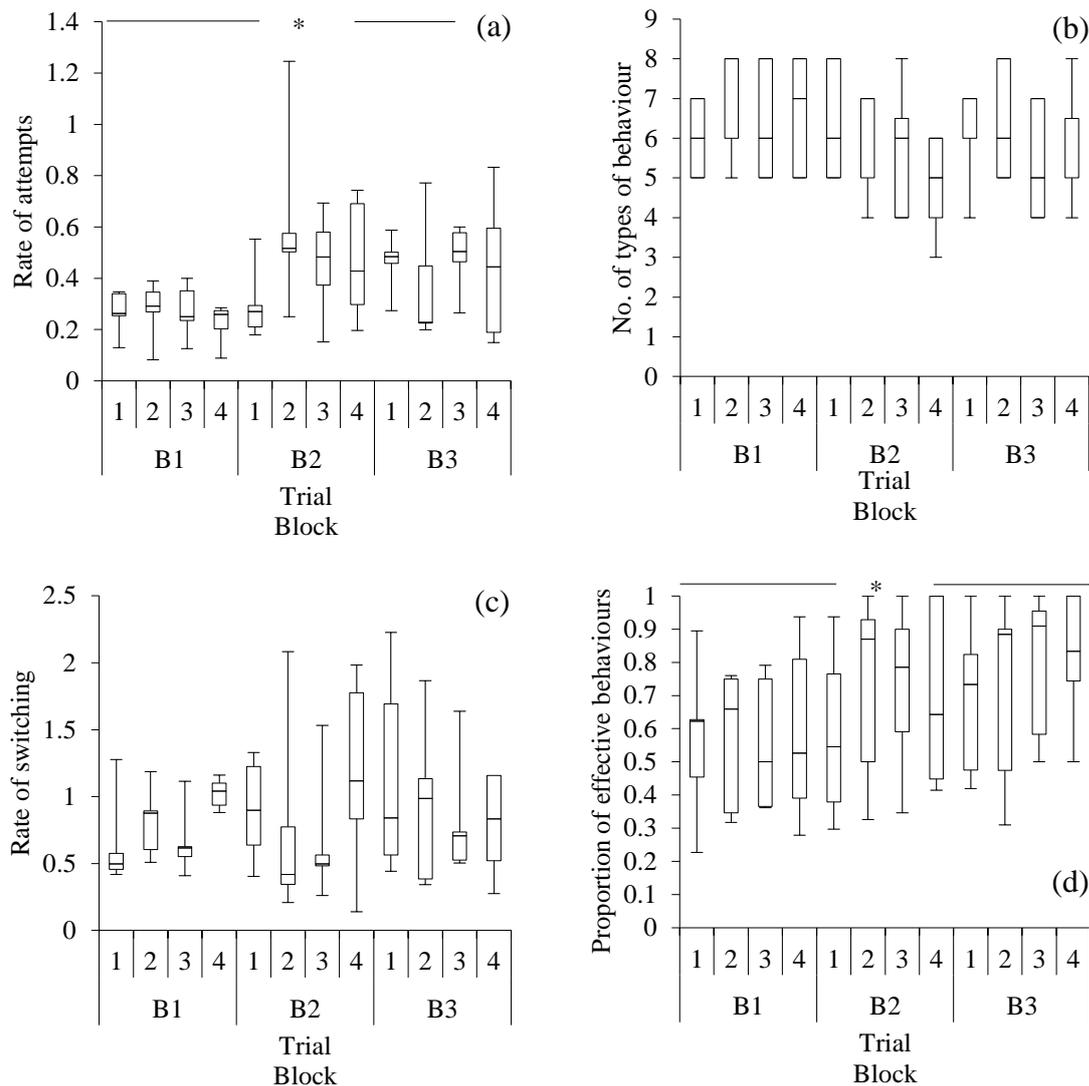


Figure 4.4. Boxplots show how each behavioural trait varied across the 12 trials. The values of the traits for the five squirrels tested in each trial are shown by the line in the box, the top and bottom of the box and the upper and lower vertical lines; thus the data of the five squirrels could be read from the graph (a) persistence, measured as the rate of attempts across the 12 trials; (b) behavioural variety, measured as the number of types of contact across the 12 trials; (c) flexibility, measured as the rate of switching between contact types across the 12 trials; and (d) behavioural selectivity, measured as the proportion of effective behaviours across the 12 trials. $N=5$. $*p<0.005$

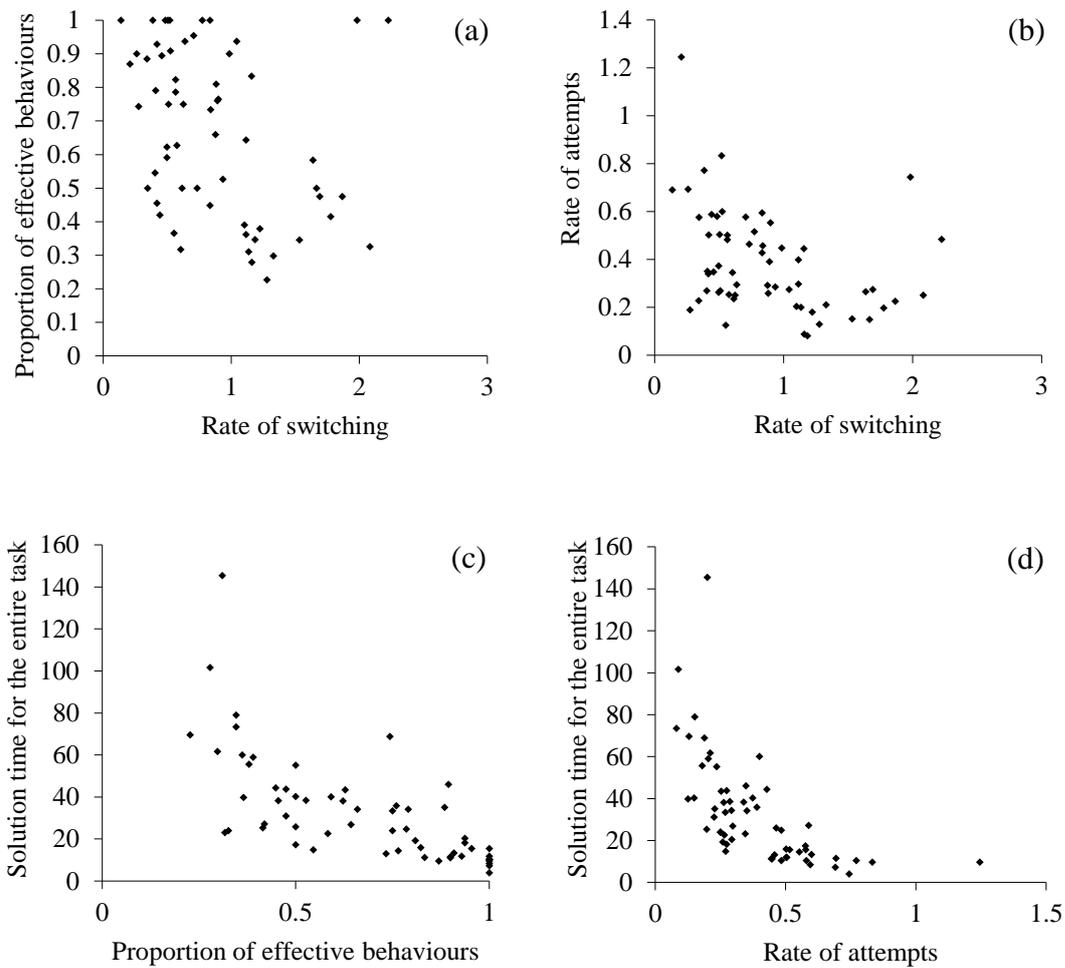


Figure 4.5. Scatter plots showing relationships between (a) flexibility (rate of switching) and behavioural selectivity (proportion of effective behaviours); (b) flexibility (rate of switching) and persistence (rate of attempts); (c) behavioural selectivity (proportion of effective behaviours) and the solution time for the entire task; (d) persistence (rate of attempts) and the solution time for the entire task. Noted that each plot uses the raw data across all subjects and trials to show the general trends between variables.

Predicting problem solving efficiency

The GEE model for the predictors of solution time (Table 4.2 Path 1) showed that two factors, persistence ($\chi^2_1=7.48$, $P=0.006$) and behavioural selectivity ($\chi^2_1=3.98$, $P=0.046$), were significant predictors of solution time. Specifically, persistence was negatively related to the solution time, indicating that increased rate of attempts led to lower solution time.

Behavioural selectivity was also negatively associated with solution time, with a higher proportion of effective behaviour associated with lower solution time. However, flexibility ($\chi^2_1=0.51$, $P=0.474$) was not a significant predictor of solution time. Moreover, with the component skill variables included in the model, trial number ($\chi^2_1=0.20$, $P=0.656$) was not a significant predictor of solution time either.

Table 4.2. This table shows the summary of three GEE models. **Path 1.** *This table shows the summary of the GEE model that examines the covariates for solution time. The model shows that only persistence and behavioural selectivity are the significant covariates for efficient problem solving.* **Path 2.** Summary of the GEE model that examines the covariates for persistence. **Path 3.** Summary of the GEE model that examines the covariates for behavioural selectivity. The table shows estimated coefficients, χ^2 , df , Z values and P values. Values are based on an adjusted variance for small sample size.

Path no.	Predictors	DV	Estimates	χ^2	df	Z	P
1	Trial numbers	Problem solving efficiency	0.27	0.20	1	0.44	0.656
	Persistence		-51.38	7.48	1	-2.74	0.006
	Flexibility		-3.30	0.51	1	-0.72	0.474
	Behavioural selectivity		-41.15	3.98	1	-2.00	0.046
2	Trial numbers	Persistence	0.03	9.10	1	3.02	0.003
	Flexibility		-0.19	6.26	1	-2.50	0.012
3	Trial numbers	Behavioural selectivity	0.03	41.68	1	6.46	<0.001
	Flexibility		-0.10	5.01	1	-2.24	0.025

Table 4.3. *The total effects, shown as standardised beta weight (β), of each predictor on solution time.*

Predictors	Total effect (β)
Trial numbers	-0.28
Persistence	-0.43
Flexibility	0.20
Behavioural selectivity	-0.40

Relationships between independent variables: mediation analysis

Although trial number was individually correlated with solution time (see Fig. 4.3b), the lack of any effects of trial number and flexibility on problem solving efficiency in the full model suggests that persistence and behavioural selectivity might mediate the effects of experience, and perhaps flexibility (Fig. 4.5a and 4.5b), on solution time (Fig. 4.5c and 4.5d). Therefore, we ran two further GEE analyses to test these mediational analyses, including trial number and flexibility as covariates factor and predict persistence for one model (Path 2) and behavioural selectivity in the other model (Path 3). Results confirmed that persistence (Path 2) and behavioural selectivity (Path 3) were both significantly predicted by trial number and flexibility, with opposite directions of effect, such that persistence increased across trials and decreased with higher flexibility whereas behavioural selectivity increased across trials and decreased with higher flexibility. We then ran the final analysis to assess whether trial number and flexibility were independent from each other. Results showed no evidence that they were dependent on each other ($\chi^2_1=0.33, P=0.565$). This is the pattern of results that would be expected if persistence (Path 2) and behavioural selectivity (Path 3) mediate the effects of trial number and flexibility on solution time. Fig. 4.6 shows the causal structure corresponding to those results, and the standardised beta weights (β) of the indirect paths from the GEE analyses. According to this analysis, two routes were involved in the improvement of problem solving efficiency across learning trials. First, persistence increased across learning trials, leading to reduced solution time ($\beta = 0.41 \times -0.43 = -0.18$), and secondly behavioural selectivity increased, increasing the proportion of effective behaviours and again reducing solution time ($\beta = 0.36 \times -0.40 = -0.14$). The total effects of each factor on problem solving efficiency are shown in Table 4.3.

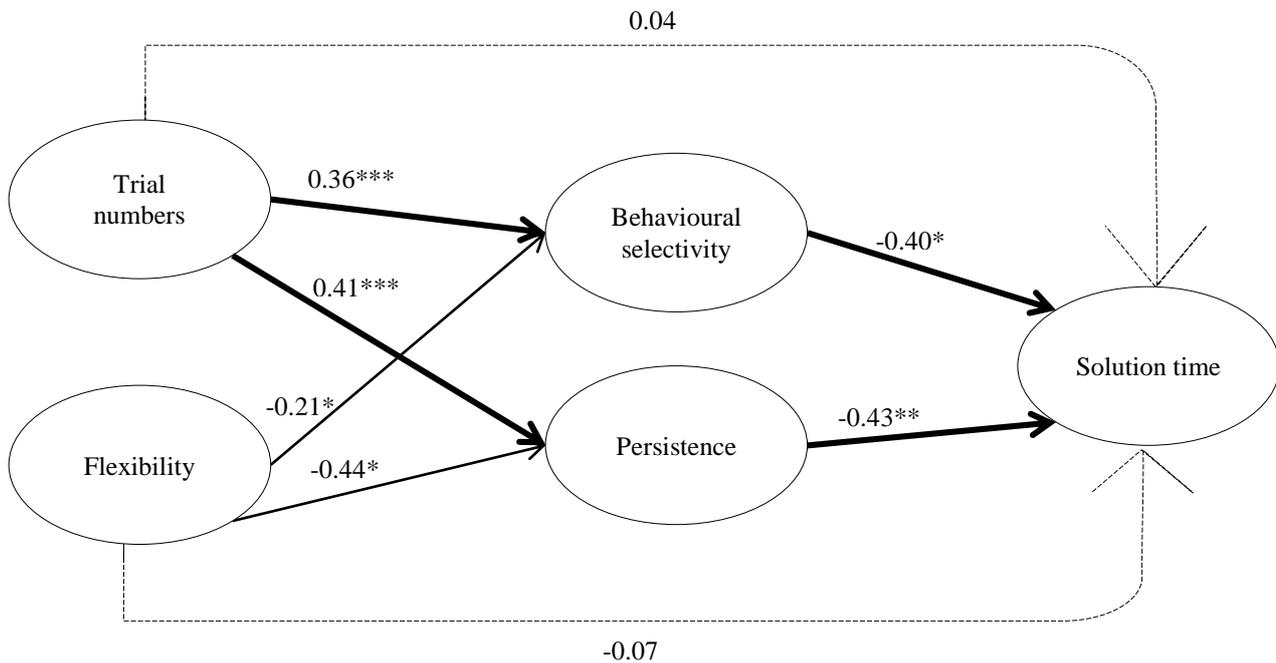


Figure 4.6. Standardised beta weights (β) of all the direct and indirect effects between factors. The dependent variable is the total solution time (including solving both functional and non-functional levers) of each trial for each squirrel. Covariates are trial numbers, flexibility, persistence and behavioural selectivity. Thick solid lines show the route to achieve efficient problem solving. Solid lines indicate significant effects while dash lines indicate non-significant effect. * <0.05 , ** <0.01 , *** <0.005

4.4 DISCUSSION

In the present experiment, we have been able to fully account for the improvement in problem solving efficiency that occurred with experience in terms of two intermediate factors, persistence and behavioural selectivity. We have also shown that an additional relevant factor, flexibility, may be an independent cognitive process, since it was unaffected by experience on the task.

A priori, the first of the two key mediating factors, persistence, could have either positive or negative effects on problem solving. But, at least in the present task, persistence (measured, following Biondi et al. 2008, as the rate of attempts), increased across trials and was an important positive factor in learning, as it was related to decreased solution time (Path 1). This is consistent with the results of Sol, Griffin, & Bartomeus, 2012, who showed that an increased attempt rate over trials was associated with an increased probability of task completion: here, we extend their result by showing that such increased persistence mediates the improvement of performance that occurs over learning trials. This result reflects that squirrels showed more attempts with improved solution time to complete the task, which is what should be expected in an instrumental task once at least some success has been achieved: the reward delivered on success in the task should reinforce both the specific effective behaviour, and also the more general behaviour of interacting with the apparatus, which we recorded as attempt rate. In addition rewards will increase incentive motivation. The present task was solved (albeit inefficiently) on the first trial by all squirrels, allowing reinforcement to take effect. In a task where some animals are not unsuccessful in initial trials, persistence might have more negative effects.

The other key mediating factor, behavioural selectivity, was measured as the proportion of effective behaviours observed on a trial. This would be unambiguously expected to be associated with increased efficiency, and it was (Path 1). Again, operant conditioning provides a straightforward account of this change, with reinforcement increasing the rates of effective behaviours and extinction decreasing the rates of ineffective behaviours. The cognitive factors underlying such reinforcement processes are likely to include attention to the subject's own behaviour. Heightened attention to relevant exteroceptive cues has been shown to be important for successful problem solving (St Clair & Rutz, 2013), as has attention to movement cues (Overington et al., 2011), but attention to the individuals' own movements has not been investigated and would be a promising area for future study. Experiments on stimulus-response overshadowing (e.g. Roberts, Tarpy, & Lea 1984) show that common attentional processes apply to both exteroceptive and interoceptive cues.

In our experiment, flexibility, measured as the rate of switching between contact types as a result of failure to solve the current problem, did not vary as a function of the squirrels' experience at the task (Fig. 4.4*b*). Increased flexibility was not associated directly with greater efficiency at problem solving (Path 1): instead, it was related to both decreased persistence (Path 2) and lower behavioural selectivity (Path 3). These results reflect that the ability to learn is not the same thing as showing flexibility within a single encounter with a problem solving task and such flexibility could be an independent cognitive process in problem solving that does not involve learning, but it facilitates animals to cope with the demand with an instant modification of behaviours after a failed attempt. Our finding of flexibility is contrary to other studies that have suggested that flexibility brings clear advantages for animals, through its contribution to successful innovation (e.g. Benson-

Amram & Holekamp 2012). Under natural conditions, individuals who spend a long time with a given problem also put themselves at predation risk, perhaps for little nutritional gain. A resolution of this apparent paradox is possible if increased flexibility is associated with lower competitive ability in the wild. Indeed, it has been shown that the individuals that commit time to solve problems are those that have lower competitive ability in foraging than their counterparts or are subordinates in their group (Cole & Quinn 2012; Thornton & Samson 2012). Thus increased flexibility might be an adaptive foraging strategy for individuals who have no alternative, enabling them to access food sources that more dominant individuals are less bothered with. Such plasticity of behaviour can bring individuals adaptive advantages in various fitness measures such as learning ability in harsh environments (Roth, LaDage, & Pravosudov 2010), species richness (Nicolakakis, Sol, & Lefebvre 2003), and mating success (Keagy, Savard, & Borgia 2009; but also see Isden et al. 2013); see Dukas (2013) for a review.

A limitation of the present study is that it is based on a small sample size, so only limited degrees of freedom were available for exploring how other interaction effects might have contributed to problem solving efficiency. We also need to be cautious in generalising the results to the whole species. Nevertheless, the study provides insights into the underlying mechanisms in problem solving. Given that grey squirrels have successfully invaded several European countries and the Western United States, future research should use a larger sample size and investigate their flexibility and problem solving ability under conditions where innovative foraging is essential to survival; this might help us to gain a better understanding of the basis for their invasive success and give insight into the success of other invasive species. Like other scatter hoarders, grey squirrels undoubtedly have unusual capacities for

spatial cognition (e.g. Smulders, Gould, & Leaver 2010). It remains an open question whether this exceptional ability is domain-specific. Their capacity for exploiting anthropogenic food sources suggests that grey squirrels may also be unusually good problem solvers; if in fact they have high cognitive capacity over a range of domains, this would be a good candidate as an explanation for their invasive success.

CHAPTER 5: DO GREY SQUIRRELS (*SCIURUS CAROLINENSIS*) OUTPERFORM NATIVE REDS (*S. VULGARIS*) IN PROBLEM SOLVING?

ABSTRACT

Flexibility varies across species and is particularly crucial for invasive species in establishing new habitat. Although flexibility does not predict extinction rate of a population, evidence shows that low flexibility indirectly leads to a series of sub-optimal adjustments in native red squirrels (*Sciurus vulgaris*) when they co-exist in the same habitat with the invasive grey squirrels (*S. carolinensis*). It raises a question whether invasive grey squirrels have suppressed red squirrels' flexibility or red squirrels are inherently less flexible. Here, we addressed this question in a field experiment at UK sites where only red squirrels or only grey squirrels existed. We examined flexibility using two food-extraction tasks. We also examined four behavioural traits persistence, behavioural variety, flexibility, and behavioural selectivity in relation to problem solving performance. The level of difficulty for the two tasks varied: squirrels were required to lift up a lid to solve a hinged box task (easy problem) or make a lever drop to obtain a hazelnut in a puzzle box (difficult problem). Results showed that all the red ($N=17$) and grey squirrels ($N=14$) solved the easy task whereas fewer red squirrels (13/21) than grey squirrels (20/22) solved the puzzle box task. Despite this, red squirrels problem-solvers were also more efficient than the grey squirrels. Detailed analyses showed that red solvers showed a higher rate of flexibility and higher rate of behavioural variety than the grey squirrels. Red solvers also increased their behavioural selectivity across successes. These findings show that 1) grey squirrels, as an invasive species, are more successful in solving a difficult task; and 2) at least a small portion of red squirrels are as capable as grey squirrels in solving a difficult task, and outperform their grey congeners in efficiency.

5.1 INTRODUCTION

Direct evidence has already shown that invasive avian species show high flexibility as innovation or successfully solving novel food-extraction tasks (Griffin, Diquelou, & Perea, 2014). Flexibility is important for invasion success, establishment in new habitats, survival in harsh environment or adaptation to urban life (Sol, Timmermans, & Lefebvre, 2002; Sol, Duncan, Blackburn, Cassey, & Lefebvre, 2005; Sol, Bacher, Reader, & Lefebvre, 2008; Sol, Lapedra, & González-Lagos, 2013). Despite this, low flexibility does not predict the decrease or extinction rate of a population (Nicolakakis, Sol, & Lefebvre, 2003). This evidence reflects that both invasive and non-invasive species possess capacity to solve problems, but the level of flexibility varies between invasive and non-invasive species.

Although a lack of flexibility does not have a direct effect on population decreases, its indirect effects on population survival have been suggested. The decrease of a population has been shown to be related to the number of members in a population (Nicolakakis, Sol, & Lefebvre, 2003) or fitness for reproduction (Grunell, Wauters, Lurz, & Tosi, 2004). These outcomes may be related to the fact that flexibility in problem solving success and mating success are correlated. Individuals that have lower problem solving ability have lower reproduction success than those with higher problem solving ability (Cole, Morand-Ferron, Hinks, & Quinn, 2012; Keagy, Savard, & Borgia, 2009; but also see Isden, Panayi, Dingle, & Madden, 2013). These outcomes could be due to a series of sub-optimal behavioural adjustments when non-invasive species co-exist with invasive species. A direct illustration for this possibility is that when non-invasive Eurasian red squirrels (*Sciurus vulgaris*) share the same habitat with invasive Eastern grey squirrels (*S. carolinensis*), red squirrels spend less time foraging and consume less high-energy food than habitats without the greys (Wauters, Gurnell, Martinoli, & Tosi, 2001; Wauters, Tosi, & Gurnell, 2002). These

behavioural adjustments suggest that red squirrels employ a low energy expenditure strategy when they encounter the presence of grey squirrels. However, these adjustments appears to induce a vicious cycle: they may cause red squirrels, especially juveniles, to become less physically fit than sites without grey squirrels during the mating season, and in turn, lead to lower reproductive success in red squirrels (Grunell, Wauters, Lurz, & Tosi, 2004). But a question that remains to be answered is whether grey squirrels in some ways inhibit the expression of flexibility in red squirrels, or whether red squirrels are just inherently less flexible than grey squirrels?

Both the invasive grey squirrels and the non-invasive red squirrels belong to the same family, *Sciuridae*, and same genus, *Sciurus*, and have a higher brain-to-body size than other rodents (Mace, Harvey, and Clutton-Brock, 1981). The two species share many ecological characteristics, see Koprowski (1994) for grey squirrels and Lurz, Gurnell, & Magris (2005) for red squirrels. Despite these similarities, grey squirrels expanded their population quickly and have replaced red squirrels in their habitats since the 19th century in UK (Grunell, 1987) and more recently in Europe (Huxley, 2013). Grey squirrels are regarded as one of the ‘100 World’s Worst Invasive Alien Species’ (Global invasive species database, 2015) and especially in UK, the population of grey squirrels is still increasing. We chose sites that were inhabited either exclusively by grey squirrels (Exeter) or exclusively by red squirrels (Isle of Arran) and squirrels are introduced than less than a century in both sites. We obtained detailed problem solving performance in two tasks: a hinged box task and a puzzle box task. Compared with the puzzle box task, the hinged box task was easier, as the behaviour required for squirrels to employ was not counterintuitive as in the puzzle box task. The puzzle box task was exactly the same as used in Chow, Lea and Leaver (*in press*). We predicted that if grey squirrels are inherently more capable than the red squirrels to solve problems, then there

would be more successful individuals in solving the problem tasks than the congeneric red squirrels. We also predicted that grey squirrels would outperform red squirrels in problem solving efficiency. However, if grey squirrels inhibit the flexibility of red squirrels, then without the greys co-exist, red squirrels should show a similar number of successes as grey squirrels as well as similar problem solving efficiency.

We also aimed to examine whether the two species differ in their behavioural traits during problem solving. Traits are a more reliable predictor than using brain size or biological characteristics such as sex or age (e.g. Overington, Cauchard, Côté, & Lefebvre, 2011). Behavioural traits included persistence, flexibility, behavioural variety and behavioural selectivity. These traits have been shown to relate to successful problem solving (e.g. Benson-Amram & Holekamp, 2012, Griffin, Diquelou, & Perea, 2014; Overington, Cauchard, Côté, & Lefebvre 2011, Thornton & Samson, 2012) and some evidence for problem solving efficiency (e.g. Chow, Lea, & Leaver, *in press*; Griffin & Diquelou, 2015). The difference between behavioural traits and problem solving performance could be a useful indicator to understand the differences between invasive and non-invasive species in problem solving.

5.2 METHODS

Study sites and study populations

Data were collected on two populations of free-ranging squirrels. The study with grey squirrels was conducted in two parts from Oct, 2013-Jan, 2014 and Dec, 2014 to Feb, 2015, at eight locations around the University of Exeter campus and parkland (Figure 5.1 upper panel). The red squirrel study took place from Sept to Nov, 2014, at seven locations that were around the Brodick castle and country park at Brodick, Isle of Arran (Figure 5.1 lower panel).

Although squirrels are active throughout the year, the season and the time that were chosen for this study was based on the most active period that squirrels were present on the ground for caching and foraging in order to increase the probability for engaging with our tasks. Locations were selected based on number of squirrels present, the density of trees and bushes, the number of humans and dogs present in each site and the distance from the nearest main road (this ranged from 280 m to 400 m).



Figure 5.1. Upper panel shows eight locations for grey squirrels data collection. Lower panel show seven locations for red squirrels data collection. Scale 1:100m



Both sites were mixed woodlands with beech trees, pine trees, and hazel trees. Although squirrels at both sites were habituated to the presence of humans and dogs, we observed that squirrels fled and stayed on the trees when they were coming into the area.

Data were collected between 0700 and 1900 in late summer to mid-autumn and between 0730 and 1645 in the mid-autumn to winter. We chose to use hazelnuts because they contain high levels of tannin and squirrels like to store them during the caching season rather than to eat them immediately. Hazel trees were not in season during both data collection periods. Each study site had three to five squirrels foraging, allowing easy identification of individuals and avoiding high competition between conspecifics (e.g. aggressive behaviours) during problem solving.

Animal identification. Prior to the first grey squirrel observation in 2013, we used trapping, PIT tagging and marking methods to identify squirrels. We live-trapped 43 squirrels around the campus from late April to early September, 2013 under the DEFRA Non-Native Species Release Licence WCA/02/11. Collapsible traps (Tomahawk Live Trap, Wisconsin, U.S.A., model #202) were baited with peanut butter and were set an hour before dawn till one hour before dusk. Traps were checked at least every 1.5-2 hours. Upon capture, a squirrel was immediately transferred to a mesh cone, weighed, sexed, tagged with a Passive Integrated Transponder (Trovan, ID 100), and then was marked with a distinctive pattern with black hair dye (Boots™ permanent hair dye, Ebony Black). So far as we could determine, no deaths or health problems resulted upon release. However, the use of a PIT reader and trapping appear to deter the squirrels from approaching novel objects, since only 3/43 marked squirrels came back during the habituation. Accordingly, the identification of red squirrels and subsequent data collection for grey squirrels relied on individual's unique characteristics based on the intensity of their coat colour, tail shape, body size, ear shape, paw colour, marks on face and body from the videos. This procedure successfully increased the sample size but required intensive observer training, typically for around two months for each species.

Apparatus and equipment

Hinged box task. Figure 5.2 shows the hinged box (12 cm x 12 cm x 4.5 cm) with four plastic wells at each corner (4.5 cm x 3 cm x 1.5cm). Each well was attached with a transparent lid (3 cm x 3 cm).



Figure 5.2. A hinged box that is constructed with four containers, one at each corner.

Squirrels could lift up a lid using their front paw, nose or teeth to obtain a hazelnut.

Puzzle box task. Following Chow, Lea, and Leaver (*in press*), we used the same puzzle box to examine the ability to solve a difficult task. Chapter 4 Fig. 4.3a shows the puzzle box. It was a Plexiglas box, which had ten holes randomly located on each side, and a pyramid-shaped base. The dimensions of the box were 25 cm x 25 cm x 19 cm, while the base measured 25 cm x 25 cm x 3 cm (Length x Width x Height). The ten holes (2 cm x 0.9 cm, W x H) on each side of the box were horizontally but not vertically aligned with holes on the opposite side (Chapter 4 Fig. 4.3b-d), so that levers could be inserted across the box through holes roughly opposite to each other. The box was secured above the base by four wooden legs and this created a 4.5 cm gap between them where a squirrel could obtain hazelnut rewards when it had solved the problem. The shape of the base allowed the hazelnuts to roll down to the squirrel during the testing phase. In the habituation phase, only the transparent box without any levers was presented at each location. During the test phase, ten plastic levers were

inserted through holes across the box, protruding from the box by 2.5 cm at each end (Chapter 4 Fig. 4.3c). Each lever (1.5 cm x 29.8 cm x 0.5 cm; Length x Width x Thickness) had a 3-sided Plexiglass nut container (back: 2 cm x 1.5 cm; side: 1.5 cm x 1.5 cm) at one end; this was positioned just inside the box. The thickness of each lever was less than the size of the hole, allowing squirrels to smell the hazelnuts when the lever was inserted into a hole. The back of the nut container was transparent and its sides were white, so the squirrel could view the nut from two opposite sides of the box. The design of the apparatus meant that the squirrel could cause the lever to drop, and thereby obtain a nut if there was a nut in the nut container, by pushing the lever end that was near to the nut (henceforth, 'near end'), or by pulling it from the opposite end (hereafter, 'far end'), but not by pulling at the near end or pushing the far end.

Procedures

Initial site-baiting. Prior to the experiment, we baited each location with 16 shelled and 8 unshelled hazelnuts twice per day (once before dawn and once before dusk) for three consecutive days, in order to attract squirrels to come to the location regularly. The use of hazelnuts was based on squirrels' preferences, but also because the size of shelled hazelnuts mostly can be carried by squirrels but not by other small animals such as robins or shrews. We checked the locations twice a day to ensure that squirrels were visiting the targeted location.

Hinged box task. We set the box on the ground at a random position to minimise squirrels using box position as a cue to open a lid. However, squirrels could use the length and the width of the lid to indicate which side to lift.

Puzzle box task. We first carried out a habituation period to minimise neophobic responses to the task apparatus. During the habituation period, we placed only the cover at the targeted locations. To encourage the squirrels to get close to the cover, 40 shelled hazelnuts were placed around the apparatus (10 on each side of the cover) and squirrels could take the hazelnuts freely. We checked the box every one or two hours and refilled the hazelnuts.

The main experiment started once squirrels were coming regularly to obtain the hazelnuts. We placed the puzzle box either 50 cm away from a tree or inside the bushes to minimise the predatory risk for squirrels. The same puzzle box was presented with ten levers in place. Of these, five levers contained hazelnuts (hereafter, ‘functional’ levers) and five levers were empty (hereafter, ‘non-functional levers’). Squirrels were free to visit the puzzle box without any interference and thus, the nut that each individual obtained varied daily as well as throughout the experiment. To avoid squirrels using the positions of the holes or the direction of the cover relative to the tree to solve the task, we randomised the side to insert the levers. We also randomised the direction of the nut container and whether or not a lever was functional or non-functional in a trial.

For both tasks, we counter-balanced the presentation of tasks. Apparatus was placed in the field for the whole day from dawn to dusk regardless of the weather condition. Accordingly, we were able to carry out 5-8 trials each day daily depending on the available day light and temperature. We checked and re-baited the apparatus at intervals that varied between 1- 2 hours; this could increase squirrels’ participation based on individual active periods. Each trial started after a check. In both tasks, detailed behaviours of squirrels during the task were captured by a video camera (Panasonic SWD HD-90) that was mounted on a tripod and placed 60 cm away from the apparatus. During the first season of data collection

with grey squirrels, an experimenter (the first author) was present 25 m away from the site; this was done with grey squirrels which were relatively habituated to the presence of humans on campus (3 out of 5 chosen locations).

Measurements

Performance on the hinged box task. Problem solving success in the hinged box task was defined as when a squirrel used its mouth, nose or front paw to lift up the transparent lid. We counted the number of squirrels in each species that solved the hinged box task. We also recorded the solving duration for each success.

Performance on the puzzle box task. Success in puzzle box task was defined as when a squirrel used any of its body parts to manipulate a lever and causing a lever or a nut to drop.

We recorded whether a squirrel successfully solved the task at the first encounter (hereafter ‘first success’ or ‘first failure’). First encounter was defined as when a squirrel first appeared in the video and it manipulated a lever till it left the view of the video for two minutes. We also recorded whether the same squirrel successfully solved the task when it returned (hereafter, ‘subsequent success’ or ‘subsequent failure’). Only squirrels that solved the task more than three times were considered as ‘problem solvers’ and others were considered ‘non-problem solvers’.

We followed Chow, Lea and Leaver (*in press*) in measuring problem solving performance and the factors that are associated with problem solving success and problem solving efficiency. Measures included flexibility, behavioural variety, persistence (all measured as rates) and behavioural selectivity (measured as a proportion). The use of rates

and proportion rather than absolute numbers aimed to minimise the confounds with problem solving efficiency.

Problem solving efficiency. This measurement aimed to examine whether learning existed in solving the problem. We examined this by measuring the time to solve a lever across trials. We recorded the duration between the time started to solve the problem and ended with either obtaining a nut or causing a lever to drop, and whether it was a functional or a non-functional lever.

Flexibility. To measure flexibility, we recorded the switches between the tactics that a squirrel exhibited during problem solving that are listed in Table 4.1. Flexibility is indicated when the squirrels changed from one type of tactic to another type of tactic. We first summed up the number of changes of tactic in each trial and divided this score by the time that the squirrel used to solve the levers in that trial to obtain the rate of switching.

Behavioural variety. Behavioural variety was measured as the number of *types of contact* that a squirrel used to solve the task. Table 4.1 lists all the behavioural types that squirrels used to solve the problem task. We then calculated the rate of behavioural variety using the total number of types of contact by the total solution time for each success.

Persistence. Persistence was measured as the rate of attempts at solving the task. An attempt was defined as a squirrel using any of its body parts to contact a lever until squirrels stopped contacting the same lever. An attempt was also counted if the squirrel changed to manipulate another lever. We recorded the total number of attempts and then divided this number by the duration that the squirrel used to solve the task to obtain the rate of attempts.

Behavioural selectivity. We measured behavioural selectivity as the proportion of effective behaviours. This was calculated by the number of effective behaviours divided by the sum of effective and ineffective behaviours. Effective behaviours were either pushing the ‘near-end’ or pulling the ‘far-end’ whereas ineffective behaviours were either pushing the ‘far-end’ or pulling the ‘near-end’.

Data analysis

Behavioural data were analysed frame-by-frame using Premiere Pro CS6. SPSS V22 was used to run all data analyses. Given that data was not normal, we used non-parametric tests including Chi-square was used to examine whether there was a difference in the number of individuals of each species in first success and subsequent successes. Mann-Whitney U tests were used to examine at the first encounter, the differences in flexibility, behavioural variety, persistence and behavioural selectivity between problem solvers at the *between*-species level, between non-problem solvers at the between-species level, and between problem solvers and non-solvers at the *within*-species level.

A Generalised Linear Model with binominal logit link was used to examine the factors that determined the first success or failure in the first encounter. Covariates included flexibility, behavioural variety, persistence and behavioural selectivity.

Generalised Estimating Equation (GEE) was used to examine the performance on efficiency. For hinged box task, we included individuals that completed 20 successes (8 grey squirrels and 10 red squirrels) for analysis whereas we included problem solvers that have completed 60 successes on the puzzle box task (8 grey squirrels and 5 red squirrels). GEE was further

used to examine the differences in behavioural traits among the two species across 60 successes. Factors included species and covariate included success number and their interaction whereas dependent variables were the solution time, flexibility, behavioural variety, persistence and behavioural selectivity. When analysing the varied contribution of each behavioural trait to efficiency in each species, we used GEE with adjusted variance (Wang and Long, 2011). Significance level was considered as Alpha less than 0.5. Results reported here are two-tailed.

5.3 RESULTS

General information

We collected data from 34 squirrels (14 grey squirrels and 17 red squirrels) for the hinged box task, and all these squirrels also participated in the puzzle box task. A further eight grey squirrels and four red squirrels participated in the puzzle box task, which yielded a sample size of 43 squirrels (22 grey squirrels and 21 red squirrels) for this task.

Among the 17 red squirrels that solved the hinged box task, only 6 solved the puzzle box at the first encounter whereas among the 14 grey squirrels that solved the hinged box task, 10 also solved the puzzle box at the first encounter.

Hinged box task: between species

All grey (14/14) and red squirrels (17/17) solved the hinged box task. On average, the first lid opening took 8.3 seconds (S.E.±3.18 seconds) for grey squirrels and 6.5 seconds (S.E. ±1.17s) for red squirrels. However, this difference in first lid opening time was not significant ($t_1=0.60$, $p=0.554$). Figure 5.3 shows that when we analysed squirrels (8 grey squirrels and 10 red squirrels) that completed 20 successes, the species did not show a significant difference in

solving duration (GEE $\chi^2_1=0.003$, $p=0.957$). Increased efficiency is shown with increased experience ($\chi^2_1=22.64$, $p<0.001$) and the interaction between species and success numbers also did not reach significant ($\chi^2_1=0.004$, $p=0.949$).

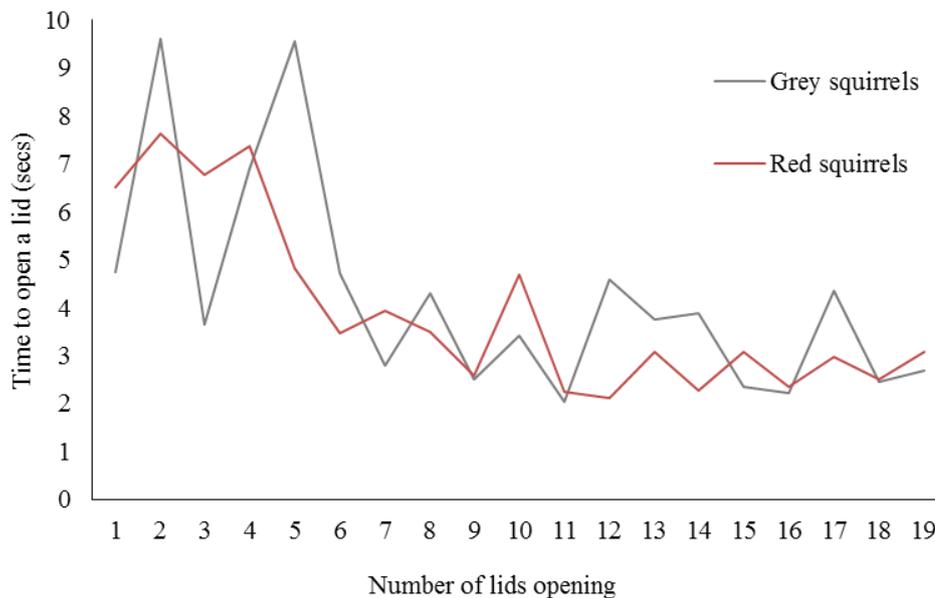


Figure 5.3. Hinged box task: averaged solution time for 18 squirrels ($N_{\text{grey}}=8$, $N_{\text{red}}=10$) that completed 20 successes.

Puzzle box task: between species comparison of problem solving success

Figure 5.4a shows that at the first encounter, 6/21 (29%) red squirrels and 14/22 (64%) grey squirrels obtained first success in the puzzle box task (total $N=43$). This between species difference in problem solving success was significant ($\chi^2_1=5.3$, $p=0.021$). Figure 5.4b shows that in subsequent encounter with the puzzle box, between species difference in problem solving success was also significant ($\chi^2_1=6.93$, $p=0.039$); more grey squirrels solved the task than red squirrels.

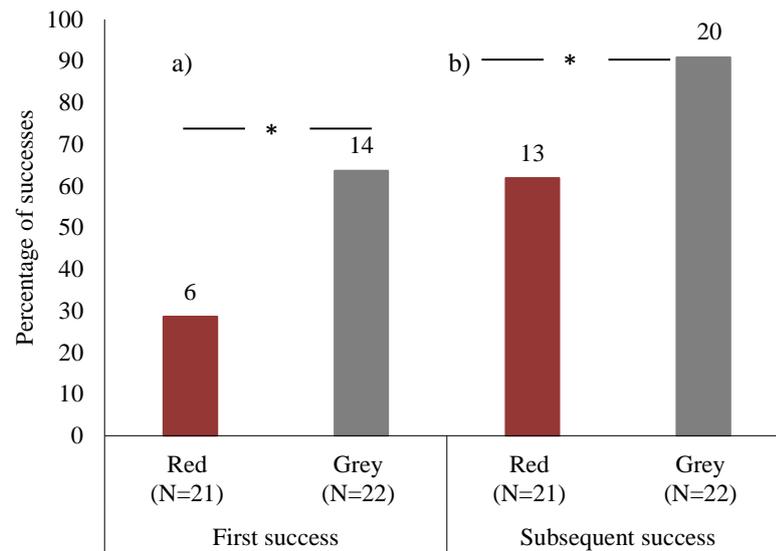


Figure 5.4. Puzzle box task: percentage of problem solvers obtained first success in each species, a) on their first encounter, and b) at subsequent encounter. The number above each bar indicates the actual number of squirrels. N gives the total numbers of observed squirrels. $*p < 0.05$

Puzzle box task: within species, behavioural traits and the first encounter

At *within-species* level, Table 5.1 shows the differences in behavioural characteristics between problem solvers and non-solvers on the first encounter. The left panel shows that red squirrels that are problem solvers showed less behavioural variety than non-solvers. This difference was significant ($p=0.016$). The middle panel shows the results for grey squirrels, problem solvers and non-solvers were only significantly different in behavioural selectivity; problem solvers showed higher proportion of effective behaviours than non-problem solvers ($p=0.011$).

Table 5.1. Puzzle box task: left and middle panel shows *within*-species level differences in behavioural traits and problem solving performance at the first encounter for problem solvers and non-problem solvers. Right panel shows *Between*-species differences in first success.

	Red					Grey					Red vs. Grey					
	Non- problem solver (N=15)	Problem solver (N=6)	U	Z	P	Non- problem solver (N=8)	Problem solver (N=14)	U	Z	P	Problem solvers (6 reds and 14 greys)			Non-problem solvers (15 reds and 8 greys)		
	Mean (S.E)	Mean (S.E)				Mean (S.E)	Mean (S.E)				U	Z	P	U	Z	P
Solving duration	3.5 (0.78)	7.0 (2.48)	29.0	-1.25	0.213	9.9 (0.90)	13.9 (6.63)	35.0	-1.43	0.152	39.0	-0.25	0.804	7	-3.42	0.001
Flexibility	0.70 (0.22)	0.90 (0.27)	29.5	-1.22	0.222	0.72 (0.20)	0.84 (0.20)	52.0	-0.27	0.785	36.5	-0.46	0.649	50	-0.65	0.517
Behavioural variety	6.89 (2.77)	0.82 (0.20)	14.0	-2.41	0.016	0.69 (1.32)	0.73 (0.15)	52.0	-0.27	0.785	30.5	-0.95	0.343	15.5	-2.87	0.004
Persistence	1.63 (0.40)	0.70 (0.14)	35.0	-0.78	0.436	0.57 (0.09)	0.79 (0.21)	47.0	-0.62	0.539	36.5	-0.45	0.650	25	-2.26	0.024
Behavioural selectivity	0.59 (0.11)	0.64 (0.12)	36.5	-0.25	0.822	0.27 (0.05)	0.70 (0.09)	15.5	-2.55	0.011	37.5	-0.39	0.697	23.5	-1.76	0.078

Puzzle box task: between species differences at the first encounter

At between species level, the right panel of Table 5.1 shows the behavioural characteristics of red ($N=6$) and grey solvers ($N=14$) on the first success. At their first success, results showed that red solvers and grey solvers did not differ in their behavioural traits. The right panel of Table 5.1 also shows that, at between species level, non-problem solvers showed significant different in persistence ($p=0.024$), behavioural variety ($p=0.004$) and solution duration ($p=0.001$). Red squirrels that were non-problem solvers showed higher rate of attempts, higher rate of behavioural variety but less time spent on solving the problem than grey squirrels that were not problem-solvers.

Contributors for problem solving success

Overall, Table 5.2 shows two GLM analyses for the factors in relation to first success and failure, two models were ran separately due to behavioural variety and persistence were highly correlated ($r=0.81$), results showed that behavioural selectivity is the only significant predictor for first success in both models; higher behavioural selectivity increases first success rate.

Dependent variable	Independent variable	First model				Second Model			
		B	df	χ^2	P	B	df	χ^2	P
First success or failure	Flexibility	0.88	1	2.07	0.150	0.82	1	1.98	0.160
	Behavioural variety	-	-	-	-	-0.14	1	0.20	0.654
	Persistence	-0.61	1	1.66	0.197	-	-	-	-
	Behavioural selectivity	2.97	1	5.52	0.019	2.75	1	4.84	0.028

Table 5.2. Puzzle box task: GLM analyses examine the covariates that are related to problem solving success or failure at the first encounter ($N=40$). The first model excluded behavioural variety whereas second model excluded persistence.

Puzzle box task: between species differences in behavioural traits across successes

Figure 5.5 shows the mean solving duration for squirrels ($N=13$, 8 grey squirrels and 5 red squirrels) that had completed 60 successes. Results showed that species had a significant main effect on efficiency (GEE $\chi^2_1=22.31$, $p<0.001$); red squirrels were more efficiency than grey squirrels. Success number also had an effect on efficiency ($\chi^2_1=40.27$, $p<0.001$); increased efficiency is shown across 60 successes. However, the interaction between species and success number was not significant ($\chi^2_1=1.71$, $p=0.191$). Table 5.3 shows between species differences in behavioural traits, species only significantly differed in behavioural variety ($p=0.017$); red squirrels showed higher behavioural variety than grey squirrels across 60 successes. Behavioural selectivity also showed significantly differed across trial; squirrels increased behavioural selectivity with increased successes ($p<0.001$).

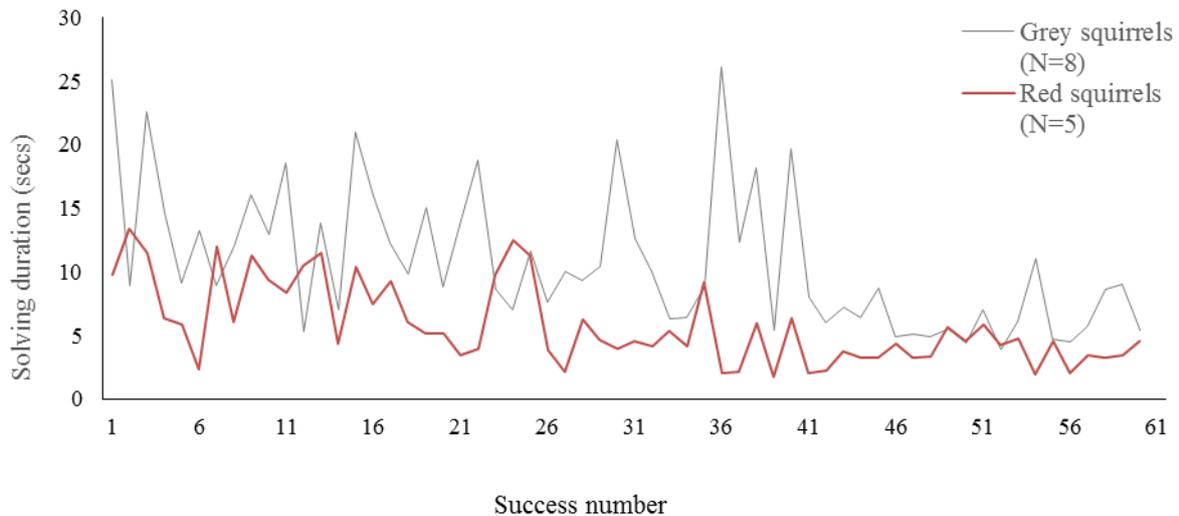


Figure 5.5. Puzzle box task: a) Mean solution time in the puzzle box task for individuals that had completed 60 successes ($N=13$, $N_{\text{grey}}=8$, $N_{\text{red}}=5$).

We also performed a detailed analysis, by breaking down the 60 successes into first 30 successes and last 30 successes to understand the behavioural differences at the between species level. In the first 30 successes, species showed a significant difference in efficiency (GEE $\chi^2_1=6.54$, $p=0.011$); red squirrels were more efficient than grey squirrels. Success number also showed a significant main effect ($\chi^2_1=4.26$, $p=0.39$); increased efficiency is shown across the first 30 successes. But interaction between species and success number was not significant ($\chi^2_1=0.01$, $p=0.917$). In terms of the differences in behavioural traits at the first 30 successes, the left panel in Table 5.3 shows that species showed a main effect on behavioural variety ($p=0.009$) and persistence ($p=0.026$); grey squirrels showed lower behavioural variety and lower persistence than red squirrels. The interactions between species and success number on behavioural variety and persistence were also significant. Behavioural variety showed a decrease in grey squirrels whereas an increase in red squirrels ($p=0.019$). Persistence also showed a decreased in grey squirrels but increased in red squirrels ($p=0.029$). Behavioural selectivity also significantly increased across the first 30 successes ($p<0.001$).

Middle panel of Table 5.3 shows that in the last 30 successes, species did not have a main effect on all behavioural traits. Flexibility increased across the last 30 successes ($p=0.015$).

Table 5.3. Puzzle box task: GEE models examining *between*-species differences in the first 30 successes, last 30 successes and across 60 successes ($N=13$, $N_{\text{grey}}=8$, $N_{\text{red}}=5$). Covariates include species, success number and their interaction. Dependent variables include four behavioural traits, flexibility, behavioural variety, persistence and behavioural selectivity.

Dependent variables	Independent variables	<i>First 30 successes</i>				<i>Last 30 successes</i>				<i>All 60 successes</i>			
		B	<i>df</i>	χ^2	<i>P</i>	B	<i>df</i>	χ^2	<i>P</i>	B	<i>df</i>	χ^2	<i>P</i>
Flexibility	Species (Grey squirrels)	-0.83	1	1.71	0.192	-0.48	1	1.11	0.293	-0.57	1	2.54	0.111
	Trial	0.30	1	0.42	0.516	-0.27	1	5.95	0.015	0.09	1	1.67	0.196
	Grey squirrels*Trial	-0.33	1	0.73	0.393	-0.06	1	0.05	0.822	-0.01	1	0.01	0.924
Behavioural variety	Species (Grey squirrels)	-2.02	1	6.78	0.009	-3.45	1	3.52	0.061	-1.07	1	5.75	0.017
	Trial	1.37	1	0.28	0.600	-2.08	1	3.75	0.053	0.41	1	0.45	0.505
	Grey squirrels*Trial	-2.10	1	5.46	0.019	1.89	1	2.56	0.110	-0.54	1	2.47	0.116
Persistence	Species (Grey squirrels)	-1.47	1	4.96	0.026	-1.51	1	1.32	0.250	-0.46	1	1.31	0.253
	Trial	0.85	1	0	0.987	-1.05	1	1.42	0.234	0.02	1	0.06	0.802
	Grey squirrels*Trial	-1.55	1	4.74	0.029	1.05	1	1.49	0.222	-0.12	1	0.10	0.749
Behavioural selectivity	Species (Grey squirrels)	-0.05	1	0.19	0.663	-0.08	1	1.79	0.181	-0.01	1	0.01	0.944
	Trial	0.21	1	13.79	<0.001	0.03	1	3.25	0.071	0.14	1	80.01	<0.001
	Grey squirrels*Trial	-0.12	1	2.64	0.104	0.03	1	0.26	0.610	-0.5	1	3.55	0.059

Puzzle box task: core factors for problem solving efficiency in each species

To understand the factors that contribute to efficient problem solving in each species, we ran a GEE model separately for each species. Factors included flexibility, behavioural variety, persistence, and behavioural selectivity. However, because behavioural variety and persistence were highly correlated ($r=0.75$), we avoided collinearity by selecting the behavioural variety instead of persistence, given that between species results showed there was a difference in behavioural variety across 60 successes but not persistence (Table 5.3 right panel).

The upper panel of Table 5.4 shows the results for grey squirrels. Two behavioural traits, behavioural variety ($p<0.001$) and behavioural selectivity ($p<0.001$) were significantly related to efficiency (Path 1). Grey squirrels increased behavioural variety and showed higher behavioural selectivity to achieve efficiency. The non-significant effects of success number and flexibility suggested that their effects may have been mediated by behavioural variety and behavioural selectivity. Accordingly, following Chow and colleagues (*in press*), we ran three mediational analyses. The first two analyses used success number and flexibility as covariates, the response variable was behavioural variety in one model and behavioural selectivity in another model. Results showed that success number and flexibility had opposite effects on behavioural selectivity (Path 2); grey squirrels increased behavioural selectivity with increased successes but decreased with increased flexibility. Flexibility, but not success number, was also positively related to behavioural variety (Path 3); higher flexibility is related to higher behavioural variety. A final analysis examined whether there was a correlation between success number and flexibility and this result was not significant ($\chi^2_1=1.11, p=0.293$).

The lower panel of Table 5.4 shows the results for red squirrels. All behavioural traits, apart from behavioural variety, were associated with efficiency (Path 1). Efficiency was positively associated with successes number, flexibility and behavioural selectivity. We also ran two mediational analyses to examine whether red squirrels showed a similar problem solving process to grey squirrels. Results showed that success number was positively related to behavioural selectivity (Path 2), but behavioural variety was not significantly associated with any other traits (Path 3). Finally, success number and flexibility showed that they were not correlated with each other ($\chi^2_1=0.35$, $p=0.554$).

Puzzle box task: total effect for each behavioural trait

Table 5.5 shows the total effect for each behavioural trait. For both species, success number and behavioural selectivity show higher total positive effects on efficiency, compared with flexibility and persistence. Figure 5.6 shows all standardised coefficients (β) for each path for each species. Amongst the paths, behavioural selectivity had the highest positive effects on achieving efficiency both for grey ($\beta = -0.41$) and red squirrels ($\beta = -0.28$).

Table 5.4. Puzzle box task: GEE models examining the varied contribution of four behavioural traits to problem solving efficiency for each species. Factors include success number, flexibility, behavioural variety and behavioural selectivity.

Species	Path	Dependent variable	Independent variable	<i>60 successes</i>			
				B	<i>df</i>	χ^2	P
Grey squirrels (<i>N</i> =8)	1	Efficiency	Success number	-0.068	1	2.41	0.121
			Flexibility	-0.076	1	0.08	0.782
			Behavioural variety	-0.655	1	16.50	< 0.001
			Behavioural selectivity	-21.132	1	71.41	< 0.001
	2	Behavioural selectivity	Success number	0.005	1	21.87	< 0.001
			Flexibility	-0.011	1	9.35	0.002
	3	Behavioural variety	Success number	-0.010	1	0.78	0.378
			Flexibility	0.783	1	35.13	< 0.001
	Red squirrels (<i>N</i> =5)	1	Efficiency	Success number	-0.055	1	6.59
Flexibility				-0.561	1	6.34	0.012
Behavioural variety				-0.120	1	1.56	0.212
Behavioural selectivity				-6.618	1	9.99	0.002
2		Behavioural selectivity	Success number	0.008	1	22.47	< 0.001
			Flexibility	0.006	1	0.30	0.585
3		Behavioural variety	Success number	0.020	1	0.93	0.334
			Flexibility	0.405	1	2.72	0.099

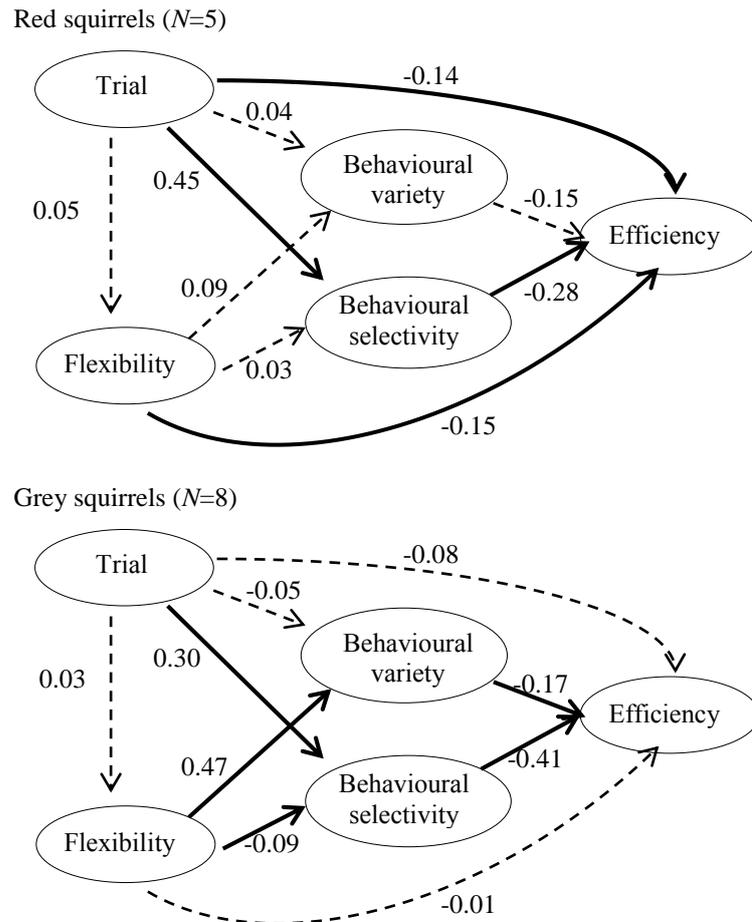


Figure 5.6. Puzzle box task: standardised coefficients (β) for all direct and indirect paths among the variables for each species.

Table 5.5 Puzzle box task: total effects of each factors on efficiency for each species.

Total effect (β)	Red squirrels	Grey squirrels
Success number	-0.28	-0.21
Flexibility	-0.17	-0.04
Behavioural variety	-0.15	-0.17
Behavioural selectivity	-0.28	-0.41

5.4 DISCUSSION

This study aimed to examine whether grey squirrels and red squirrels differ in their problem solving performance; using two standardised problem solving tasks in two populations. We also examined four behavioural traits that are associated with problem solving performance. We provide evidence that there are capacity differences between the two populations in problem solving, depending on the task difficulty. Our results showed that both red and grey squirrels solved a simple problem task, but grey squirrels were more likely to solve a difficult puzzle box task. Despite this, problem solvers among the red squirrels were more efficient than the grey squirrels in the puzzle box task.

The use of food-extraction problem-solving tasks is a standardised way to record behavioural flexibility (Griffin & Guez, 2014). Here, our result shows that, at the population level, the invasive grey squirrels in the U.K resemble evidence found in invasive bird species that behavioural flexibility as in using novel foraging techniques is crucial for invasive species (Sol, Timmermans, & Lefebvre, 2002); grey squirrels obtained more first success as well as in the subsequent success in solving the puzzle box than red squirrels (Figure 5.5). The success of grey squirrels in problem solving could be explained by the adaptive flexibility hypothesis (Wright et al., 2010), which proposes that flexibility varies depending on the stage of establishment in new habitat, with early stages of invasive process requiring higher behavioural flexibility whereas low behavioural flexibility should be seen once the species established in new environment. However, grey squirrels invaded Exeter since 1920s whereas the red squirrels on Arran Island were first introduced there in 1950s. In this case where both species are expanding their population in areas without each other's competition for ecological niches, behavioural flexibility should be similar, but this is not the case in this

study. Accordingly, this hypothesis may not completely explain the results. Another possible explanation could be from selective pressure on high behavioural flexibility for grey squirrels during the invasive process that could shape them to become inherently more flexible and more capable of solving difficult problems.

At the individual level, our results showed that a small proportion of red squirrels are as capable as grey squirrels at solving the puzzle box task and showed no differences among the behavioural traits that we measured (Table 5.1). These red squirrels even outperformed the grey solvers in efficiency (Figure 5.6). Detailed analyses across 60 successes showed that these red squirrels showed higher behavioural variety than grey squirrels. With an additional high persistence, measured as the rate of attempts in this study, than the grey squirrels at the first 30 successes (Table 5.3 left panel). Both persistence and behavioural variety are important for problem solving success (Benson-Amram & Holekamp, 2012; Griffin, Diquelou, & Perea, 2014; Thornton & Samson, 2012) and increased persistence has also been shown to increase efficiency (Chow, Lea, Leaver, *in press*). Here, we further showed that these two behavioural traits could be particularly important at the early stage of the problem solving process (first 30 successes) to achieve efficiency.

Although these two traits are important for problem solving performance, they could not completely differentiate problem solvers and non-solvers. Our *within*-species results (Table 5.1) showed that solvers and non-solvers showed no difference in their persistence. This result is in line with other evidence such as Benson-Amram and Holekamp (2012) who showed that hyenas that could not solve a puzzle box spent a similar amount of time on the puzzle box as those hyenas that successfully solved the task. Non-solvers also showed higher

behavioural variety at their first encounter, on average, non-solvers showed 6 types of tactics (a mean of 6.25 types of tactics for red squirrels and a mean of 6 types for grey squirrels), but still failed to solve the task in red squirrels or similar rate of behavioural variety in grey squirrels (Table 5.1). Thus, our results showed that neither pure persistence nor behavioural variety is sufficient for first success. Instead, our results (Table 5.2) showed that behavioural selectivity, the proportion of effective behaviours, is a crucial factor for the first success; non-problem solvers showed lower behavioural selectivity than problem solvers (Table 5.1). This result reflects that with the same motivation and number of types of tactics used to solve a problem, only those showed more effective behaviours solved problem successfully.

Model comparisons between red and grey problem solvers reveal there are some similarities of cognitive process as in achieving problem solving efficiency. Both species showed that behavioural selectivity with increased experience provides a powerful route to increase efficiency. This finding is in line with Chow, Lea and Leaver (*in press*) showing increased behavioural selectivity increases problem solving efficiency. On the other hand, we also found some differences regarding the role of behavioural flexibility. In this study, we measured flexibility as changing to a different type of tactic after a failed attempt. Chow and colleagues (*in press*) showed that the role of flexibility could increase time cost and decrease effective behaviours in problem solving. Here, we showed flexibility showed a positive effect on efficiency, but the effect was minimal because the positive effect of flexibility was negated by the negative relationship with behavioural selectivity in grey squirrels (Table 5.4 upper panel path 2). In contrast to the grey squirrels, red squirrel problem-solvers showed high rate of flexibility to achieve efficiency. This result shows that flexibility is an alternative

means for improving problem solving performance or at least the use of flexibility brings an advantages in this sample (Table 5.5).

Overall, we showed that some red squirrels possess a certain level of flexibility that is comparable with the invasive greys. Our findings also provide evidence that the red squirrels are inherently less flexible than the grey squirrels; even without sympatric grey squirrels, the difference in problem solving success between red and grey squirrels still exists. Four directions of future research are suggested to strengthen the conclusion: 1) investigators could conduct a series of different problem tasks to examine whether there are consistent differences or similarities between non-invasive reds and invasive grey squirrels; 2) urbanization and flexibility are positively related (e.g. Sol, Lapiedra, & Gonz, & Gonz flexibi). Although I have tried to control the data collection in U.K, the chosen study sites are still not completely comparable (Arran Island is a relatively rural environment whereas Exeter is a city); 3) given that recent studies have shown that the genetic diversity of the grey squirrels in U.K are limited, (e.g. Signorile, Reuman, Lurz, Carbone, & Wang, 2016), future studies should replicate the study in other populations, perhaps outside the U.K such as Italy where the red and grey squirrels co-exist; and 4) if possible, study could identify the ancestry of the grey squirrels that were introduced in U.K and examine whether the origin population also possess similar capacity in problem solving; this would allow us to see whether the heightened problem solving ability of UK grey squirrels is a cause or consequence of their successful invasive of a new environment.

CHAPTER 6 GENERAL DISCUSSION

In this thesis, the central aim has been to explore the ‘how’, the missing linkage between the ‘what’ and problem solving performance. I have highlighted in the Introduction that this process requires an understanding of learning and behavioural flexibility. Throughout the thesis, I have been addressing these two types of processes in problem solving; while learning provides a gradual change in behaviour with accumulated trial-and-error information or experience, flexibility provides a more spontaneous and instantaneous form of change when obstacles occur. Specific type of learning that I examined was instrumental conditioning and the type of behavioural flexibility that I examined was motor flexibility. I examined how these two processes varied their contribution to different problem solving performance (success/failure and efficiency). The use of serial reversal learning task and novel problem solving tasks are the standard ways to assess flexibility in animals (Griffin & Guez, 2014) and I have applied these tasks in Chapter 2, 3, and 4 with laboratory grey squirrels. In chapter 5, I conducted a field study to compare red and grey squirrels using two standardised problem tasks. In this section, I will first summarise the main findings across the experiments in this thesis **(6.1)** followed by study limitations **(6.2)**. Then, I will answer the question that was posed in the Introduction **(Section 1.4)**: the ‘How’, focusing on the role of flexibility in problem solving processes and in relation to problem solving performance **(6.3)**. I will also address the implications of findings to wider ecological contexts **(6.4)**. Finally, I will highlight three potential areas for future studies **(6.5)** and give a general conclusion **(6.6)**.

6.1 SUMMARY OF THE MAIN FINDINGS

In Chapter 2, I used a serial spatial reversal learning task to examine whether squirrels would be flexible under a recurring change if we used an ecologically appropriate design,

testing their spatial capacity in laboratory. This design was different from the two-stimulus reversal learning tasks that have been used previously, in that I provided four choices for squirrels; this allowed me to analyse whether their improvement in the task was rule based or not. This chapter has two key messages: 1) squirrels were flexible under a recurring change, as indicated by decreasing the number of errors across reversal phases; and 2) such increased efficiency is not achieved purely through increased experience, but also through a change in the tactics used. Squirrels gradually came to use more integrative rather than sequential tactics, as a result of increased experience (one or two trials error). This tactic change is related to the use of extra-apparatus cues to locate hidden food rewards diagonally, and requires a more holistic understanding of the problem than the sequential tactics used initially.

In Chapter 3, I used a colour reversal task to further investigate the roles of two underlying learning mechanisms, inhibitory control and attention, in supporting the squirrels' ability to increase efficiency in the laboratory. Two core messages from this study are: 1) both mechanisms were responsible for the squirrels' achieving efficiency when a change occurred; and 2) inhibitory control towards the incorrect stimuli had a greater effect on increasing learning efficiency before the squirrels reached the criteria of successful learning than afterwards. Increased inhibitory control also contributes to increase efficiency after reaching the criteria and squirrels showed higher attention to relevant cues.

In chapter 4, I examined how learning, along with other behavioural traits such as persistence, flexibility and behavioural selectivity, contribute to problem solving efficiency, using a novel food-extraction task, a puzzle box, in the laboratory. To do this, I attempted to disentangle various behavioural traits, and examine the varied contribution of each trait to

increased efficiency. The design of the puzzle box required squirrels to exhibit effective (pushing the near end of a lever or pulling the far end) and inhibit ineffective behaviours (pulling the near end or pushing the far end). Three key messages from this experiment are: 1) the effect of experience was mediated by other behavioural traits: individuals increased behavioural selectivity (proportion of effective behaviours) or increased persistence (attempt rate) to achieve efficiency; 2) flexibility, measured as a change of tactic after a failed attempt, was independent from learning; and 3) flexibility is not always positive, given that it increases time costs, and also has to be traded-off against behavioural selectivity, at least in grey squirrels in the task that was used.

With the knowledge that has been gained in these chapters, we know that grey squirrels are flexible in adapting to change. However, how do we know whether they are relatively more or less flexible in problem solving than other species? Accordingly, in Chapter 5, I examined this question in native free-ranging red squirrels and invasive wild grey squirrels in the U.K. I explored the capacity and performance in problem solving of the two populations by designing two problem tasks that varied with the level of difficulty, a hinged box (an easy problem task) and the puzzle box used in Chapter 4 (a difficult problem task). Four key findings are: 1) all the squirrels solved the easy task, but more grey squirrels solved the difficult task; 2) although grey squirrels showed higher problem solving ability than red squirrels, a small proportion of the red squirrels were as capable as grey squirrels in problem solving success; 3) these problem solvers among the red squirrels outperformed the grey solvers in efficiency; and 4) red and grey squirrels were possibly using different cognitive processes in solving the difficult problem.

6.2 LIMITATIONS

Across the four experiments, one limitation has to be highlighted. The small sample size across laboratory studies (Chapter 2-4) with only five laboratory squirrels means that the power of the experiments is low and it is not clear that the results could be generalised to the whole population. To try to increase the sample size throughout these years, I received two juveniles from the local vet hospital, as well as tried to recruit more squirrels from rescue centres. However, rescue centres failed to reply and the two ex-hospitalised squirrels proved to be in poor health condition (one suffered from internal bleeding and the other had constant seizures when received), so they were not suitable to start any testing. To mitigate this impact of the small sample size, throughout the thesis I have used advanced statistical techniques, the GEE with adjusted variance (recently developed by Wang & Long, 2011) to explore my data as much as possible. As well as this, I also conducted field work, to obtain a larger sample and also to make sure that my data from the laboratory (Chapter 4) shows ecological validity.

6.3 BACK TO THE 'HOW': LEARNING AND FLEXIBILITY IN PROBLEM SOLVING

6.3.1 LEARNING IN PROBLEM SOLVING

In the Introduction (**Section 1.4.1**), learning was defined as a process of acquiring information or knowledge or skills through practice or experience. Learning is clearly involved in problem solving throughout all my experiments: squirrels showed a gradually decreasing number of errors in the spatial reversal learning task across reversal phases (Chapter 2), a decreasing number of errors in the colour reversal learning task (Chapter 3), and increasing efficiency across trials in the problem solving tasks (Chapter 4 and 5). Another factor that improved with increased experience was tactic change across phases (Chapter 2); squirrels gradually changed their tactics from sequential to integrative, increased

persistence across trials (Chapter 4), increased behavioural selectivity (Chapter 4 and 5), and decreased the number of behavioural variety (Chapter 5: $\chi^2_{\text{red}}=56.62$, $p<0.001$; $\chi^2_{\text{grey}}=20.84$, $p<0.001$). Another definition of learning is ‘the acquisition of neuronal representations of new information’ and it also requires retention of information both in a long term and a short term period (Dukas, 2009, pp. 7-26). This is shown when squirrels remembered at least one of the two rewarded locations as their first choice in serial reversal task (Chapter 2).

The role of learning in problem solving is shown as a direct effect to increase efficiency in serial reversal learning task (Chapter 2). While we tried to record three of the possible factors, positive interference, tactic change, and other interference, that were expected to vary their contribution during learning, the direct effect of learning reflects that there may be other mediating factors that are outside the scope of study. The simplest explanation for this direct effect could be individuals becoming more familiar with the experimental set up, and context or an object. But this effect could also reflect the involvement of underlying cognitive mechanisms such as attention and inhibitory control that are not easy to be measured, but support the reversal learning task (Chapter 3). Squirrels had to increase inhibitory control in order to choose the correct stimulus before reaching the learning criterion and increase attention to relevant information for a task after reaching the learning criterion. These results show that learning is not simply a process of acquiring information through practice, but also involves a collection of underlying mechanisms that facilitate this process.

In contrast, learning shows an indirect effect on problem solving efficiency in novel food-extraction tasks for grey squirrels (Chapter 4 and Chapter 5). The fact that learning was mediated by other behavioural traits indicates that I have identified the factors that would

affect problem solving performance, at least, in my experiments and thus, taken the first step to understand the 'How'. Increased persistence and behavioural selectivity with increased experience; squirrels progressively show more attempts (Chapter 4) and exhibit more effective behaviours (Chapter 4 and 5) as problem solving continues. These results are simply explained by positive reinforcement - when an individual's behaviour is directly leading to a positive outcome (food reward) in a short period of time.

6.3.2 SPONTANEOUS FORM OF FLEXIBILITY IN PROBLEM SOLVING

The spontaneous form of flexibility is seen in the changes of tactic that occur after a failed attempt at solving a novel problem (Chapter 4 and 5). The rate of change of tactics during problem solving can be used to assess how spontaneous flexibility could affect problem solving performance. Presumably, this measure reveals how an individual sought alternative solutions to solve a task instead of the current ineffective tactic. Using this measure, my results show that the role of behavioural flexibility in problem solving performance appears to be different between species: for grey squirrels in the laboratory study (Chapter 4) and field study (Chapter 5), the total effect of flexibility provided little advantage as well as reducing behavioural selectivity, whereas for red squirrels in the field study (Chapter 5), increased flexibility provided a direct positive effect on increased efficiency. These opposite effects can possibly be understood by considering whether flexibility is directly or indirectly in relation to problem solving efficiency. In general, the direct effect of flexibility on efficiency is positive, with higher flexibility increasing efficiency (Chapter 4 and Chapter 5). When the effect of flexibility on efficiency is mediated by other behavioural traits, however, its effect on efficiency is less straightforward: it depends on which behavioural trait had mediated the effect of flexibility. For example, a negative effect on efficiency is seen when increased flexibility is related to low behavioural

selectivity, whereas a positive effect on efficiency is seen when increased flexibility is related to increased behavioural variety.

The specific relationship between flexibility and each behavioural trait requires an understanding of the way each trait is measured. While my measurement of flexibility recorded a change after a failed attempt, it did not record whether the change was effective or ineffective. Accordingly, the fact that high flexibility decreases behavioural selectivity (the proportion of effective behaviours) may reflect squirrels changing to use more ineffective behaviours than effective behaviours. Conversely, the positive relationship between flexibility and behavioural variety could be because high flexibility facilitates the use of different types of behavioural variety during the problem solving, and thus, increases the likelihood of exhibiting a tactic that is effective for the task. It follows that, the context of problem solving, a refined concept of behavioural flexibility is not only ‘change’ (West-Eberhard, 2003), but the change also has to be effective. For example, near-end pulling is ineffective in solving the puzzle box, and hence, squirrels have to change from near-end pulling to near-end pushing (an effective behaviour) to solve the task, but not from near-end pulling to far-end pushing, which are both ineffective behaviours.

6.3.3 NON-PROBLEM SOLVERS AND BEHAVIOURAL INFLEXIBILITY

In the Introduction (**Section 1.4.2.4**), I mentioned that non-problem solvers could provide useful information about behavioural flexibility. For example, non-problem solvers have been shown to have lower persistence than problem solvers (Cauchard, Boogert, Lefebvre, Dubois, & Doligez 2013; Overington, Cauchard, Côté, & Lefebvre 2011). However, along with other evidence we not only showed that problem solvers and non-problem solvers showed similar persistence levels as did, for example, Benson-Amram &

Holekamp (2012), but also obtain a similar result for other behavioural traits such as flexibility and behavioural variety (Table 5.1). The GLM results in Table 5.2 show that behavioural selectivity (the proportion of effective behaviours) is the key factor for success. This is in line with studies (e.g. Leal & Powell, 2011) that show that individuals failed to solve reversal learning task because they persistently make the incorrect choice. Therefore, the key point here is that persistence could have failed in individuals that are not motivated or that decided not to spend time and energy cost on solving a problem, but this does not necessarily mean that their failure in problem solving is due to their being inflexible. Instead, only those individuals that do not show the ‘change’ in their behaviours reflect behavioural inflexibility, and hence, are ‘true’ non-problem solvers.

6.3.4 LEARNING AND BEHAVIOURAL FLEXIBILITY: INDEPENDENT OR VARIATION ALONG AN AXIS?

As I have mentioned in the Introduction, learning and spontaneous flexibility could be on a continuum (**Section 1.4.2.6**), or be connected in some other way. Authors have suggested that learning provides additional flexibility (Mery, 2012), is a minor form of flexibility (van Schaik, 2013) or that flexibility is the end product of learning (Dukas, 2013). So far, my discussion has treated learning and behavioural flexibility as two independent processes in problem solving, as this is what we found in Chapter 4 and 5. In addition to this, learning and flexibility showed opposite relationships to all behavioural traits that I have studied. This may imply that even if learning and flexibility do lie on a continuum, it is highly likely that they are at its extreme ends. With this in mind, here, I used the data from grey squirrels from the laboratory and field data ($N=13$) to explore the relationships between learning, behavioural flexibility, and each studied behavioural trait on a graph. Here is the result:

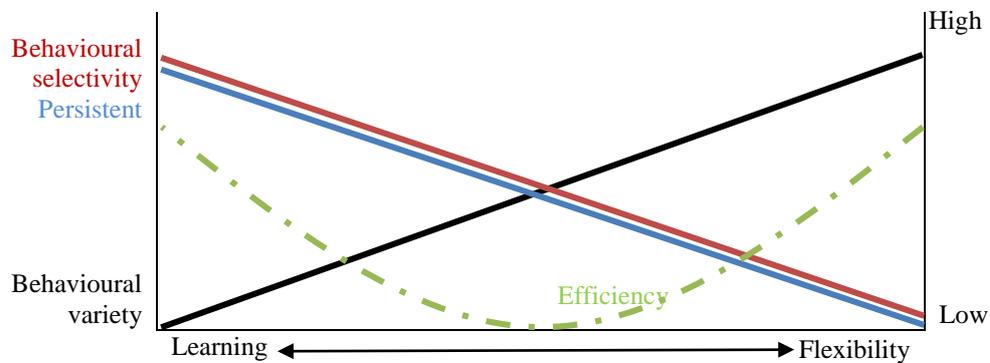


Figure 6.1. Relationship between learning, behavioural flexibility and each behavioural trait on high efficiency.

Figure 6.1 shows that when learning and behavioural flexibility are shown as a continuum, trait variations along this continuum also occur. The plotted efficiency line was based on the three routes that led to increased efficiency: 1) increased persistence with increased experience; 2) increased behavioural selectivity with increased experience; and 3) increased flexibility increased behavioural variety. The result shows that efficiency is achieved at the extreme ends, either by being highly flexible or by increasing experience, along with specific behavioural trait. Because both processes showed opposite effects on each trait (Chapter 4), they cannot affect the same trait simultaneously during problem solving. For example, the positive effect of behavioural selectivity on solving efficiency is low if it increases with experience but decreases with increased flexibility at the same time.

Following the above arguments, here, I further showed how traits vary at two different stages of problem solving (Figure 6.2 left and middle panel), using the data from

wild grey and red squirrels (Chapter 5). Recall the results in Chapter 5 that red squirrels that actually solved the puzzle box were more efficient than grey squirrels (Figure 6.2 right panel). Figure 6.2 (left panel) shows the pattern of effects in the first 30 successes for each species. If we look at how success number and flexibility are correlated with other traits, we see that success number and flexibility were not correlated with the same behavioural traits when red squirrels were solving the problem whereas it is the case for grey squirrels at both solving stages.

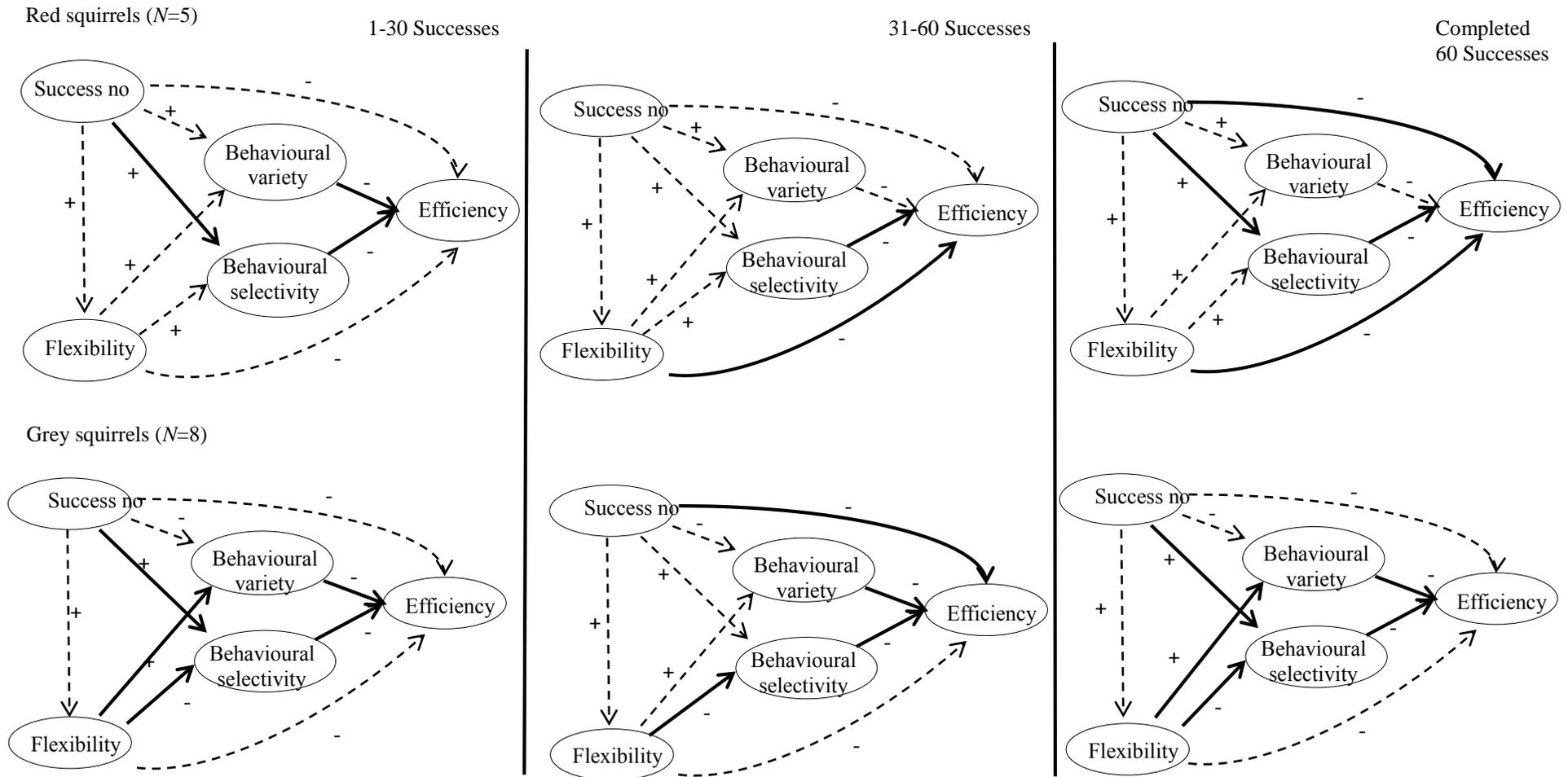


Figure 6.2. GEE path models analyse the varied contribution of each covariate, success number, flexibility, behavioural variety and behavioural selectivity on problem solving efficiency. Problem solving process broken down into two stages: First 30 successes (left panel) and successes 31-60 (middle panel). Significant relationships are highlighted in bold whereas non-significant results are shown as dash lines.

6.4. WIDER IMPLICATION OF FINDINGS

Taking the results across the thesis, here I will revisit the ‘why’ that has been highlighted in **Section 1.1.** I showed that learning and flexibility are facilitating the problem solving process, in a way that is expected to be adaptive and could increase efficiency to maximise the food reward gain. These traits are hence expected to be selected for animals advancing other ecologically related aspects. In this section, I will address a few.

6.4.1 LEARNING AND SPONTANEOUS FLEXIBILITY IN PROBLEM SOLVING TO OTHER ECOLOGICAL ASPECTS

As has been discussed in Chapter 3, learning and its mechanisms such as attention and inhibitory control are important for squirrels in adapting to their natural environment. In the colour reversal learning task (Chapter 3), these mechanisms have been shown to support them in increasing efficiency, but they are highly likely to have wider ecological implication and there is already evidence to show that they do. For example, the use of inhibitory control and attention can be seen when squirrels are caching in the presence of conspecifics. Given that squirrels consider their conspecifics as competitors (Hopewell, Leaver, & Lea, 2008) and they show more curl-tail digs when the conspecifics observers are around (Hopewell & Leaver, 2007), these behaviours may relate to increased inhibitory control to withholding themselves to start caching in front of conspecifics and in turn, minimise the pilferage risk of caches.

Spontaneous flexibility is more likely to be demonstrated when a challenge occurs. For example, field studies have shown that squirrels show their backs more often than facing their conspecifics during caching, increase the distance between caches (Leaver, Hopewell, Caldwell, & Mallarky, 2007), make false caches (Steele et al., 2008) or cache suboptimal

food types to minimise the pilferage risk (Schmidt & Ostfeld, 2008). Spontaneous behavioural adjustments is also seen when visual access to the environment is obstructed; grey squirrels adjust their body position to increase the visibility of the surroundings (Makowska & Kramer, 2007; Partan, Fulmer, Gounard, & Redmond, 2010).

6.4.2 THE ROLE OF FLEXIBILITY IN INVASION SUCCESS

In this thesis, I have shown that grey squirrels adapt rapidly to recurring changes (chapter 2). There are also more successful problem solvers among grey squirrels than red squirrels (Chapter 5). These results lead to some suggestions about how grey squirrels have become such successful invaders. There are various factors that could affect invasion success, to name a few, factors include habitat suitability, food sources availability, intra- and inter-specific food competition level, predator risk. But one advantage of possessing higher behavioural flexibility, associated with large brain relative to body size, has been established (e.g. Sol, Duncan, Blackburn, Cassey, Lefebvre, 2005); successful invaders showed more innovative foraging, including consuming novel food and using innovative foraging techniques, than unsuccessful invaders, which lead to invasive success (Sol, Timmermans, & Lefebvre, 2002).

Although Sol and colleagues (2002) showed that increased behavioural flexibility facilitates 52% of invasive species that studied, they also showed that around 38% of successful and unsuccessful invaders showed similar behavioural flexibility and 10% of unsuccessful invaders possess higher behavioural flexibility in foraging than successful invaders. Our results in Chapter 5 reveal a similar pattern, but in invasive grey squirrels and native red squirrels: there is a small proportion of red squirrels that are not only as capable as grey squirrels, but also outperform grey squirrels in problem solving efficiency. This result

suggests that although behavioural flexibility could provide advantages for invasive species, there are other factors beside flexibility in novel foraging that facilitate them in becoming a successful invader. For example, grey squirrels exploit and pilfer the caches made by red squirrels which in turn, decreases the fitness of red squirrels, especially juveniles (Grunell, Wauters, Lurz, & Tosi, 2004; Wauters, Tosi, & Gurnell, 2002). These pilfering techniques are not novel but it has been suggested as a potential cause for population decrease for red squirrels (Grunell, Wauters, Lurz, & Tosi, 2004).

6.4.3 GENERAL INTELLIGENCE ('g') ACROSS TASKS

General intelligence ('g') assesses to what extent that individuals show similar variation in performance across tasks. It includes a series of tasks that tap into a variety of sensory -motor aspects of learning performance and problem solving is one aspect of 'g'. Increasingly, studies have indicated that some forms of general intelligence exist within species (e.g. Isden, Panayi, Dingle, & Madden, 2013; Matzel et al., 2003; Shaw, Boogert, Clayton, & Burns, 2015) and between species (e.g. Bond, Kamil, & Balda, 2007; Galworthy, et al., 2005; Reader, Hager, & Laland, 2011). Following this line of research, I also observed that a possible 'g' exists among the free ranging squirrels (Chapter 5) and the laboratory squirrels across the three experiments discussed here (Chapter 2-4). As I have shown in Chapter 5, wild squirrels that solved the puzzle box could also solve the hinged box task but individuals that solved the hinged box task (easy) may not solve the puzzle box (difficult). This result shows that there is individual variation in problem solving ability. In Chapter 2-4, each experiment examined a different modality; spatial learning ability (Chapter 2), visual discrimination (Chapter 3) and motor ability (Chapter 4). Figure 6.3 shows the ranked performance of the five squirrels that participated all three experiments. Ranked performance was broken down into discrimination phase and the first reversal phase in the serial reversal

learning task (Chapter 2), the discrimination and the reversal phase in the colour reversal task (Chapter 3), and the solution time on the first block and last block of the problem solving task (Chapter 4). I ranked the squirrel that had the fewest number of errors before reaching the criterion as '5' whereas the squirrel that had the highest number of errors to reach criterion was ranked as '1'. For the puzzle box task, I took the average of the total solution time across four trials in the first block and average across the four trials in the last block. Similar to the assigned rank for each individual in the reversal learning tasks: the squirrel that has the lowest solution time was assigned as '5' and the squirrel that has the longest solution time was ranked as '1'. The results appear to give some correlations in performance between tasks (Friedman test: $\chi^2_4=9.87$, exact $p=0.032$), thus, suggesting that 'g' is a useful concept.

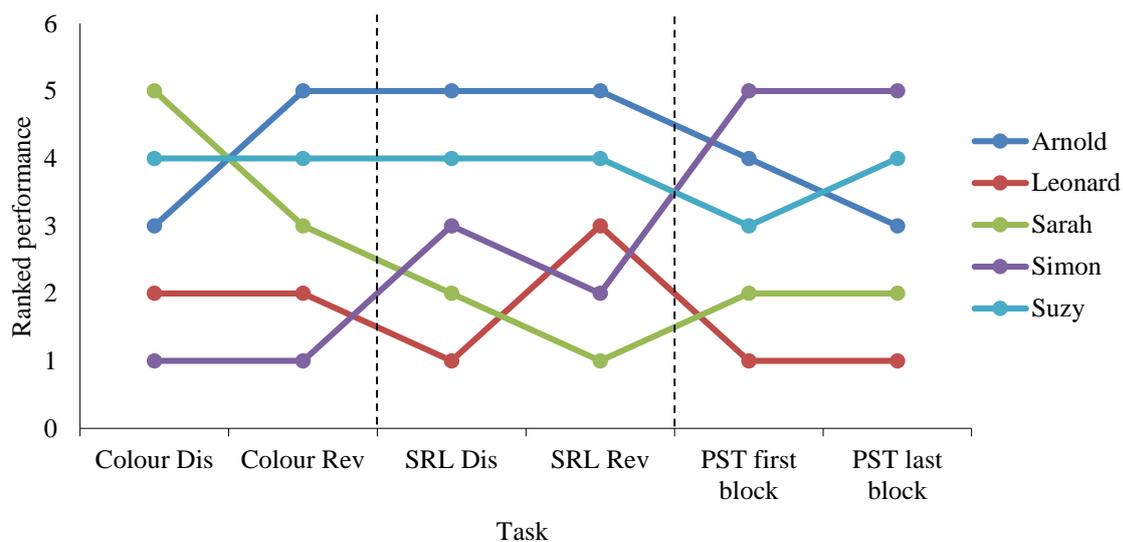


Figure 6.3. Ranked performance across three tasks (in chronological order), colour reversal learning, serial reversal learning and puzzle box. In both reversal learning tasks, assigned rank is based the number of errors before reaching the learning criteria in discrimination phase and reversal phase. Colour discrimination phase (Colour Dis); colour reversal phase (Colour Rev); Serial reversal learning discrimination phase (SRL Dis); Serial reversal learning reversal phase (SRL Rev). Ranked performance for problem solving task was based

on the average solution time in the first block (four trials) and last block (four trials). The individual showed lowest solution time ranked as '5' whereas individual took the longest solution time was ranked as '1'.

6.5 SUGGESTED FUTURE STUDIES

6.5.1 PROBLEM SOLVING ABILITY IN UK INVASIVE VS U.S NATIVE GREY SQUIRRELS

In Chapter 5, we used an invasive grey population and a group of native red squirrels to examine problem solving ability and performance differences. Although I have shown some evidence that, on average, the native red squirrels are weaker in problem solving than the invasive grey squirrels, it does not follow that this is true for grey squirrels in general (see **Section 6.4.2**). If we want to exclude the possibility that selective pressure has increased the behavioural flexibility for grey squirrels during the invasive process, instead of their being inherently flexible, then it is obvious that investigation should trace back to the native environment of grey squirrels in North Carolina, North America. If grey squirrels inherently possess higher capacity to solve problems, then one would expect that the native greys would show similar problem solving ability to the invasive greys in UK. However, if the native grey squirrels showed poorer problem solving than the invasive greys, then the results would support the ‘adaptive flexibility hypothesis’ (Wright, Eberhard, Hobson, Avery, & Russello, 2010).

6.5.2 GENERAL INTELLIGENCE IN THE FAMILY *SCIURIDAE*

As mentioned in **Section 6.3.3** it appears that our laboratory grey squirrels show some indications of general intelligence across tasks. But to strengthen the conclusion, an extended investigation should be done to assess to what extent that learning performance is shared within these individuals across various tasks (e.g. Isden, Panayi, Dingle, & Madden, 2013; Shaw, Boogert, Clayton, & Burns, 2015). Ideally, an investigation would compare the variations of cognitive ability among squirrel family members as has been done in avian species, for example, Bond, Kamil, & Balda (2007) compared three related species of North

American corvids with varied sociality: pinyon jays (*Gymnorhinus cyanocephalus*), Clark's nutcrackers (*Nucifraga columbiana*), and western scrub-jays (*Aphelocoma californica*). New investigation could be carried both in laboratory and in the field. For field study, the set up requires pre-baiting, but as long as the apparatus is baited, grey squirrels will visit regularly (personal observation for red and grey squirrels, for fox squirrels). With these data, we could understand how cognitive ability varies between species and possibly, the factors that underlie the differences.

6.5.3 COMPARATIVE STUDIES WITH DISTANT SPECIES THAT SHARE SIMILAR ECOLOGICAL NEEDS

Although I have explored the difference in problem solving performance within the family *Sciuridae*, to what extent that the flexibility of grey squirrels could be comparable to that of other species is largely unknown. It is notoriously difficult to design a standardised task for different species, given that the perceptual modality and physical characteristic of each species may pose a constraint in manipulating the same task (Holekamp, Swanson, van Meter, 2013). Despite this, an attempt could be taken to consider some ecological similarities in assessing their flexibility. For example, grey squirrels could be compared with other *Corvidae* family members such as ravens or scrub jays based on their ecological needs as scatter-hoarders. Similar to grey squirrels, corvids have been shown to be sensitive to their conspecific during caching, for example, in ravens, *Corvus corax* (e.g. Bugnyar, 2011; Bugnyar & Kotrschal, 2002; Bugnyar & Heinrich, 2005; Heinrich & Pepper, 1998) and western scrub-jays (e.g. Clayton, Dally, & Emery, 2007; Dally, Emery & Clayton, 2004) and Eurasian jays, *Garrulus glandarius* (Legg & Clayton, 2014; Shaw & Clayton, 2013). One way of examining flexibility could be to design a caching study including different levels of challenges, for example, presence of conspecific competitors in which cachers could see and

hear the conspecific competitor (visual + auditory cues available condition), provide auditory but not visual cues for cachers to indicate the presence of competitor (only auditory cues available condition) compared with when cachers could cache alone (control condition). Under these conditions, we could examine the similarity of caching strategies and post-caching management seen in different species. Such examination ideally is taken in a laboratory set up, which investigators could avoid some difficulties posed in the field (e.g. individual identification) and increase the possibility of following the cache location and especially follow the post-caching management. Under such a standardised set up, we could assess to what extent that scatter-hoarders, across widely different taxa, share similar flexibility in responding to different levels of challenge and whether such flexibility provides adaptive responses or optimal behavioural reactions.

6.6 GENERAL CONCLUSION

In conclusion, I have shown how learning and behavioural flexibility are involved in problem solving mostly in grey squirrels and a study with red squirrels. Through four experiments, I accumulated evidence to show two distinct forms of process that are important in problem solving. While learning plays a gradual role, flexibility is shown as an instant adjustment to encounter challenges. These results show that both mechanisms are needed in problem solving, and suggest that the activation of either mechanism is associated with varied problem solving performance. Both forms of flexibility are directly and indirectly interacting with other behavioural traits such as effective behaviours or persistence.

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