Individuality and consistency in foraging behaviour of the Bumblebee *Bombus terrestris*

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This thesis incorporates work carried out in collaboration with Dr. Heiko Grossmann at Queen Mary University of London. The collaboration related to the statistical analysis of the experiment 2 chapter 5 section 5.2.2: individual consistency in response to novel colours.

I certify that, with the above qualification, the work carried out in this thesis is entirely my own and that ideas and quotations from other sources are fully acknowledged.

Hélène Müller, August 2011

Abstract

Many vertebrates and a few invertebrates are known to show individual-specific consistency in their behaviour across time and situations, sometimes in ways that can be paralleled with human personality. Despite their relatively small brains, bees show remarkable cognitive abilities. It is therefore not unreasonable to speculate that, as other animals with such cognitive abilities, they too would be able to show some form of animal personality.

The first three chapters of this work are theoretical and discuss relevant concepts and controversies in the field of animal personality. Chapter 4 explored the possibility of individual bees differing in their ability to learn to associate stimuli with reward. While some bees learned to differentiate between two stimuli with a high degree of accuracy, others made frequent mistakes, independently of the modality or dimension of the stimuli considered. Bees therefore appeared to differ individually in their ability to discriminate between stimuli. Chapter 5 of this work aimed at answering the question of whether individual bees consistently differ in their behaviour, which is a prerequisite to establishing the existence of personality in any animal. Individual bees' response to novelty (neophobia-neophilia) was found to be relatively predictable within a short time scale but not on the long term. Neophobia-neophilia is therefore an episodic personality trait. Chapter 6 was concerned with individual responses to a simulated predation threat. Individual bees were found to vary widely, both qualitatively and quantitatively. These responses were consistent through time and so were other features of their foraging behaviours.

Taken together, my findings provide an insight into individual variations in foraging behaviour in the bumblebee *Bombus terrestris* and represent good evidence for the existence of individual consistency, thus paving the way for further research into personality traits in this species.

Publications arising from this thesis

Research papers

Muller H, Grossmann H, Chittka L (2010) 'Personality' in bumblebees: individual consistency in responses to novel colours? Animal Behaviour 80:1065-1074

Muller H, Chittka L (2011) Consistent inter-individual differences in discrimination performance by bumblebees in colour, shape and odour learning tasks (submitted)

Commentaries

Muller H and Chittka L (2008) Animal personalities: the advantage of diversity. Current Biology 18:R961-R963

Review article

Chittka L and **Muller H** (2009) Learning, specialization, efficiency and task allocation in social insects. Communicative & Integrative Biology 2(2): 151-154

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Figures and photographs

Chapter 1: Introduction

Individual variation in behaviour is commonly observed by behavioural biologists or behavioural ecologists in their subjects of study and there is a general consensus that individual variation plays a key role in evolution (e.g. see Darwin, 1859). Despite this, consistent individual variation in behaviour, i.e. personality, has long been under-studied in non-human animals. Genetic and physiological studies have shed light on some mechanisms which are at the origin of individual variations in behaviour, but genetics and physiology cannot alone explain all the variation observed (Dingemanse et al., 2009). Traditionally, individual variation in behaviour was seen as noise surrounding the mean optimal behaviour (Chittka and Dornhaus, 1999; Thomson and Chittka, 2001; Bergmüller and Taborsky, 2010). Stochastic processes may well account for some of the variation; however, they are unlikely to account for all the remaining variation observed, especially when the behaviour appears to be repeatable and predictable (Sih et al., 2004; Réale et al., 2007) or even detrimental to the individual’s fitness (Johnson and Sih, 2007). The existence of such repeatable/predictable and sometimes apparently detrimental behaviours raise many questions, which can be summarised along three lines: Why do individuals consistently vary in their behaviour? Why are individuals limited in their behavioural range? Why do individuals repeat themselves in their behaviour?

In the past few decades, the emerging field of animal personality research has developed an evolutionary framework in order to answer these questions. Because of their widespread use as models in cognition and behaviour studies, bees make an ideal model on which to investigate the existence and consequences of personality in a social insect. The present work will attempt to shed some light on the questions of the existence of personality traits in *Bombus terrestris* and whether these traits could be linked with individual cognitive ability.

Before examining the case of bumblebees, it is important to discuss and clarify the various concepts and controversies of the field relevant to this work so that the terminology and concept developed in the experimental chapters will be understood. Hence, the first three chapters are dedicated to important terminology and conceptual issues in the field of animal personality and the application of animal personality concepts to eusocial insects.

The present chapter (Chapter 1), is a general introduction to the field of animal personality. It includes the definitions of the terms used throughout this work. Chapter 2 is concerned with a discussion of the controversies arising from applying human terms and concepts to non-human animals as well as an overview of the current research in the evolution and maintenance of personalities in both human and non-human animals. Chapter 3 comprises a brief introduction to task specialisation in eusocial insects and its relationship to personality. The next three chapters are experimental chapters. Chapter 4 investigates individual differences in bumblebees' ability to solve discriminating tasks and whether the individual's response to novelty has bearings on their abilities and *vice versa*. Chapter 5 is concerned with measuring individual responses to novelty and assessing whether bumblebees neophilia-neophobia can be considered as a personality trait. Chapter 6 will deal with another potential personality trait: risk-taking (in a predation context). And finally, Chapter 7 is a general discussion of all the findings from Chapter 4, 5 and 6 and how these findings relate to the issues raised in Chapter 2 and 3.

1.1 What is Animal Personality?

The word ‘personality’ and ‘person’ originated from the Latin *persona*. ‘*Persona*’ were masks used in the theatre to represent a particular character in a play. The word ‘personality’ may take different meaning. For example, personality in common usage might refer to an individual’s charisma or assertiveness (e.g. ‘this candidate has a strong personality’). In psychology, there is no consensus on the definition of personality and this term might refer to observable phenotypic traits (Nettle, 2006a) as well as affective and cognitive features of human subjects, including beliefs, motives, life histories and intellectual characters (Anestis, 2005; Aiken, 1999).

Terms associated with human personality have long been applied to the individual behaviour of some animals: apes, cats, dogs and horses are commonly referred to as ‘bold’, ‘fearful’, ‘aggressive’, ‘curious’, ‘sociable’ or even ‘laid back’ and ‘restless’ (Careau et al., 2008). The use of such terms, which are recognised components of personality in humans, is typically justified by the similarities with human behaviour. One could therefore argue that the use of the term ‘personality’ for non-human animals is also justified. However, the use of this term remains controversial. The mechanisms and evolutionary processes behind animal and human personalities might differ (Dingemanse et al., 2009): more work needs to be done on the mechanisms and functions of these behavioural traits in both humans and animals before this issue can be resolved. It is therefore prudent to refrain from claiming continuity between animal and human personality. However, one can argue that in as much as a ‘wing’ can refer to a bat or an ostrich’s forelimbs or to an insect’s appendages, the term personality and terms applying to personality traits could be used to describe behaviours found in non-human animals.

As some of the behaviours studied are very evocative of the ones observed in humans, I believe their use is justified. However, when defining the meaning of the term used, one should stress that there no implied continuity with human psychological processes.

The use of the term personality in the biological literature implies that two conditions are fulfilled (Biro and Stamps, 2008; Réale et al., 2007; Careau et al., 2008; Dingemanse et al., 2010b):

> 1) Individuals vary from one another in the behaviours they express. This means that an individual does not express the full range of behaviour available in the population (Dingemanse et al., 2009) and that the observed inter-individual variation is significantly greater than the measurement error.
>
> These personality traits can vary qualitatively or quantitatively between individuals. Individuals might differ in the nature of their responses (or strategy; Nettle, 2006a) to environmental stimuli (e.g.: one individual reliably responds to a new object by investigating it whereas another individual avoids it) or they might differ in the degree of their response (e.g. one individual consistently flees 2m away from a predator whereas another individual reliably flees 6m away).
>
> 2) Personality also implies that the individual variation (i.e. personality trait) observed is consistent across time and situations (i.e. within a given context; see below). This means that the variation in behaviour observed within an individual is lower than the variation in behaviour between individuals of the population. Consistency is usually assessed statistically by comparing the within- and between-individual variance or by comparing the ranks of individuals relative to one another. An individual's behaviour is consistent if it is significantly repeatable (time

consistency) and predictable (situation consistency). Being consistent does not mean that the behaviour will remain unchanged through time or across situations. Individual consistency is always assessed in relation to the behaviour of other individuals. For example, in a fictional population, if all individuals greatly increased their aggressivity level (say following an increase in temperature), consistency would still be found where the individuals keep the same relative rank within the population. Personality can be dynamic and therefore might change through an individual's lifetime. It is therefore important, when assessing the consistency of behavioural traits through time, to choose an appropriate time scale relevant to the animal's life history (see Sinn et al., 2008a and section 1.3 and chapter 5). The terms context and situation are often used interchangeably in this definition (e.g. Biro and Stamps, 2008) although I believe they refer to different concepts. Generally, a context is 'a functional behavioural category' (Sinn et al., 2010) consisting of different situations. Contexts can be social (e.g. care for offspring, competition for food), linked to the habitat (e.g. predation risk), phenological (e.g. seasonal) and physiological (e.g. hormonal, metabolic; Dingemanse et al., 2009). Each personality trait is associated with one context (Sinn et al., 2010; Réale et al., 2007) although personality traits might be correlated to one another (cross contexts correlations) as behavioural syndromes (see section 1.2). I believe it is inaccurate to define personality as consistent individual behaviour across contexts in the sense that an individual's personality will indeed cover many contexts but each of the traits composing the personality affects only one context (though several situations within a context) unless some traits are correlated with each other (but this is not compulsory).

Personality traits can be innate[1] or result from experience. Hence personality is dynamic and might change throughout an individual's life. The innate components of personality are sometimes referred to as 'temperament' and treated as a separate concept. I share Réale et al. (2007)'s view that justifications for such distinction do not however withstand critical examination and both terms should be considered as synonymous. Behavioural style (Anestis, 2005) and behavioural profile (Groothuis and Carere, 2005) have also been used as synonyms for personality.

Idiosyncrasies, motivational states (such as hunger) and ability traits are not considered as personality traits (Réale et al., 2007). Nevertheless, they are likely to impact personality: they certainly play an important role in determining an individual's behaviour (e.g. individuals with a higher hunger threshold could tend to be more aggressive in order to acquire more food through predation or competition). Being unobservable, motivational states and ability traits cannot be considered as behaviours and therefore do not fulfil the requirements for personality traits. Idiosyncrasies, being found in a single individual, are difficult to study and therefore will not be developed here.

1.2 Behavioural syndrome

The present work will not be concerned with behavioural syndromes. However, there will be many references to behavioural syndromes studies throughout this work and it is important to disentangle the two concepts. Unlike personality, a behavioural syndrome is a property of a population, not of an individual (Sinn et al., 2010; Bell, 2007b; Réale et al.,

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[1] Here a flexible behaviour is not meant as the opposite of an innate behaviour but rather as the ability to change behaviour in responses to changes in the environment (or situation).

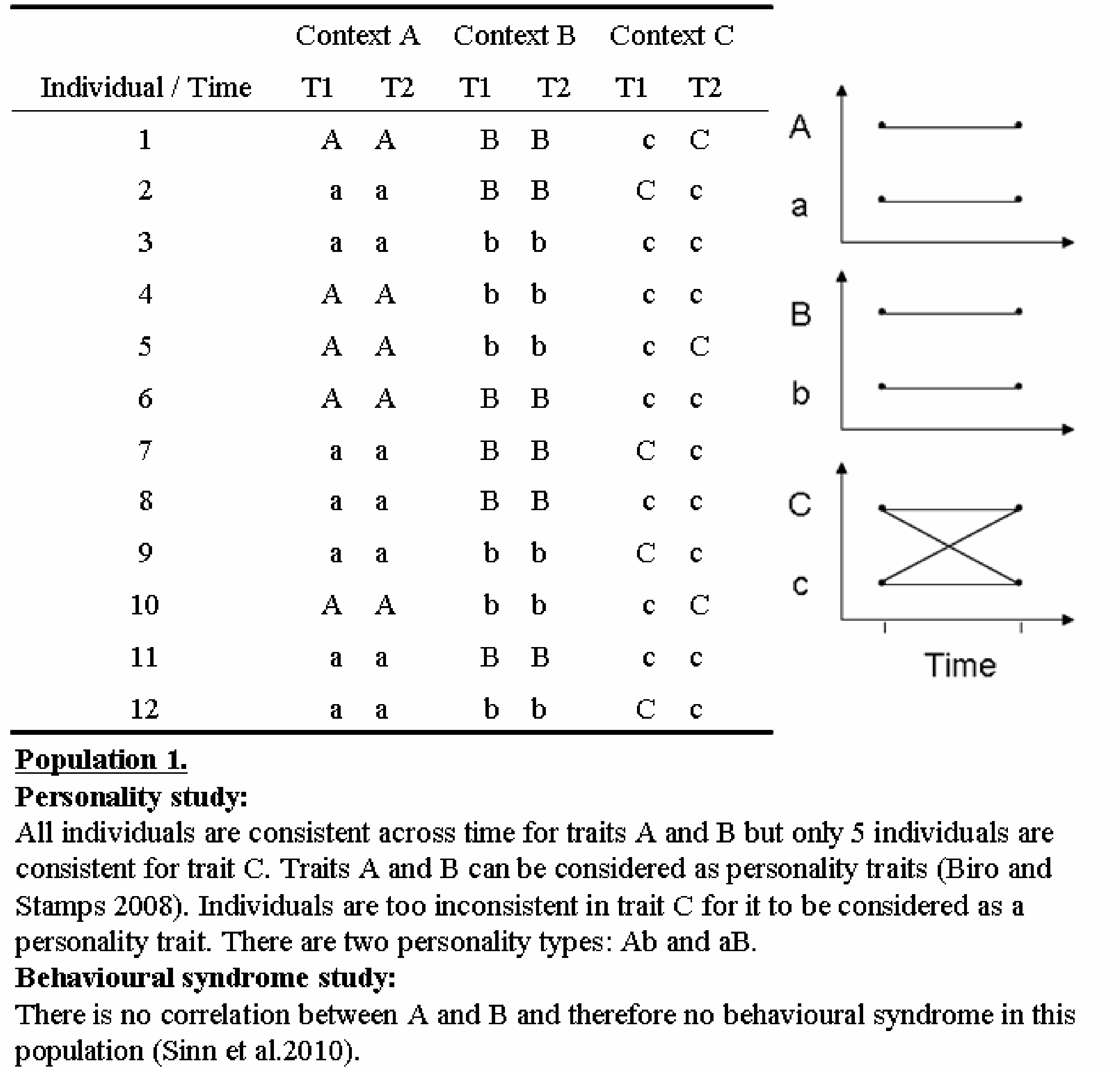
2007), although individuals can be classified by behavioural types. A behavioural syndrome is a correlated suite of behaviours (Sinn et al., 2010; Bell, 2007b; Réale et al., 2007). For example, Johnson and Sih (2007) found a ‘boldness syndrome’ in female fishing spiders *Dolomedes triton*: risk-proneness in presence of a predator was positively correlated with aggression as females with an aggressive behavioural type tended to be more risk-prone than less aggressive females (correlation across contexts). Importantly, a given behavioural syndrome might only be found in a specific population as different environmental conditions in different populations might lead to different behavioural syndromes (as exemplified by Sinn et al.’s study of *Euprymna tasmanica* squids; Sinn et al., 2010).

As pointed out by Logue et al. (2009), the definition of behavioural syndrome does not require the demonstration of repeatability for the constituent behaviours. Johnson and Sih, (2007)’s study of juvenile spiders highlighted this issue: a behavioural syndrome might be stable but this does not imply that individuals’ behavioural types remain stable through time (e.g. all individual may change behavioural types over time but all the different behavioural types are still present in the population).

Several behavioural syndromes studies did not explicitly demonstrate repeatability of behaviour across time (Logue et al., 2009) and because of this, these studies run the risk of finding spurious or ephemeral correlations which would then be extrapolated to the species or population (Sinn et al., 2010). In order to mitigate this caveat, behavioural syndromes studies often include retesting of individuals (which technically makes them equivalent to personality studies, e.g. Wilson and Godin, 2009) or population measurements repeated in time (Sinn et al., 2010).

The concepts of personality and behavioural syndromes are often regarded as equivalent (Hollander et al., 2008; Careau et al., 2008; Dingemanse et al., 2009; van Dongen et al., 2010 and Wolf et al., 2007). Dingemanse et al. (2009) even argued that the existence of personalities implies that interindividual correlations between contexts exist, and therefore that behavioural syndromes are an obligatory component of personalities. I disagree with this view. A personality type can be common to many individuals (a group within a population for example) and therefore be treated as a behavioural type. However, the existence of behavioural constraints within individuals (personalities) does not necessarily result in correlations across individuals (behavioural syndrome) and vice versa (see Fig. 1.1a and 1.1b). Work by Sinn and his team showed that in the dumpling squid *Euprymna tasmanica*, individuals did appear to be consistent (i.e. they had personalities; Sinn et al., 2006) but they failed to find any meaningful behavioural syndrome linking these personality traits (Sinn et al., 2010).

Nevertheless, personality and behavioural syndrome concepts are intimately linked. The study of personality is frequently the first step towards the study of behavioural syndromes. Personality studies tend to be descriptive and then used in a behavioural syndrome framework which will explore potential causal links between personality traits (resulting in behavioural types) and/or between personality traits and fitness or physiology (e.g.: coping style studies). The study of behavioural syndromes can therefore be considered as the study of personality in a broad sense (Réale et al., 2010) although, throughout this work, personality will refer to the more restrictive (exclusive of behavioural syndromes) meaning.



	Context A		Context B		Context C	
Individual / Time	T1	T2	T1	T2	T1	T2
1	A	A	B	B	c	C
2	a	a	B	B	C	c
3	a	a	b	b	c	c
4	A	A	b	b	c	c
5	A	A	b	b	c	C
6	A	A	B	B	c	c
7	a	a	B	B	C	c
8	a	a	B	B	c	c
9	a	a	b	b	C	c
10	A	A	b	b	c	C
11	a	a	B	B	c	c
12	a	a	b	b	C	c

Population 1.

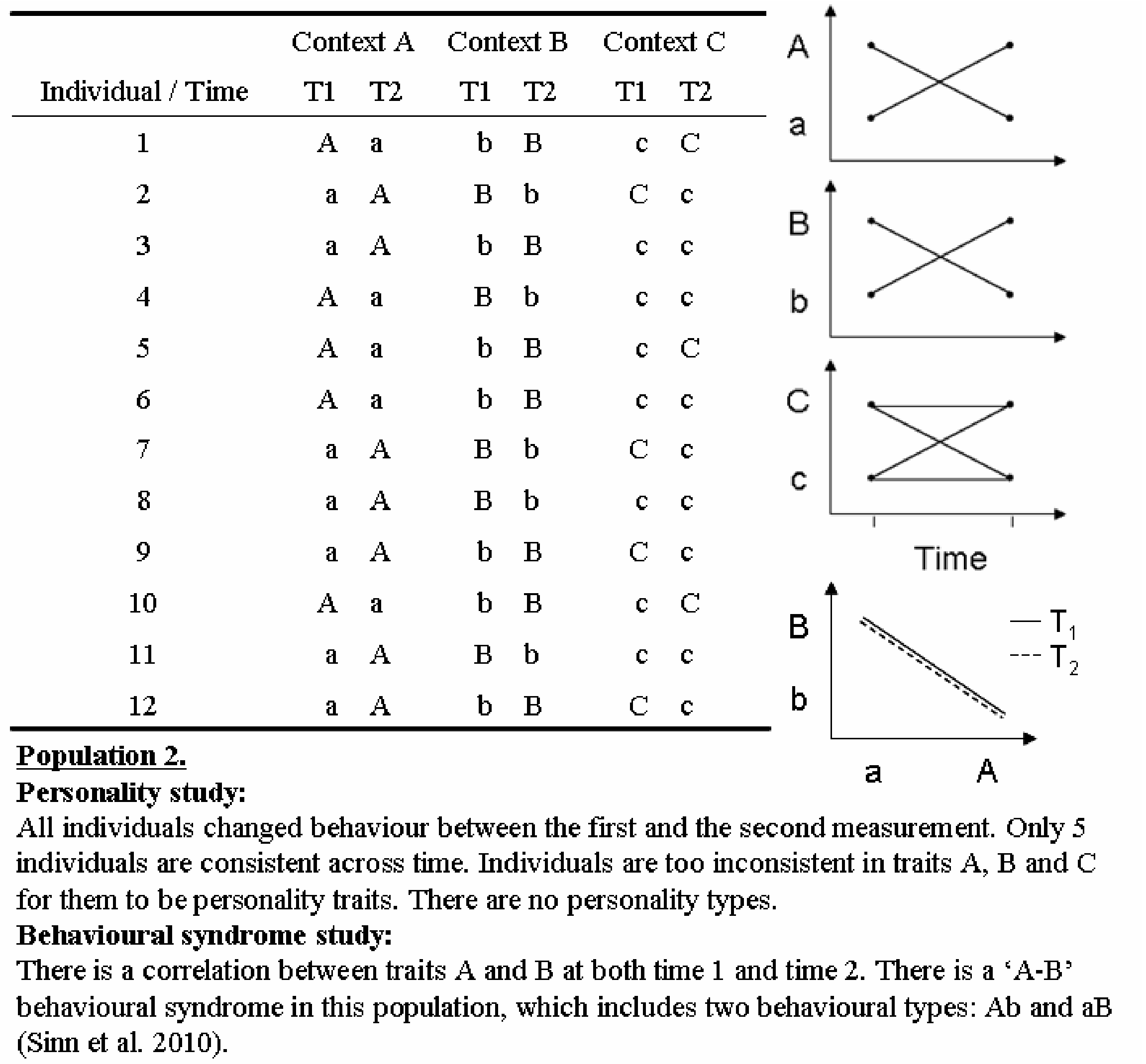
Personality study:

All individuals are consistent across time for traits A and B but only 5 individuals are consistent for trait C. Traits A and B can be considered as personality traits (Biro and Stamps 2008). Individuals are too inconsistent in trait C for it to be considered as a personality trait. There are two personality types: Ab and aB.

Behavioural syndrome study:

There is no correlation between A and B and therefore no behavioural syndrome in this population (Sinn et al.2010).

Figure 1.1a. *A fictional study of a population of individuals (population 1) analysed through both personality and behavioural syndromes approaches, thus highlighting each approaches' limitations.*



	Context A		Context B		Context C	
Individual / Time	T1	T2	T1	T2	T1	T2
1	A	a	b	B	c	C
2	a	A	B	b	C	c
3	a	A	b	B	c	c
4	A	a	B	b	c	c
5	A	a	b	B	c	C
6	A	a	b	B	c	c
7	a	A	B	b	C	c
8	a	A	B	b	c	c
9	a	A	b	B	C	c
10	A	a	b	B	c	C
11	a	A	B	b	c	c
12	a	A	b	B	C	c

Population 2.

Personality study:

All individuals changed behaviour between the first and the second measurement. Only 5 individuals are consistent across time. Individuals are too inconsistent in traits A, B and C for them to be personality traits. There are no personality types.

Behavioural syndrome study:

There is a correlation between traits A and B at both time 1 and time 2. There is a 'A-B' behavioural syndrome in this population, which includes two behavioural types: Ab and aB (Sinn et al. 2010).

Figure 1.1b. *A fictional study of a population of individuals (population 2) analysed through both personality and behavioural syndromes approaches . Note: it could be argued that the individuals here do have a personality in the sense that they systematically adopt the opposite behaviour from T1 to T2 for traits A and B. However, this example is limited to only to measurements in time (T1 and T2). If more measurements were taken it would be apparent that the change is not systematic.*

1.3 Consistency - Plasticity

Behavioural consistency is a key concept in animal personality and will be of paramount importance in chapter 5. It is therefore important to clarify the meaning of consistency in animal personality studies and how it translates in terms of individual behaviour. Appropriate behavioural response to changes in the environment assumes great flexibility. One would expect individuals to tune their behaviour to the current situation they are experiencing. However, it is often found that individuals lack flexibility. For examples, aggressive female fishing spiders (Johnson and Sih, 2007) are very successful predators in as much as they will readily attack prey and kill them. These aggressive females however do not experience the same success when it comes to mating as they also tend to attack and kill potential mates. This ‘spill-over’ of aggressivity is counterintuitive in as much as one would expect females to adapt their behaviour to the situation in which they find themselves. Similarly, individuals which are born in captivity may still show variation in their tendency to spend time in exposed habitat while the risk of predation is naught (Sneddon 2003). Clearly these and many other examples (see Bergmüller and Taborsky, 2010; Biro and Stamps, 2010; Wolf et al., 2008) reflect a lack of behavioural flexibility as well as the existence of variability between individuals, which is the basis for personality.

Consistent individual behaviour has now been found in a wide range of species (Wolf et al., 2007) and is paramount to the concept of personality. The term consistency can be rather confusing as an individual’s behaviour can be described as consistent and plastic at the same time (Biro and Stamps, 2008; Dingemanse et al., 2009), which implies some flexibility within the consistency. One has to consider that the term consistency is applied to describe the behaviour both across time and across situations. Consistency across time refers to repeatability (Biro and Stamps, 2010) or stability (Bergmüller and Taborsky, 2010) of behaviour, implying that there is little unexplained (i.e. stochastic) variation between two measurements of the behaviour in the same situation at different times. Consistency across situations has a different meaning: cross situation consistency in behaviour means that one can predict the behaviour of an individual in situation B from its behaviour in situation A. Responses to the two different situations maybe very similar qualitatively and/or quantitatively. For example, a 'predation-risk averse' fish would not only be expected to seek refuge when confronted with a dummy predatory fish but also to act similarly when a bird-like shape (another potential predator) flies over it.

The fact that individuals show consistency across situations rather than plasticity appears to be suboptimal in terms of fitness. By being consistent, an individual risks responding inappropriately to some situations (as in the 'spill-over' case of the aggressive females of the fishing spider; Johnson and Sih, 2007). On the other hand, obtaining up-to-date and accurate information about one's environment is costly (Briffa et al., 2008; McElreath and Strimling, 2006), which could restrict the plasticity of an individuals' behaviour. In a social context, consistency in behaviour is also thought to be advantageous: consistency might diminish conflicts amongst conspecifics (Bergmüller and Taborsky, 2010). Predictable behaviour is necessary to the establishment of stable hierarchy and cooperation (Dall et al., 2004). On a proximate level, consistency can be explained by physiological or sensory/cognitive limitations (e.g. pollen foraging and sucrose sensitivity in honeybees (*Apis mellifera*); Page and Scheiner, 2006; emotional responsiveness and aggressive behaviour in rodents David et al., 2004). Metabolism (Biro and Stamps, 2010; McElreath and Strimling, 2006) and hormonal regulation of behaviour (Cavigelli, 2005) are both known to constrain an individual's behavioural repertoire. Conversely, plasticity might also be selected for: one could imagine that flexible individuals would get an edge on more consistent individuals in a changing environment (Wilson et al., 1994; Pronk et al., 2010) and there is evidence for the existence of genetic variation in the degree of plasticity (Dingemanse et al., 2009). Due to the simultaneous existence of consistency and plasticity for the same behavioural trait, assessing an individual's consistency for the purpose of studying its personality can be arduous. However, assessing individual consistency is essential to establishing the existence of personality traits (see section 1.1). The following section will introduce some of the difficulties encountered when attempting to do so, and these difficulties will be further discussed in chapter 5.

1.3.1 How to quantify consistency and plasticity

Checking for individual consistency in behaviour is, paradoxically, often overlooked in studies of animal personality or behavioural syndromes (Kurvers et al., 2009; Logue et al., 2009). As Sinn et al. (2010) points out: “Most work to date has been taken from limited ‘snapshots’ in space and time, with the implicit assumption that a behavioural syndrome is an invariant property, fixed by evolutionary constraints or adaptations”. Assessing consistency requires that repeated measures of individuals' behaviour are taken. Ideally, this would be done by measuring an individual's behaviour several times both within each situation and across situations. However, this is rarely done, mainly because collecting repeated measurements is very time consuming and sometimes impractical (as is often true for studies of animals in the wild). Some studies draw conclusions about personality from measuring individual's behaviour once for each trait (e.g. Dochtermann and Jenkins, 2007; Hollander et al., 2008; Minvielle et al., 2002; Logue et al., 2009 and see many more examples in Gabriel and Black, 2010). As highlighted by Sinn et al. (2010), assessing consistency is essential and such incomplete studies can lead to spurious results. In addition, careful consideration has to be given to the time intervals between two measures when assessing time consistency (see Chapter 5).

Consistency has traditionally been assessed through various statistical tools such as intra-individual correlation between repeated measures for a particular individual (Bergmüller and Taborsky, 2010) and comparison of within- and between-individual variance (e.g. Kurvers et al., 2009; Dingemanse et al., 2009; Magnhagen and Bunnefeld, 2010). The usefulness of the latter approach is debatable as the variance is a property of the population and therefore comparing two variances together says little about individual repeatability (Bell et al., 2009). Alternative methods are used such as Kendall's W (Biro and Stamps, 2008; Wilson et al., 1994) or the consistency model (Müller et al., 2010).

The degree of individual consistency can vary between individuals and classes of behaviour (e.g. aggression has been found to be highly repeatable in several species; Bell et al., 2009). Measuring consistency can be difficult, especially if one considers all the factors which can potentially bias or affect its measurement. For example, the experimental settings (i.e. laboratory versus field; Bell et al., 2009) in which the study is conducted can impact on the measurement of consistency. Measurement errors might interfere with estimates of consistency (Bell et al., 2009) and learning (Wilson and Godin, 2009; Sinn et al., 2008a) is also thought to influence consistency measures.

1.3.2 When is it plasticity or a new personality trait?

The question of whether one or two personality traits were measured will arise in chapter 6. Readers should therefore be warned of the pitfalls which might lie in studies where several behavioural traits are evaluated as potential personality traits. A recurrent issue with the study of personality is to define a cut-off point between personality traits, in the sense that each personality trait is expressed in a particular context and that contexts are sometimes hard to define. Indeed, the necessity for repetition of measures in personality studies and experimental constraints can lead to several contexts being thought of as one or of one context being artificially divided into many. In the first scenario (several contexts taken for one), one could wrongly conclude a lack of consistency in behaviour as the several personality traits measured might not be correlated to one another. For example, Boogert et al.’s (2006) evaluation of an individual’s response to novelty in starlings (*Sturnus vulgaris*) comprised two tests: one involving a novel environment and the other involving a novel object. Although these two tests might appear to be similar and therefore to belong to one context (response to novelty), Boogert et al. (2006) did not find any relationship between the results for the two novelty response tests. Whereas it is possible, as Boogert et al. (2006) suggested, that this finding can be explained by a low statistical power, one could also consider the possibility that the novel environment test included a ‘risk-taking’ component (in terms of predation) as well as neophobia (it was the first time the individuals was isolated from its social group and therefore it might have felt vulnerable as in predatory context), which would make of the novel environment test an unreliable way to evaluate response to novelty. In the second scenario (one context artificially split into several), the conclusion will be that the two personality traits are correlated while they are in fact the same trait measured twice. For example, the phenomenon of ‘carry-over’ (Sih et al., 2003), i.e. correlation between behaviours from seemingly different contexts, could be explained in this way. Johnson and Sih (2007)'s 'bold' female spiders (i.e. a female eating both prey and potential mates) would simply fail to detect the reproductive opportunity, mistaking it for a foraging opportunity (possibly due to a heightened ability to detect potential prey, leaving too little time to the males to initiate courtship). One way of disentangling the two contexts (foraging and mating) would be to make sure that all females are satiated prior to introducing the courting male.

I would argue that the debate of context-dependant variation within a personality trait, such as the one surrounding the 'shy-bold continuum' (Wilson and Godin, 2009; and section 1.4) stems from the incorrect perception that personality traits exist across contexts, while in fact, each personality trait corresponds to one context and personality traits are consistent across *situations*. It is true that personality traits might be correlated to one another (as found in personality-behavioural syndromes studies; e.g. Wilson and Godin, 2009), thus resulting in 'cross-context personality traits'. I believe this view to be inaccurate and confusing. Because of such inaccuracies, the correlated traits are often referred to using a single 'umbrella' term (e.g. shy-bold see section 1.4). This can be rather confusing, especially if this term is the same as the one referring to one of the constituting personality traits. 'Bold' can refer to a single personality trait or to the entire behavioural type (see section 1.4). I believe it would be more rigorous to refer to such assemblages of personality traits as personality 'groups'. The 'shy-bold group' would therefore refer to several correlated personality traits (each corresponding to a different context such as foraging, social or reproductive).

1.4 Boldness, neophobia and exploration

As discussed in chapter 2, the use of terms usually applied to human behaviour is controversial. However, and in accordance with the majority of preceding studies, some of such terms will be used in the present work. It is therefore important to define them as accurately as possible. This section aims to do so as well as resolving other controversies surrounding the misuse of terms within the field of animal personality and to justify the use of alternative terms in the present work.

Human personality traits are commonly considered to fall into five categories: extraversion, agreeableness, conscientiousness, neuroticism and openness (Nettle, 2006b). Human personality traits routinely refer to emotions or to concepts related to consciousness. Using the same terminology for animal personality traits would be highly controversial and often unjustifiable (as some traits cannot yet be assessed without direct questioning). Animal personality researchers tend to use their own five categories. For example, Réale et al. (2007) refers to: shyness-boldness, exploration-avoidance, activity, aggressiveness and sociability. I will develop below the three categories relevant to this work.

Shyness-boldness is commonly used to refer to approach/avoidance behavioural responses with regards to a predator and/or a novel object and/or to a novel environment (Sinn et al., 2008a). However, these three responses are potentially very different in functional/evolutionary terms. A stimulus identified as a predator represents an immediate threat to the individual. The individual’s response to it is therefore likely to have a high impact on its fitness. While the response to a predator is necessarily one eliciting avoidance/defensive behaviours, the response to novel objects and environments can elicit avoidance as well as attraction behaviours. Indeed, novelty is not necessarily dangerous to the individual and might even be beneficial (e.g. novel food sources). Not only are ‘bold’ and ‘shy’ used to refer to these different functional contexts, but they are also used to refer to groups of behaviours covarying with one another under the name of ‘shyness-boldness syndromes’ (Sinn et al., 2008a; Wilson and Godin, 2009; Ward et al., 2004; Briffa et al., 2008). Such syndromes usually cover responses towards predators as well as responses towards novelty.

I believe that the attribution of the label ‘bold’ or ‘shy’ to various behaviours is problematic. As discussed above, these terms might encompass functionally and evolutionarily different behaviours, and this fact is often ignored when comparisons are made across studies. For example, Wilson and Godin (2009) state that “Individual variation in boldness has been documented for a variety of taxa, including birds, mammals, reptiles, fishes, insects, and cephalopods”. A closer look at the references cited for each taxonomic group reveals that some of these references were concerned with responses to predatory threats, others with responses to novelty and some with whole behavioural syndromes. Given that the same term is used to refer to different processes and phenomenon, extra care should be taken when comparing different studies. In the following sections, I will attempt to produce working definitions for each of the two aspects generally gathered under the label ‘boldness’, namely risk-taking and response to novelty (neophilia-neophobia).

1.4.1 Risk-proneness and risk-aversion

An individual can be categorised as risk-prone or risk averse depending on its propensity to take risks when confronted by a predator or predator cues. ‘Risk-taking’ in the non-human literature can also be meant as the propensity to exploit unreliable food sources in a foraging context (e.g. Cartar and Dill, 1990), but here, I will only refer to risk-taking in relation to predators or predator cues (see chapter 6). Risk-prone individuals tend to increase, as a result of their behaviour, the likelihood of predator-encounter or of dying as a result of predation. Following this definition, risk-taking is a synonym for boldness-shyness in the following studies: Smith and Blumstein (2010), Johnson and Sih (2007), Sinn et al. (2008a), Sinn et al. (2010), Biro and Stamps (2008), Réale et al. (2007), Wolf et al. (2007), Dochtermann and Jenkins (2007), Dingemanse et al. (2007) and Magnhagen and Bunnefeld (2010). Predation-risk-aversion is also regarded by some authors as a synonym for 'fearfulness' (Hedrick, 2000), 'docility' and 'tameness' (Réale et al., 2007).

Risk-taking (in terms of predation) may appear straightforward to test experimentally but for the potential confounding factor of environmental novelty. As mentioned previously, novel situations or objects can be perceived as a threat (i.e. potential predators of hiding places for predators) as well as potential food sources. If a naïve individual is exposed to a novel stimuli and responds by avoiding it, then it will be classified it as risk-averse, whereas if the individual perceives novelty as potentially rewarding, it will be classified as risk-prone. When testing for risk-taking, it is therefore paramount that the individual associate the stimulus with a predation threat, otherwise risk-prone individuals could simply be individuals which fail to perceive the risk.

1.4.2 Neophilia-neophobia and exploration

Neophilia is commonly defined as attraction to novelty whereas neophobia is repulsion from novelty (see chapter 5). Neophilia and neophobia are sometimes regarded as part of ‘exploration’ (Réale et al., 2007; Brown and Nemes, 2008; Heinrich, 1995) and ‘exploration’ has also been used as a general term when no distinction was made between attraction and repulsion behaviours (e.g. Smith and Blumstein, 2010; Gabriel and Black, 2010; Dingemanse et al., 2004). In my opinion and following the principle of similarities with human psychology as described above, exploration can be regarded as a synonym for neophilia (as in Mettke-Hofmann et al., 2002).

In neophilia, the individual investigates the new object/environment by approaching it, in order to acquire sensory information about it. In neophobia, the individual ‘fears’ the new object/environment which is reflected by the individual avoiding proximity with it or seeking a refuge. Neophobia is therefore another name for risk-avoidance whereas neophilia appears to stem from a different process. Brown and Nemes (2008) argued that neophilia and neophobia should be considered as two orthogonal factors rather than the two extremes of a continuum. However, I would argue that this view confuses processes and outcomes. The behaviour that is classified as neophobic or neophilic is the product of both fear and propensity to investigate a new object/environment. An individual categorised as neophilic could be seen as lacking any fear towards a novel object or as displaying so much investigative behaviour that fear cannot be detected. Conversely, a ‘neophobic’ individual could be deprived of any investigative motivation or be fearful of the novel situation. In this view, neophobia and neophilia are the products of two different responses: fear and investigative motivation. Because of the composite nature of neophilia and neophobia (Dingemanse et al., 2004), and because it is difficult to isolate the fearful and investigative behaviour composing them experimentally, it makes sense to consider neophilia and neophobia as two extremes of the same continuum, along which the amount of fear and investigative behaviour may vary in an uncorrelated manner. In the present work, neophilia/neophobia will refer to the behaviour (attraction/repulsion) observed in response to novel (non-predatory) stimuli (Müller et al., 2010).

As stressed by Réale et al. (2007), greater care should be taken when designing experiments assessing ‘boldness’ (i.e. predation-risk-taking, exploration or neophilia-neophobia). The conclusion that the measured behaviours are correlated might be due to the fact that the same behaviour was measured in different ways and interpreted wrongly as representing different traits, or that the measure used might reflect another behavioural feature such as general locomotor activity (Brown and Nemes, 2008).

Now that the terms to be used have been defined, I can discuss in the following two chapters the controversies arising from applying human terminology and concepts to non-human animals, as well as provide an overview of the current research in the evolution and maintenance of personalities and a brief introduction to task specialisation in eusocial insects and its relationship to personality. These chapters shall highlight the main issues surrounding the study of animal personality in bumblebees and will introduce the empirical part of this thesis. The empirical work will be concerned with three main questions: (1) do individual bees vary consistently in abilities? (2) Do individual bees vary consistently in their behaviour (i.e. do bees have personalities)? And (3) Is there a relationship between consistent individual variation in ability and behaviour? The findings from this work will then be drawn together to examine their potential implications for the study of personality in social insects.

Chapter 2: Controversy, challenges and evolution

As highlighted in the previous chapter, the study of animal personality poses many conceptual and experimental challenges. Extra care must therefore be taken in defining the terms used and in designing the experiments in order to answer the question asked. In addition to these ‘practical’ challenges, biologists studying animal personality must take great care when using terms and paradigms usually applied to humans, as highlighted in this chapter.

2.1 Can animal personality be compared to human personality?

Most dog or horse owners would not feel uncomfortable with the use of the word ‘personality’ to describe the behaviour of these animals. However, many psychologists are still reluctant (if not averse) to use it for non-human animals, especially if the animals concerned are not mammals. This reluctance can be partly accounted for by the fact that definitions of personality in human-psychology include terms like ‘thoughts’, ‘emotions’, ‘beliefs’, ‘motives’, ‘intentions’ and ‘expectations’ (Funder, 2001; Anestis, 2005; Cervone, 2005), which can only be assessed indirectly in non-human animals (emotions are frequently assessed through self-reports in psychology; Alvergne et al., 2010; Nettle, 2006a). Indeed, the attribution of emotions and thoughts to non-human animal is a much debated topic and would only fuel controversy in the study of animal personality as well as claims of anthropomorphism. Many animal personality researchers distance themselves from making any comparison with human personality. For example Dingemanse et al. (2009) stresses that “The term ‘animal personality’ […] does not imply a link with human personality”.

Nevertheless, there have been ingressions of biological approaches into domains traditionally reserved to human personality and vice-versa and psychologists and biologists appear to converge on some aspects of human/animal personality. For example, biological methods were applied to the study of human personality (e.g. Chapple, 1940 and Nettle, 2006b) and psychological methods to the study of animal personality (e.g. Capitanio, 1999). A major problem with many psychology studies of human personality is the subjectivity of the measurements obtained (Cavigelli, 2005; Funder, 2001). Indeed, the subjects of the study often rate themselves through questionnaires. The use of biological methods to assess personality could help to make the measurements more objective (as done by Chapple, 1940) and Funder (2001) stresses that there is a need for greater control over experimental conditions in human personality studies as well as for more descriptive studies. Reciprocally, the use of more ‘psychological’ methods to assess personality in animals would be helpful to compare human and non-human personality (see Capitanio, 1999).

There are various examples of convergence between biological and psychological approaches. On conceptual grounds, human personality definitions frequently emphasise the importance of individual differences and consistency: Cervone (2005) states that “a basic goal for a personality theory is to explain […] enduring tendencies in experience and action” and Funder (2001) refers to individual-specific “patterns” and “stable and distinctive qualities”. Both non-human and human personality research strives to describe and explain personality (Funder, 2001) although human research for fitness-related traits is hampered by the ethical concerns with experimentation (methods to estimate the impact of various factors on fitness are more limited than in non-humans as experimentation is prohibited) and by the possibility that the post-demographic transition environments (i.e.

the western culture where the vast majority of studies have been conducted; see Alvergne et al., 2010) no longer reflects the selection pressures which gave rise to human personality (Alvergne et al., 2010).

In some cases, the findings of personality studies in both domains are comparable. For example, the repeatability of personality traits is similar between human and non-human studies (correlation coefficients of 0.4 to 0.6 for both, see Nettle, 2006a; Funder, 2001; Cavigelli, 2005; Bell et al., 2009) and the personality traits studied in animals are often strikingly reminiscent of the ones found in humans (e.g. Careau et al., 2008 and Cavigelli, 2005).

Funder (2001) highlights that a great proportion of personality psychology is concerned with pathological psychology. Though this is still a domain dominated by psychologists, biologists are encroaching on it with the development of animal models for psychological traits (e.g. vulnerability to stress; Koolhaas, 1999).

The discovery of convergences or synapomorphies between human and non-human animals is very valuable, but to ensure that the field of animal personality retains its credibility, comparisons between human and non-human personality (and the use of common terminology) must be done with great care.

2.2 Evolution and maintenance of personality

Personality is defined as a consistent individual variation in behaviour across time and situations (see section 1.1). Behavioural consistency implies that individuals might be unable to adjust their behaviour to respond optimally to a particular situation. For example, a neophobic individual will avoid proximity with new objects: such new objects might well turn out to be good hiding places for predators but they could also be valuable food sources. At first sight, behavioural consistency appears to lower an individual’s fitness by limiting its behavioural range. So how did the diversity of personalities evolve and how are these personalities maintained?

To account for the maintenance of the diversity of personalities, one can invoke the action of frequency-dependent selection (Hawk-Dove strategies, etc; Dall et al., 2004; Bergmüller and Taborsky, 2010; Wolf et al., 2008), differences in individual states (e.g.: hunger, reproductive status, age), spatial/temporal variation in the environment (e.g. predation levels: Réale and Festa-Bianchet, 2003; O' Steen et al., 2002; Mettke-Hofmann et al., 2002; Smith and Blumstein, 2010) or resource availability (Dingemanse et al., 2004; Boon et al., 2007), tradeoffs (e.g. Johnson and Sih, 2007), life-history (Wolf et al., 2007; Boon et al., 2008), multiplicity of fitness peaks on the fitness landscape (Dochtermann and Jenkins, 2007) and constraints (e.g. genetic and physiological; Sinn et al., 2008a; Bergmüller and Taborsky, 2010; Biro and Stamps, 2010; Müller and Chittka, 2008).

It is important to keep in mind that any behavioural trait can be both target and agent of selection (there is now ample evidence suggesting that personality traits influence an individual’s fitness in both human (Alvergne et al., 2010) and non-human animals (e.g. Boon et al., 2008; Réale et al., 2007; Sinn et al., 2008a; Logue et al., 2009; Dingemanse et al., 2004; Cavigelli, 2005) but also that any such trait might be adaptive or non-adaptive (e.g. due to pleiotropic selection; van Oers et al., 2011).

While studying the ultimate mechanisms underlying personality, the prevalence of proximate mechanism should not be underestimated. Some personality traits are known to have a heritable component (e.g. Dingemanse et al., 2002; Sinn et al., 2006; Drent and Van Noordwijk, 1997; Bolivar and Flaherty, 2004) but as Nettle (2006b) points out, "stable individual differences are by no means wholly attributable to genetic polymorphisms". Indeed, as for any phenotypical trait, the influence of the environment during key stages of an individual's development may have a deterministic impact on its behaviour (e.g. Capitanio, 1999; Kurvers et al., 2009; Roulin et al., 2010).

2.2.1 Maintenance of behavioural strategies in the honeybee

As discussed previously, one of the major challenges in research on the individuality of animals, from insects to humans, is understanding its adaptive significance (Raine et al., 2006a; Nettle, 2006a). How can multiple 'personalities' persist, side-by-side, in the same environment, when one might expect that one particular configuration of traits might outperform all others, and should therefore be favoured by selection? One possibility is that variation is selectively neutral (Raine et al., 2006a), but in many cases, spatial and temporal heterogeneity in the environment might play important roles in maintaining diversity (Nettle, 2006a). Chittka et al. (2003)'s study demonstrated a trade-off at the individual level between foraging accuracy (proportion of correct choices in a colour discrimination task) and foraging speed (interflower interval). Such a trade-off cannot be regarded as personality *sensu stricto:* individuals are consistent in both accuracy and speed across two situations (i.e. no reward vs punishment) but their consistency across time was not assessed (which is a prerequisite of personality see section 1.1) and these traits can be thought of as ability traits (see section 1.1). It is nonetheless worthwhile to examine it in the light of the maintenance of consistent individual variation in behaviour in a population.

In Chittka et al. (2003)'s experiment, the foraging accuracy and speed of honeybee workers (*Apis mellifera*) was assessed individually in two situations within a foraging context: in situation 1, unrewarding flowers contained only water whereas in situation 2, the unrewarding flowers contained a quinine hemisulphate salt solution that is aversive to bees (i.e. incorrect choices were punished). Individual bees fell along a continuum from slow-accurate to fast-inaccurate strategies.

Moreover, Burns and Dyer (2008) discovered in a separate experiment that fast-inaccurate bees would have collected slightly more nectar than slow-accurate bees when there was a low cost associated with making mistakes. Conversely, when the accumulating cost of mistakes was higher, slow-accurate bees would have clearly out-competed the fast-inaccurate bees (Fig. 2.1). Therefore, these findings support a differential advantage for each behavioural type: when discrimination is difficult (flowers were of similar colours in this experiment), but costs of errors are low, it is advantageous (or at least, not detrimental) to be fast and inaccurate. If such individual speed-accuracy strategies were found to be maintained over a longer time-span, then this would make of this speed-accuracy tradeoff true personality trait, with important repercussions for our understanding of the persistence of multiple personalities within a population.

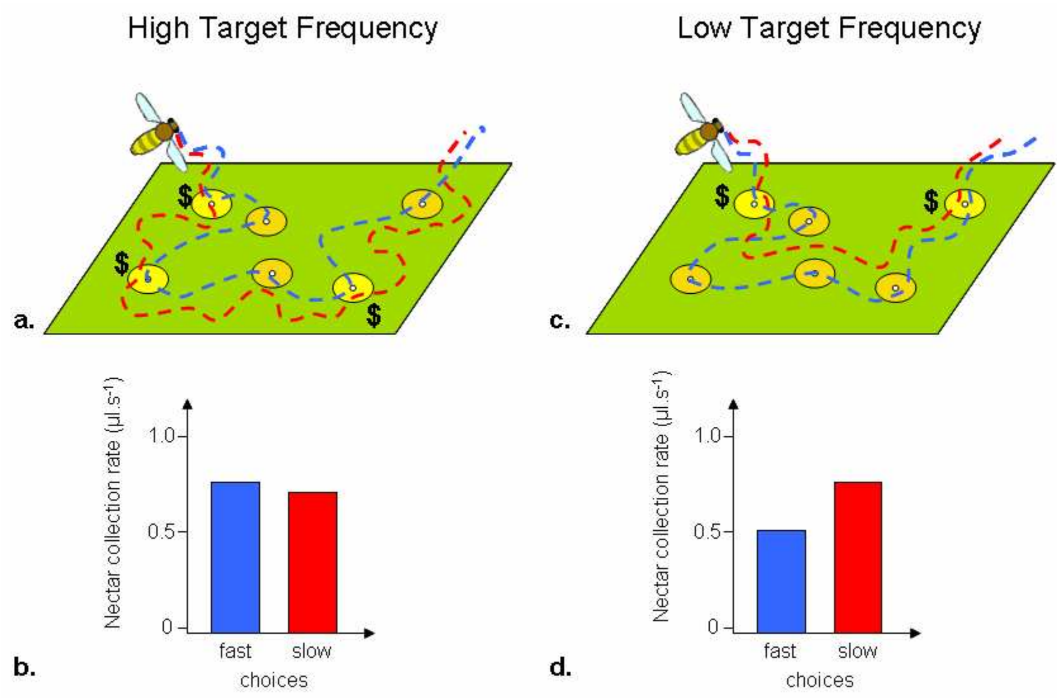


Figure 2.1. *Different strategies prevail in different foraging environments.*

Burns and Dyer (2008) tested individual foragers in artificial flower meadows where one 'species' of artificial flower contained sucrose rewards (indicated here by $ symbols) and the other, similarly coloured species did not. The two test situations differed in the percentage of unrewarding and rewarding flowers — in one condition there were equally as many (A,B), whereas in the other condition, there were twice as many unrewarding flowers as those that contained sugar solution (C,D). The colour red exemplifies the strategy of a 'careful', slow and accurate forager, and the colour blue corresponds to the performance of a fast, inaccurate 'impulsive' forager. In conditions with fewer unrewarding flowers (A, B), a slow and careful strategy does not pay off: the temporal costs of correct decisions are too high, whereas the temporal costs of erroneous probing of unrewarding flowers are low. Therefore, under such conditions the foraging rate of a careful forager does not exceed that of a 'sloppy' forager (B). Conversely, under conditions where rewarding flowers are scarcer, a careful strategy prevails (D).

Burns and Dyer (2008) propose that such intra-colony variability would be essential to colony survival in that it would enable the colony to respond flexibly to environmental variation. Indeed, the array of available flower species will vary with season and, within a flower species, the availability of nectar varies across time (Real, 1981; Seeley, 1994; Waddington, 2001). During the foraging season, or indeed in meadows simultaneously available within a hive’s flight range, a colony is likely to encounter conditions resulting in selection pressure maintaining the behavioural types.

Burns and Dyer’s (2008) findings open up several promising avenues for future research. For example, it would be interesting to investigate the proximate mechanisms resulting in the observed trade-off. Additionally, one could wonder if these interindividual differences in foraging speed and accuracy have repercussions for other aspects of the bee’s behaviour, such as predator avoidance (risk-aversion) or in response to novel flower stimuli? One could indeed conceive that slow-accurate bees might slow down even more in the presence of a predator (see chapter 6) or that they would not be attracted to potential new food sources as it might take them longer to learn to exploit them. This, however, remains to be tested empirically.

Chapter 3: Sociality and personality

The impact of personality on social behaviour is particularly salient in our species. In non-human species, personality has been shown to affect many aspects of an animal's behaviour and ecology, including its susceptibility to predation (Réale and Festa-Bianchet, 2003; Bell and Sih, 2007b) and competition for resources (e.g. mates or food; Dingemanse et al., 2004). Unsurprisingly, personality has also been found to impact non-human animals' social behaviour (Schuett et al., 2010; Kurvers et al., 2009) and use of social information (Kurvers et al., 2010). Studying the impact of personality in eusocial species (the term 'eusocial' describes a species in which individuals in a group show reproductive division of labour, overlapping generations and cooperative care of young (Wilson and Hölldobler, 2005) therefore appears very promising. The insect societies, e.g., ants, bees and termites, are arguably some of the most successful organisms across the globe. Their success has been attributed to labour division, specialization, and the resulting efficiency. Individuals of many insect colonies are indeed often highly specialized, so that animals will predominantly engage in colony defence, nursing larvae, removing debris, or foraging only for particular commodities but not other available ones. If personality was found to exist in social insects, then it would surely have an impact on task specialisation.

3.1 Task specialisation in social insects

Polyethism (individual specialisation in certain tasks within the colony) has been well researched: we know that individuals specialise in certain tasks because of the food they were given during their development (Wheeler, 1986) or because of their genetic (e.g. patrilines; Hughes et al., 2003) or physiological make-up (e.g. Robinson, 2009). However, there are still many points requiring investigation (Beshers and Fewell, 2001). With the exception of some extreme morphological castes such as egg-laying ‘queens’ or termite ‘soldiers’, individuals can often fulfil a variety of task within their caste. Specialists are often not distinct in morphology and indeed largely totipotent in terms of the tasks they can potentially perform. Indeed, even though social insect specialists might perform the same routine over and over for extended periods, with the same repetitiveness as assembly line workers, they can typically switch to other activities should these become necessary.

3.1.1 Task specialisation and efficiency

Surprisingly, there is relatively little quantitative research into the question of how specialization contributes to colony efficiency (see Jeanson et al., 2008; Trumbo et al., 1997; Langridge et al., 2008). The proverb that the ‘Jack-of-all-trades is an ace of none’ is perhaps so intuitively appealing that many scientists have not deemed empirical proof necessary. Past controversies from ecology (Waser et al., 1996) and psychology (Allport, 1980; Chittka and Thomson, 1997) however, indicate that the advantages of specialization can not be assumed a priori, and might depend fundamentally on the tasks involved, and their context. If task specialisation is associated to efficiency, one could expect the association to be based on the individuals’ variation in the ability to perform a given task (e.g. due to experience, learning ability or ability to discriminate between stimuli).

In what is perhaps the most comprehensive exploration of this question to date, (Dornhaus, 2008) marked 1142 *Temnothorax albipennis* ants from 11 colonies with paint dots (Fig. 3.1), so that she could identify individuals and measure their performance in four different tasks that became necessary as a result of various emergencies that required immediate attention.

Figure 3.1. *A laboratory colony of Temnothorax albipennis ants. While workers are not morphologically specialized for the various tasks required by the colony, individual paint marks reveal that many workers are specialists (repeatedly performing only certain activities) while others are generalists, often sequentially performing multiple tasks. This side-by-side existence of individuals that vary in their level of specialization makes it feasible to test whether specialists are more efficient than generalists. Photo by Anna Dornhaus, with permission.*

In the first treatment, the roof of the colony was removed, so forcing the entire colony to migrate to a new nest, and carrying the helpless brood along in the process. In the second treatment, ants were offered diluted honey and dead flies a small distance away from the colony after a period of starvation. Finally, the front wall of the ants' dwelling was removed, so that they had to scramble to obtain building material (small stones; Fig. 3.2).

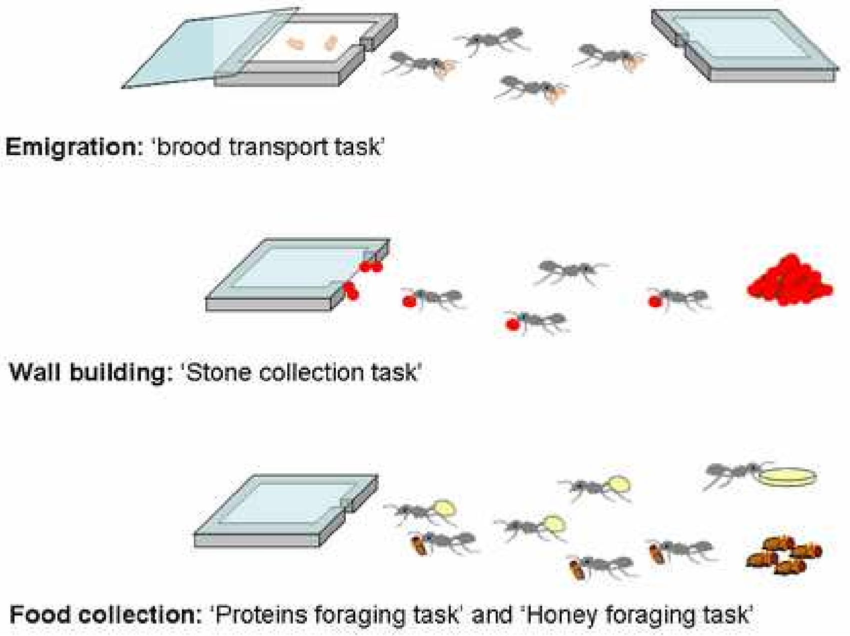


Figure 3.2. *Are multi-taskers less efficient than specialist ant workers? In a recent study testing the adaptiveness of task specialization in Temnothorax albipennis ants (Dornhaus, 2008), each colony was subjected to three treatments. (A) The glass cover of the artificial nest was removed, exposing the ants and brood. At the same time, another nest with glass cover was placed 10 cm away from the original nest. The workers had to move the brood from the unsuitable, uncovered, nest toward the new, covered, nest. (B) The front wall of the nest was removed and a pile of small stones was left at the ants' disposal in the foraging area. The workers could carry the stones to their nest entrance so as to build a wall with a smaller opening. (C) The colonies were starved for two weeks and then provided with diluted honey solution and dead Drosophila flies in the foraging area. There was no significant difference in performance between specialists (performing only one task) and generalists (performing two, three or even all four tasks), for any of the tasks tested.*

For each individual ant, performance was quantified by averaging the duration of the first two successive trips. Performance was evaluated as a function of that individual's degree of specialization (its propensity to focus activity on only one task, or two or more). Unexpectedly, specialists did not outperform generalists for any of the tasks. Also, an individual's readiness to engage in a task (as quantified by the time taken to first embark on it after the start of the experiment) did not consistently predict its performance. These results are provocative, and a healthy reminder that we should not assume that biological complexity is automatically adaptive in any situation. However, the specialization of workers in social insects must surely be adaptive in some situations, and studies as comprehensive as the one by Dornhaus should be performed to identify these conditions. Perhaps the emergency situations that were in force in this study meant that as many individuals as possible (independently of previous specialization, experience and efficiency) needed to engage, resulting in recruitment of many suboptimal performers into a task they would not otherwise perform. It might also be informative to test colonies in emergency-free situations, where they are given a choice between multiple activities that can be performed concurrently. Much research on social insect specialization has been concerned with the stimuli by which workers identify the need for a task to be performed, and the sensory thresholds at which individuals respond to these stimuli (Gordon, 1996; Beshers and Fewell, 2001; Spaethe et al., 2007; Waibel et al., 2006). Ideally, the readiness with which an individual engages in a task should correspond to its innate ability (or 'talent') at performing the task (Trumbo et al., 1997). Although such a correlation has been found in some tasks (Trumbo et al., 1997), the results of Dornhaus' (2008) study show that it should not be assumed to be general. However, a correlation between response thresholds and efficiency might be generated over an individual's lifetime, since the thresholds themselves might become gradually lower with experience (Weidenmüller, 2004), but also because, as a result of a lower threshold, an animal might perform the task more often, allowing it to polish its skills over time.

3.1.2 Task specialisation and learning

As for personality, some decisive factors that might generate task specialization could relate to learning and memory (see chapter 4). In Dornhaus' (2008) study, 'all hands on deck' were needed; thus individuals might have had little chance to familiarize themselves with a task and improve performance over time. In more day-to-day situations, experience might often be the single best predictor of performance. Learning has been shown to play a fundamental role in efficiency of many everyday tasks in social insects' lives, including food handling techniques (where performance can improve with experience by an order of magnitude (Laverty and Plowright, 1988; Raine and Chittka, 2007; Chittka and Thomson, 1997), information about the locations and identification of food sources (Srinivasan, 2006; Collett and Zeil, 1997; Saleh and Chittka, 2007; Menzel and Giurfa, 2001; Chittka and Raine, 2006), nest repair (Downing, 1992), nestmate recognition (Sheehan and Tibbetts, 2008; Châline et al., 2005) comb building (Oelsen and Rademacher, 1979), strategies in handling prey (Corbara and Dejean, 2000) and nest climate control (Weidenmüller, 2004); but not, for example, in corpse removal in honeybee colonies (Trumbo et al., 1997). For complex tasks such as natural foraging at long distances from the nest, efficiency can increase with experience over a substantial portion of an insect's lifetime (Dukas, 1995; Peat and Goulson, 2005). Just like in human labourers, there can be substantial interference if insects switch from one task to another (Chittka and Thomson, 1997; Dukas, 1995)—in one study on butterflies, feeding on a new plant species resulted in almost complete forgetting of the handling procedures for a previously visited flower species (Lewis, 1986).

In other studies, individuals seemed comfortable in juggling two tasks (Weiss and Papaj, 2003). If interference occurs, then the very mechanisms that make it preferable for an individual to work efficiently may lead to a certain inertia in switching tasks (Chittka et al., 1999). In some cases, the transition from one task to another may be orchestrated by fundamental alterations in brain structure, neuronal wiring pattern and protein synthesis, in part to generate the hardware to facilitate learning activities that come with the new tasks, but the changes can also be directly induced by new experience (Gronenberg et al., 1996; Fahrbach et al., 2003; Robinson et al., 2008; Krofczik et al., 2008). These changes have been examined primarily in the mushroom bodies of honeybees at the major transition from within-nest activities to foraging, and there might be less pronounced alterations of circuitry when switching between activities which do not involve a near-complete change of life-style. Nonetheless, this research suggests that costs of task switching can extend substantially beyond those of temporal inefficiency at a new task. Transfer is likewise important—in some cases, there might be similarities in two tasks that facilitate performance on a new job (Chittka and Thomson, 1997; Dukas, 1995). For example, in Dornhaus' (2008) study, all tasks involved locomotion, orientation within the (presumably familiar) surroundings of the nest, and three of four tasks involved carrying items with the mandibles— thus skills at these tasks would have been largely transferable, whereas more specialized activities (such as wall-building Aleksiev et al., 2007) or handling live prey (Corbara and Dejean, 2000) might involve learning (because the precise nature of the substrate is not predictable on an evolutionary scale), but skills obtained at either of these activities might not be transferable to the respective other one. The extent to which transfer and interference exist for many of the within-nest tasks of social insect colonies remains to be shown on a case-by-case basis, but they could be more important in determining an individual's efficiency at any given task than the response threshold that causes it to engage with the task in the first place. The reason is that almost any motor task, however simple, will require some fine-tuning, i.e., adjusting actions to desired outcomes, even if it is based on genetically pre-programmed templates (Laverty and Plowright, 1988; Wolf et al., 1992). Even in basic locomotion, ‘robotic’, fully hard wired motor routines would fail when load is redistributed along the body (such as when a prey item is carried) or when alterations occur to body structure (such as in insect flight when asymmetric wing wear occurs with ageing; Higginson and Barnard, 2004).

Over a social insect’s lifetime, it might come into contact with a large variety of tasks, and have a go at several of them. What are the feedback loops that ensure that individuals perform the tasks that they are good at? In humans, there is self-assessment (as well as feedback from others) of talent and the steepness of the learning curve. In insects, there is likely no feedback from others, but Ravary et al. (2007) showed that individual experience in deciding in which task an individual specializes might be decisive: previously naïve *Cerapachys biroi* ants repeatedly explored their environment for food—only for some individuals, the experimenters had made sure they never found any. Such ants gradually decreased their efforts, and in the end, stayed mostly in the confines of the nest and became specialist brood carers, whereas their more successful relatives happily continued to forage in the outside world (Fig. 3.3). In this case, the experience of success and failure determined specialization. Along with the influence of individual experience, other factors such as wing-wear (Foster and Cartar, 2010) or mandible wear (Schofield et al., 2011) are known to affect individuals’ efficiency and task specialisation in social insects. I therefore suspect that the biggest missing piece of the puzzle in our understanding of labour division in animal societies relates to the extent that individual experience and abilities contribute to efficiency.

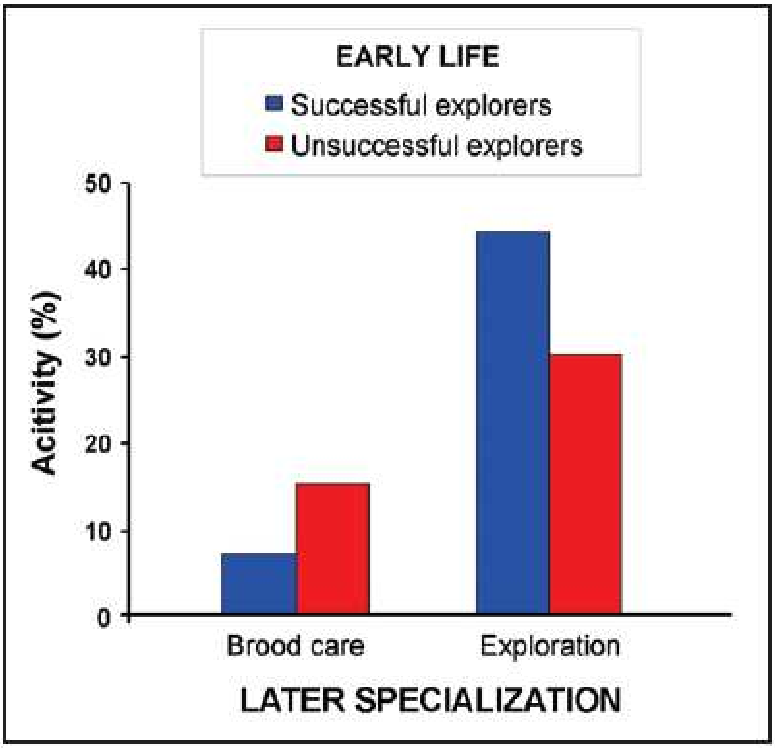


Figure 3.3. *Early success or failure determines task specialization in Cerapachys biroi ants (Ravary et al., 2007). Age matched cohorts of ants with very low genetic diversity were subdivided into two groups that differed in terms of their foraging success in early adult life. One group was regularly rewarded when exploring the nest's surrounding, whereas the other never found any food. Weeks later, individuals that had been successful explorers in early life showed a much higher propensity to continue exploring, whereas their unsuccessful sisters showed a stronger tendency to care for brood inside the nest. Data approximate; redrawn from (Ravary et al., 2007).*

3.2 Task specialisation and personality

Task specialisation and personality are conceptually very similar. Task specialisation implies that each worker restricts its behaviour range to a subset of the complete repertoire of tasks available and that this subset varies across individual workers in the colony (Beshers and Fewell, 2001). Personality equally implies restriction in the behavioural repertoire as well as inter-individual variation. Task specialization even appears to fulfil the strict requirement for individual consistency to qualify as 'personality' (and it sometimes regarded as such, e.g. Mather and Logue, in press), as task specialization tends to last for extended periods of the individual’s life. However, where a personality might comprise several personality traits (e.g. a predation-risk-prone, neophilic and aggressive individual), an individual specialized in a particular task will often be restricted to a single situation/context (e.g. nest defence) and therefore exhibit mainly one personality trait (say aggressiveness). Task specialization therefore restricts the behaviour of an individual to a small number of specific situations/contexts. Whereas restriction in range of behaviour in the definition of personality refers to the full set of behaviours *within* each situation, in task specialization it is meant at another level: the behaviour repertoire of individuals is restricted *across* situations/contexts. Put another way, the individual is less likely to express behaviours outside of the situations/context(s) in which it specialises. To illustrate this, one can consider the following example: a particular squirrel has been repeatedly observed foraging in the vicinity of potential predators and is frequently found roaming outside its main territory. Compared to other squirrels, this particular individual would be described as ‘predation-risk-prone’ and ‘exploratory’. Now if we take the case of a particular ant in a colony, one might also say that she is ‘predation-risk-prone’ (foraging incurs the risk of encountering potential predators) and ‘exploratory’ (leaving the nest) compared to other workers (nurses, guards, etc). However, some ants (e.g. nest-guards) might never leave the nest due to the task they perform and so their risk-proneness or exploratory tendencies could never be assessed. Do we then have to conclude that individuals in a colony show only ‘partial’ personalities? Not necessarily. It could simply be that one particular ant became a forager because her personality predisposed her to. By analogy, humans tend to take employment that reflects some aspects of our personality, but one’s job does not by any means represent one’s personality as a whole. The personality of an individual in a social insect colony would therefore determine the cue-threshold required for performing the task and the likelihood of performing it (Weidenmüller, 2004; Beshers and Fewell, 2001).

3.3 Could bees have personalities?

Individuality in behaviour has been documented in invertebrates and as far as eusocial invertebrates are concerned, bumblebees are also known to show consistent specialisation in foraging (Chittka et al., 1999; Heinrich, 1979) and honeybees vary individually in their foraging efficiency (amount of forage brought back to the hive and duration of foraging trips; Dukas, 1994).

Bees are a model of choice for cognition and behaviour experiments in invertebrates: bees are capable of learning complex stimuli requiring various learning mechanisms (see Giurfa, 2007 for examples). Past research has also shed light on the genetic architecture and physiology underlying individual differences (Page and Scheiner, 2006). The idea that bumblebees (and more specifically, *Bombus terrestris*) could show personalities appears realistic if one considers that, contrary to other social insects, the workers of this species are totipotent: they are able to switch between tasks in response to colony requirements (Weidenmüller, 2004; Cartar, 1992), there are only two castes (reproductives and non-reproductives, i.e. queens, males and workers), there is no strong evidence for age polyethism in this species (Jansen et al., 2009) and different patrilines cannot account for the task specialisation observed (*Bombus terrestris* queens are singly mated; Schmid-Hempel and Schmid-Hempel, 2000). Moreover, there are reports of individualised consistent behaviour as well as individual differences in terms of cognitive abilities in bumblebees. In fact, individual bumblebees are known to use traplining (e.g. individual bees follow particular, repeatable circuits when visiting flowers; Thomson and Chittka, 2001) and to show flower constancy (foraging on a preferred flower species; Heinrich, 1976; Heinrich, 1979). In terms of cognitive abilities (Chittka et al., 2003) showed that individuals differ in the time they allocate to make choices (some bees are ‘fast and sloppy’ while others are ‘slow and accurate’; Chittka et al., 2003). Could traits identified as part of personalities in other non-pollinator species be found in bumblebees? For example, could individual bumblebees be described as predation-risk-prone or neophobic? Are some bees more fearful than others? Do bees differ in their ability to solve cognitive tasks? These are the questions the present work aims to answer.

In studying individual behaviour - and potentially - personality in a social insect, one might advance our understanding of the way a colony of social insect functions, especially in the field of task division. Colonies of social insects are commonly considered as ‘super-organisms’ in which sub-groups of individuals perform complementary tasks. Often, individuals specialise in a certain task, sometimes for their entire life. Are individuals pre-programmed to be flexible only over a limited range of conditions or are they originally totipotent and then later in their development acquire preferences for certain tasks?

Chapter 4: Consistent inter-individual differences in discrimination performance by bumblebees in colour, shape and odour learning tasks

Learning can be defined as a modification of behaviour based on experience (see Lachman, 1997 for a full discussion of the definition of learning) and it is thought to enable an organism to modify its phenotype rapidly in response to shifting environmental conditions (Raine and Chittka, 2009; Johnston, 1982). Most animals, from the roundworm *Caenorhabditis elegans* (Rankin et al., 1990) to humans, are capable of some form of learning. The influence of learning on an organism's behaviour is ubiquitous and undeniable: even innate behaviours can be modulated by learned components (e.g. bees are innately attracted to blue flowers but they can learn to prefer other colours or even avoid blue flowers altogether; Giurfa, 2007; Ings et al., 2009).

4.1 Learning and personality

Given the importance of learning for the survival and reproduction of an individual, it is surprising that so few studies have investigated the potential links between personality and learning (e.g. Boogert et al., 2006; Benus et al., 1987) showed that there is a link between learning ability and aggressivity in mice and rats). It is likely that the learning type of an individual will have an impact on its personality type and vice-versa. For example, neophilic individuals are more likely to encounter new stimuli and therefore learn from them (Sinn et al., 2008b). Similarly, risk-taking individuals would be more exposed to predator cues and therefore would be more likely to learn to avoid predator efficiently.

4.2 General introduction to learning in bees

Bees are proficient learners: in order to extract nectar and pollen from their environment, bees may learn to associate landmarks, colours, odours, shapes, textures, patterns, orientation (Worden et al., 2005; Colborn et al., 1999) and even temperature (Whitney et al., 2008) with rewarding flower species (and with their nest location) and they may also learn to handle each flower species efficiently (Woodward and Laverty, 1992). Bee foragers are thought to spend a substantial amount of time and energy learning, which enable them to achieve increasing rates of food intake with experience (Ohashi et al., 2008; Dukas, 1994). Whereas learning is known to provide many benefits to organisms such as increased efficiency and behavioural plasticity, learning might also entail significant costs. For example, naïve individuals may suffer an initial lack of efficiency, greater predation risks (Dukas, 1994; Dukas, 1995; Johnston, 1982) and the risk to develop maladaptive instead of adaptive behaviour patterns (Johnston, 1982). Even experienced individuals may face costs, such as potential costs related to the maintenance and operation of the nervous system (Raine and Chittka, 2009) as well as developmental (Mery and Kawecki, 2003), reproductive (Mery and Kawecki, 2004) and ecological trade-offs (Araujo, 2007).

There is evidence that, in bees, variations in learning abilities can impact fitness e.g.: Dukas and Bernays, 2000; Ohashi et al., 2008; Raine and Chittka, 2008; Snell-Rood et al., 2011) and studies in other groups of animals showed that individuals of a species may vary greatly in their learning abilities (e.g. Dukas, 2008; Kolata et al., 2005; Kotrschal and Taborsky, 2010).

In the ant *Cerapachys biroi* (Ravary et al., 2007), differences in individual experience generated division of labour (i.e. individuals which found food while foraging remained foragers whereas unsuccessful individuals specialised in other tasks). If learning is found to play a role in division of labour for other tasks and social species, it is potentially influencing what is thought to be a pillar of social insects' success (Hölldobler and Wilson, 1990).

In honeybees, Page and Scheiner (2006) reported another individual basis for task specialisation: individual difference in sensitivity to sucrose concentration. They found that a bee's sensitivity to sucrose is not only likely to influence the nature of the tasks a bee will perform (i.e. pollen or nectar foraging; Page and Scheiner, 2006), but also it significantly correlated to her learning performance (learning speed and highest learning asymptote) during an olfactory task. This and the fact that there are anecdotal reports of individual bees failing to learn in experimental settings (e.g. individuals which were excluded from an experiment as they did not reach a learning criteria; (e.g. Worden et al., 2005) suggests that, as in other animal groups, bees may vary individually in their ability to learn. In honeybee, this individual variation might itself vary across time as it is modulated by the individual's caste (Ben-Shahar et al., 2000).

Additionally, it is known (Page and Scheiner, 2006) that sensory thresholds are correlated with each other across modalities (gustatory, olfactory and visual) but it is not known whether an individual's performance at one learning task can predict its performance at another learning task. Most of the previous observations highlighting variation within bee species involved specialised groups of bees (e.g. pollen versus nectar foragers or especially established breeding lines; Chandra et al., 2000; Page and Scheiner, 2006; Brandes et al., 1988; Brandes, 1991). Learning was quantified as product of group behaviour: individual learning performance was not typically quantified and individual consistency across task was not investigated.

4.3 Experiment

4.3.1 Introduction

For bumblebees, one of the model organisms in the study of the evolutionary ecology of learning, inter-individual differences in learning performance have been measured in a variety of tasks (Chittka and Thomson, 1997; Chittka et al., 2003), but it is not known if the learning performances are correlated with each other across modalities. Often (but see Chittka et al., 2003), individual learning performance at task 1 was used to select individuals participating in task 2, so potential correlations between individual performances at both tasks were not investigated (Worden et al., 2005). Raine and Chittka (2008) found that learning speed in a colour discrimination task correlated with fitness as assessed by foraging performance in the wild. To explore the generality of this correlation, it is essential to demonstrate that better learners are not limited to superior performance in a single task, but that performance is individually consistent across tasks. The ability to learn to discriminate between two stimuli within a particular dimension/modality might reflect a more general ability to learn (Boogert et al., 2011; Sih and Bell, 2008), independently of the dimension or modality considered. Alternatively, it is conceivable that there are tradeoffs between performance levels across tasks, so that superior performance in one task comes at the expense of poor performance in another (Mery et al., 2007; Papaj and Snell-Rood, 2007; Sih and Bell, 2008; Worden et al., 2005).

This study therefore aimed at exploring whether individual discriminatory abilities are consistent across and within sensory modalities. To achieve this, I used simple differential conditioning based on a foraging paradigm in which I could vary the type of cues which the bees had to learn. I tested each individual bee on her ability to differentiate between pairs of stimuli belonging to a given modality (visual or olfactory) or dimension (shape or colour).

4.3.2 Material and Methods

Two colonies of *Bombus terrestris* containing approximately 40 workers each were obtained from Syngenta Bioline (Weert, Netherlands). Queens of this species mate only once in their life; therefore all the foragers within a colony are full sisters (Schmid-Hempel and Schmid-Hempel, 2000). Upon delivery, colonies were transferred into bipartite wooden nest boxes (28×16×11 cm). All tested bees were individually tagged with coloured dots or Opalith number tags (Christian Graze KG, Germany). Whenever new workers emerged from the pupae, identification tags were applied within 48 hours, enabling us to determine their age at the time of experiment (age available for 7 bees from colony A and 11 bees from colony B).

The nest box in which the colony was housed was connected through a plastic tube to a foraging flight arena (120×100×35 cm) covered with a UV-transparent Plexiglas lid (Fig. 4.1a). Bees could be allowed one at a time into this arena using a system of shutters built in the connecting tube. The room in which the colonies were kept had an average ambient temperature of 21ºC. Controlled illumination for laboratory experiments was provided by high-frequency fluorescent lighting (TMS 24F lamps with 4.3 kHz ballasts; Philips, The Netherlands) fitted with Activa daylight tubes (Osram, Germany) to simulate natural daylight, and equipped with special ballasts for high frequency lighting above the bee flicker fusion frequency (Dyer and Chittka, 2004). Foragers were initially allowed to collect 50% (w/w) sucrose solution from translucent gravity feeders (Von Frisch, 1967, p19, Fig. 18) which provided unlimited supplies for two days.

Thereafter, sucrose solution was provided through ‘nectar pumps’ connected via flexible plastic tubing to artificial flowers. Each ‘flower’ consisted of a cut-out shape of laminated coloured paper (‘corolla’) mounted on a grey plastic cylinder (height: 6cm, diameter: 3.1cm). The ‘corolla’ part of the flower was pierced in its centre to allow space for a small plastic cup containing the sucrose. The cup was embedded into the plastic cylinder so that the amount of nectar available in it could not be seen by the bee. The cup was connected to a pump (Fig. 4.1b) delivering 40% sucrose (w/w) through the tubing at a rate of 0.363ml.h^{-1}. The ‘corolla’ part of each flower was cleaned with 70% ethanol between foraging bouts so as to ensure there were no scent marks left from previous visits by the bee (Saleh et al., 2007). The position of the flowers on the arena was shuffled between foraging bouts using computer-generated random spatial patterns on a 6x5 square grid (with 14 cm between positions). This shuffling of the position of the flowers was necessary to prevent the bees from associating location with reward.

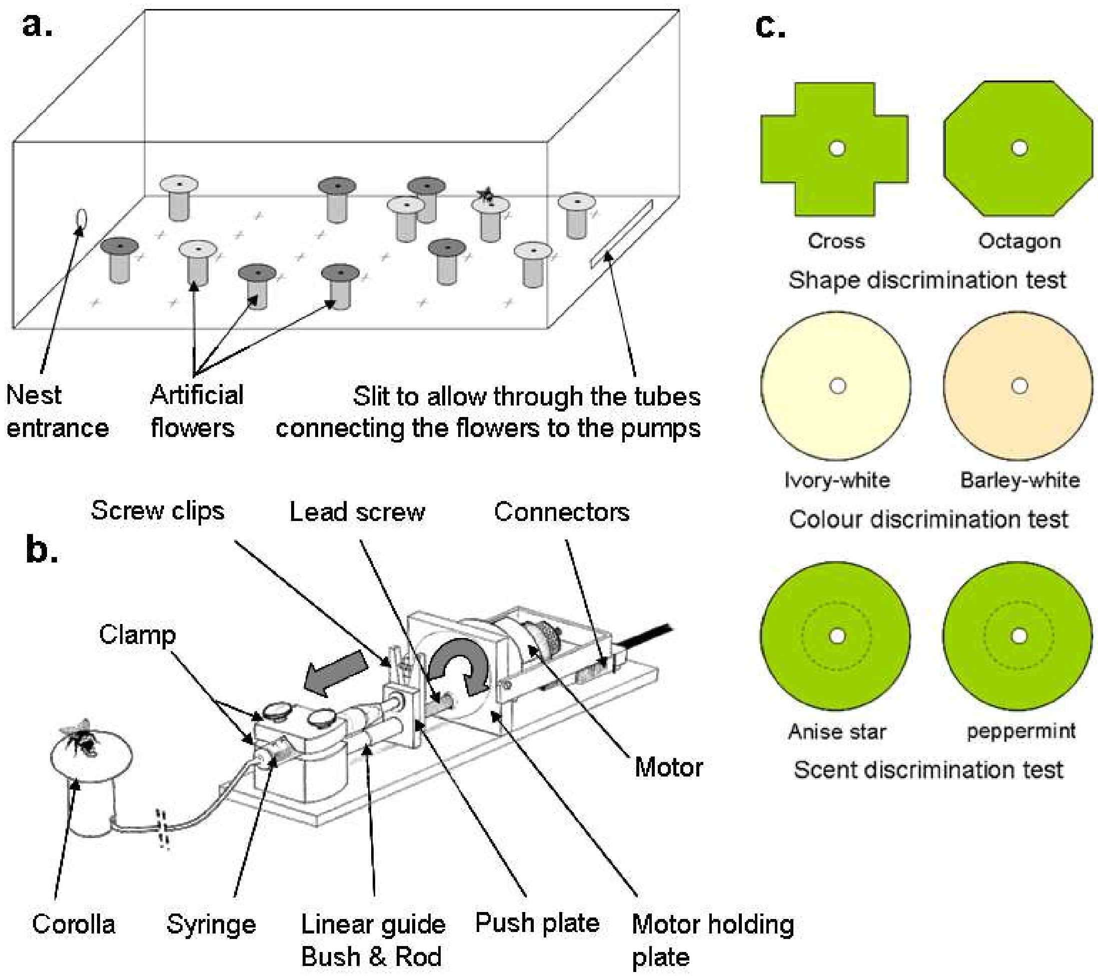


Figure. 4.1 *Experimental set-up (a.) and nectar-pump (b.) amd stimuli (c.). The bees were released one at a time in the arena containing the artificial flowers distributed in a random pattern. The sides of the arena were made of wood whereas the top lid was UV-transmitting Plexiglas. The position of the flowers was changed after every foraging bout. Each 'flower' was connected to a nectar-pump (Leadbeater and Chittka, 2008). A pump consisted of a glass syringe the plunger of which was connected to a lead screw. This screw was slowly rotated by a mains-operated motor (500rpm with gearbox set for 1rev/12hours; RS, London, UK), causing the plunger to continuously squeeze minute amounts of 40% (w/w) sucrose solution out of the syringe into the tubing and out into the cup of the flower. c. shows the different pairs of stimuli used (the dashed circle in scent stimuli represents the limits of the inner disc where the paper was left uncovered to absorb the liquid scent solution). Drawing of the pump by Sarah Blackburn.*

Bees were pre-trained collectively to obtain sucrose from the artificial flowers without any paper ‘corolla’. The grey cylinders supporting the cup and tubing were achromatic and pre-training to achromatic stimuli has been shown not to affect colour preference during subsequent exposures to coloured stimuli (Giurfa et al., 1995; Raine et al., 2006a; Raine et al., 2006b). A focal bee was then allowed alone into the arena with ‘corolla’-less flowers for one foraging bout prior to the experiment itself.

Each focal bee was subjected to three tasks, after which she was removed from the colony. Two tasks were visual (learning to differentiate between colour and shape respectively) and one task was olfactory. During the ‘shape’ task, individual bees were presented with six cross-shaped flowers (4x4cm; 4 arms, each 2cm wide) and six octagon-shaped flowers (4 cm across, sides of 1.7 cm). Both shapes of ‘corolla’ were cut from green-coloured paper (khaki, Maya coloured card; Clairefontaine, Ottmarsheim, France; Fig. 4.1c) and were covered with transparent plastic film (Frisk Coverseal Film Rolls; Artcoe, Manchester, UK) for easy cleaning. Each of the octagon-shaped flowers was linked to a sucrose pump which delivered 40% sucrose (reward) whereas the cross-shaped flowers contained saturated quinine hemisulfate salt (Sigma, UK) solution (punishment). In the ‘colour’ task, bees were presented with 12 flowers bearing ‘corolla’ (paper discs: diameter: 5cm) of two different colours: six were ivory-white and six were barley-white (Daler Canford Card; Daler-Rowney, Bracknel, England; Fig. 4.1c), covered in transparent film. In the olfactory discrimination task, the ‘corolla’ of the flowers consisted of discs (diameter: 5cm) of green coloured paper, covered in transparent film except for a small central area (disc diameter: 1.8cm), where the paper was left uncovered so as to enable absorption of the scented solution. Five microlitres of scented solution (333 µL/L solution of peppermint or anise star essential oil; Essential Oils Direct, Oldham, UK; Fig. 4.1c) were deposited on the paper surface of each flower before each foraging bout. There were six anise-star-scented flowers, connected to the nectar-pumps and six peppermint-scented flowers, containing quinine solution. The flowers containing quinine were not linked to pumps. Unconnected tubing was used to mimic the appearance of the rewarding flowers. I varied the order in which the bees were subjected to the tasks to control for order effects (colony A contributed 21 bees in total and colony B contributed 17 bees in total, order1: shape-colour-scent $N_{colonyA}$= 11 and $N_{colonyB}$= 10; order2: colour-scent-shape $N_{colonyA}$= 5 and Nc_{olonyB}= 3; order3: scent-shape-colour $N_{colonyA}$= 5 and $N_{colonyB}$= 4).

Each task presented the bee with two different artificial flower types. For each individual bee, I recorded the first 50 choices, a ‘choice’ being defined as a landing on a flower. Landing on the rewarded stimulus (sucrose solution) was considered as a correct choice whereas landing on the stimulus associated with punishment (quinine solution) was considered as incorrect. Individual bees’ performance was measured in the saturation phase of their learning curve (choices 31-50). Visual inspection of the bees’ learning curves revealed that at this stage, performance improved no further (see Fig. 4.2).

I measured the maximum thorax width three times per individual bee and took the average as an estimate of the bee’s size (thorax width is the most common measure of body size for bumblebees; Spaethe and Weidenmüller, 2002; Spaethe and Chittka, 2003). This information is essential since sensory performance in some tasks can be correlated with body size; for example the visual-spatial resolution of the eye is superior in larger workers (Spaethe and Chittka, 2003).

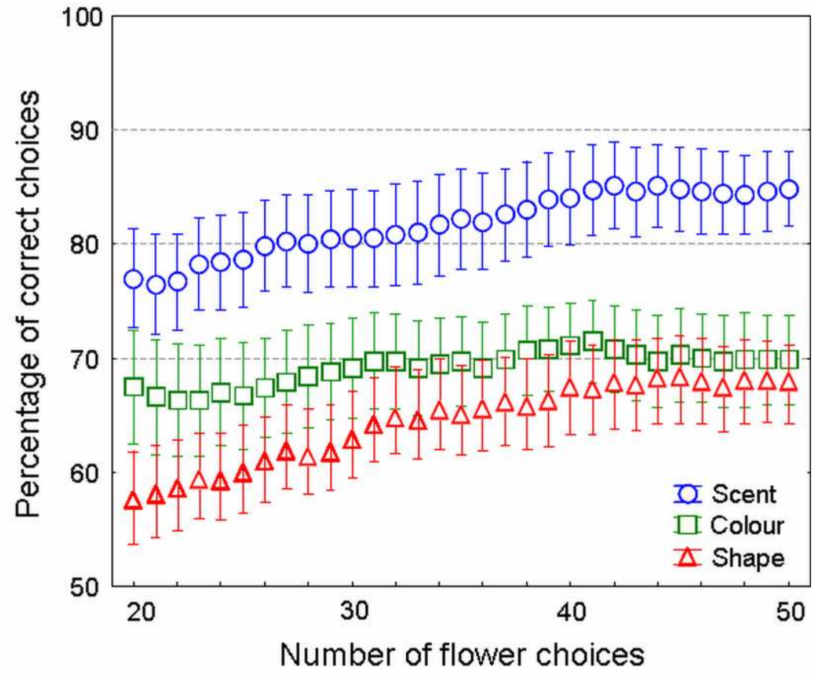


Figure. 4.2. *Learning curves for each task. Each point is the mean ± SE for all bees for the preceding 20 choices.*

Data Analysis

I used Kendall’s W (also known as Kendall’s coefficient of concordance) to assess the consistency of the bee’s performance across all tasks. Kendall’s W quantifies the agreement between ranks of two or more variables and has been used to assess the consistency of behavioural traits (e.g. Briffa et al., 2008). Kendall's W ranges from 0 (no agreement) to 1 (complete agreement).

In order to test for the effect of various factors (the nature of the task, the order of the task and the colony from which the bees originated), a Repeated Measures General Linear Model (SPSS 16.0) was built, using the number of correct choices out of 20 for choices 31-50. All tests were two-tailed and the alpha level was set to 0.05. I used task as within-subject factor with three levels, corresponding to the three tasks (shape, colour and scent discrimination). I included two between-subjects factors: colony and the order in which the tasks were performed in a full-factorial model.

Initially, age and size were also included in the model as covariates. However, neither covariates had a significant effect (size: $F_1 = 0.620$, $P=0.451$; age: $F_1 = 1.099$, $P = 0.322$) which is consistent with findings by Raine et al. (2006b). The number of bees for which age and size were available limited the total number of bees included in the model to 17. Therefore the covariates were removed in the final analysis (increasing our effective sample size to N = 40). A potential correlation between size and discrimination performances was investigated using Spearman's rank correlation coefficient.

4.3.3 Results

Individual scores for discrimination performance across tasks were significantly concordant (Kendall's $W_{38}= 0.530$, $P < 0.0001$, see Fig. 4.3), meaning that the rank of a given bee for one task was consistent with her rank for the two other tasks. There was a significant within-subjects effect of task ($F_{2, 64}= 32.89$, $P < 0.001$), demonstrating that tasks differed significantly from one another. There was a significant between-subjects effect of colony ($F_{1, 32}= 9.00$, $P = 0.005$), suggesting that the two colonies differed in their mean for discrimination performance. There was no significant between-subject effect of task order ($F_{1, 32}= 1.88$, $P = 0.16$) and no significant interaction between colony and task order ($F_{2, 32}= 0.92$, $P = 0.406$). There were no significant correlations between size and the discrimination performance for any task (Spearman correlation coefficients: all $|r| < 0.27$ all $P > 0.14$).

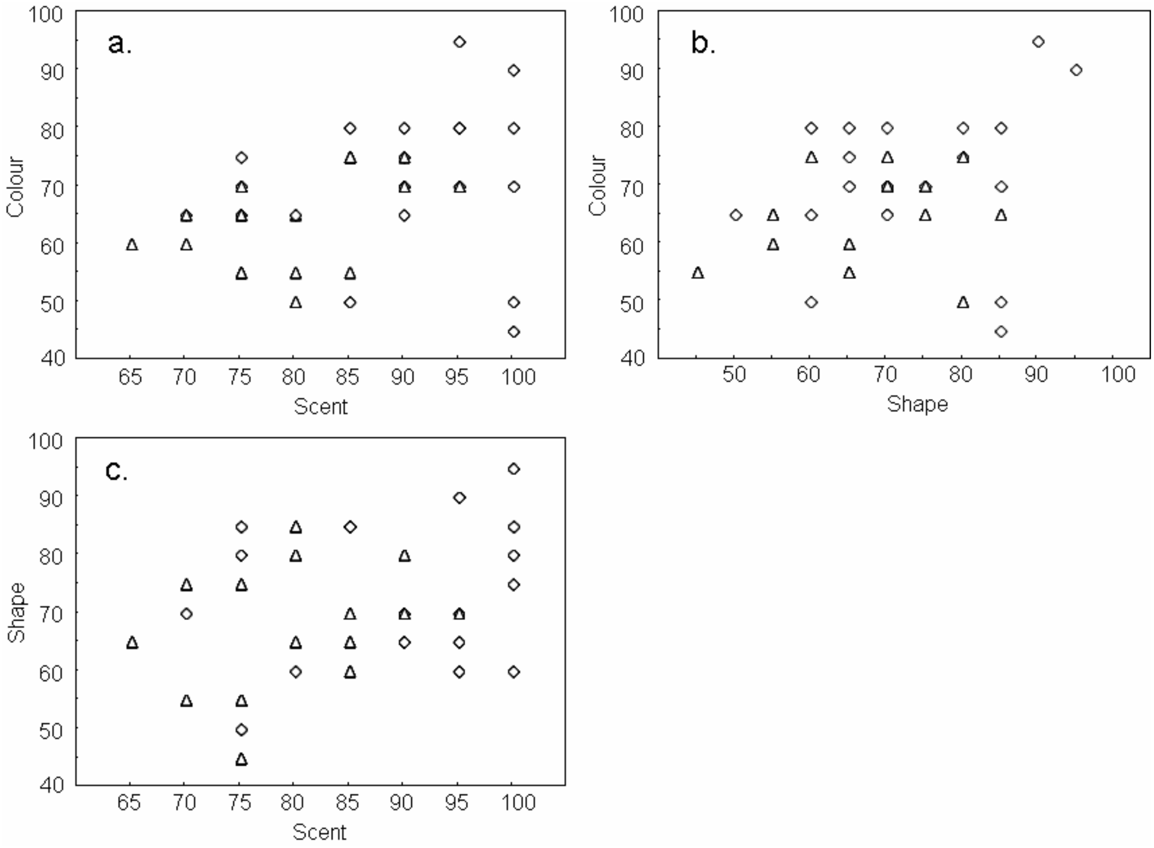


Figure. 4.3. *Pairwise correlation for discrimination performance in scent, colour and shape learning (percentage of correct choices between the 31st to the 50th choice). Spearman's rho for each pair: a. Colour versus scent:* $r_S = 0.33$, $P = 0.04$; *b. shape vs. colour:* $r_S = 0.31$, $P = 0.057$; *c. shape vs. scent:* $r_S = 0.43$, $P < 0.01$. *Circles represent bees from colony A and triangles represent bees from colony B.*

A significant interaction effect between task and colony suggests that bees originating from different colonies have different learning abilities ($F_{2,35} = 11.7$, $P < 0.0001$), with bees from colony A scoring higher on average (mean: 37.1s; standards error: 6.7s) than bees from colony B ((mean: 34.5s; standards error: 4.5s). However, bees from colony A were older (age range: 41-71 days) than bees from colony B (age range: 6-10 days). This could result in colony and age being confounding factors. I believe that age is unlikely to influence discrimination performance as previous studies in bumblebees found no correlation between learning abilities and age (Raine et al., 2006b; Raine and Chittka, 2008).

4.3.4 Discussion

I showed that there is consistent variation in discrimination performance in colour, shape and scent learning tasks between individual foragers. In other words, bees which made the highest number of correct choices during their first task were also the ones which made the most correct choices during the second and third task. It is tempting to speculate that these findings support the view that learning is domain-general (Chiappe and MacDonald, 2005) and adding support to the theory that evolution acts on ‘generalized learning processes’ (i.e. learning a across a wide range of tasks differing in nature) rather than independent ‘cognitive modules’ (Kawecki, 2010). However, the observed discrimination abilities could be the result of differences in sensory performance rather than learning. Scheiner and Erber (2009) showed in honeybee that sensory thresholds correlate across modalities. The bees which had a low response threshold to a stimulus in the gustatory modality also had a low threshold response to stimuli in the olfactory and visual modalities and (Scheiner and Erber, 2009) claim that “to date, there has been no unequivocal experimental evidence for a stimulus-specific tuning of thresholds” (but see Roussel et al., 2009). In our study, not only could bees vary in their response threshold to the visual and olfactory stimuli, but also, they could vary in their response threshold to both gustatory stimuli (sucrose and quinine). It is therefore possible that some of the individual variation in discriminating abilities observed here could be attributable to differences in response threshold for conditioned as well as unconditioned stimuli.

Whether the observed individual variation in discrimination abilities can be explained solely or partially by learning and/or sensory thresholds, the ability to discriminate stimuli in the environment is very likely to affect an individual’s foraging performance (Raine and

Chittka, 2008) and therefore colony fitness. Indeed, learning plays a major role in the development of individual behaviour and in the way an individual responds to its environment (Ravary et al., 2007; Chittka and Müller, 2009) and learning cannot occur without some form of discrimination between the stimuli available in the environment.

4.4 Implications for task specialisation and colony fitness

Polyethism, the division of tasks among workers, is thought to be the key feature underlying the ecological success of the eusocial insects (Hölldobler and Wilson, 1990). In social insects, learning and the ability to discriminate between stimuli is essential to the performance of certain tasks, such as foraging. In the honeybee *Apis mellifera*, where age is a determinant factor in task specialisation, Ben-Shahar et al. (2000) found that changes in learning and memory appeared to occur in association with the change in tasks performed. Learning and memory are thought to be important in completing foraging tasks: a forager has to learn and remember the location and features (stimuli) associated with the food sources. Other tasks appear to be much less demanding in terms of learning and discriminatory ability and may be largely governed by innate behaviour (e.g. nursing the brood). Given this difference between tasks and the fact that there is no definite age polyethism in bumblebees, it is natural to postulate that a bee’s ability to perform a given task would be related to her ability to learn the task. Indeed, in honeybees, foragers and nurse bees are known to differ in learning abilities (Ben-Shahar et al., 2000) and in ants, Ravary et al. (2007) showed that individual experience can generate task specialisation. It is premature to conclude that the likelihood to perform a cognitively demanding task is linked to an individual’s cognitive abilities (as suggested by Chittka & Müller 2009), but this study suggests that this is fruitful area for further research.

These findings showed that individual bumblebees consistently differed in their ability to learn to discriminate stimuli from the visual and olfactory modality. These results open the door to further research on the potential link between sensory/cognitive abilities and consistent individual behaviour, and more specifically, task specialisation in social insects. Studies similar to the one described here could be expended to include a broader variety of learning tasks (i.e. not restricted to discrimination, such as social learning; Leadbeater and Chittka, 2007) and other aspects of the learning process (e.g. speed-accuracy trade-offs, Chittka et al., 2003).

Chapter 5: ‘Personality’ in bumblebees: individual consistency in responses to novel colours?

Neophilia/neophobia is attraction/repulsion in response to novel non-predatory stimuli (see section 1.4.2). As the environment changes over time (e.g. new food source, new competitors, etc), individuals are constantly exposed to new stimuli. Attraction/repulsion towards these novel stimuli is likely to be pivotal to the individual’s survival: neophobic individuals might reject a new food source whereas naïve neophilic individuals might be attracted to new predator/parasite cues. Even at the species level, there is some evidence for a link between neophilia/neophobia and ecological specificity (Heinrich, 1995) as well as an organism’s ability to invade new environments (Martin and Fitzgerald, 2005).

5.1 Response to novelty in bumblebees

Bumblebees are often exposed to novel food sources in their environment as their floral food resources are far from reliable. Nectar or pollen production by a given flower species might vary within a day (Goulson, 2003) and seasonal availability as well as competition with other pollinators further complicates the task of the foragers. When given the opportunity, bumblebee foragers tend to specialise on a few flower species (Heinrich, 1979; Chittka et al., 1999). However, a particular individual’s preferred flower species might decline in profitability over various time scales, making it necessary for the forager to seek alternative food sources as more profitable flower species might become available. Resource assessment (through investigation of unfamiliar food sources) is presumably essential to efficient foraging in bees (Heinrich, 1979), and inter-individual differences in response to novelty could be of considerable relevance in bumblebees' natural foraging ecology.

In animals, neophilia/neophobia is commonly assessed through introducing novel objects/food in a setting already familiar to the individual (e.g. Bolivar and Flaherty, 2004; Logue et al., 2009). Introducing new flower 'species' and removing previously exploited food resources is easily done in the lab using artificial flowers, and hence provides us with an ecologically relevant way of assessing individual bees' response to novelty. Previous works suggest that bumblebees may respond to novelty in ways similar to vertebrates: Goulson et al. (2004) reports that bees were investigating new landmarks and Forrest and Thomson (2008) showed that bumblebees preferred to forage from familiar rather than unfamiliar flowers and noted that there appeared to be individual variation in novelty response. These observations lead to the question of how do bees keep track of the changes in their environment: do individual bees indeed vary in their tendency to investigate unknown flowers, and if so is this variation generated by consistent inter-individual differences rather than chance variation?

Assessing individual consistency is important if one is considering bumblebees' response to novelty as potentially equivalent to the phenomenon observed in vertebrate species. In these species, neophilia/neophobia is recognised as a personality trait, meaning that a given individual's behaviour will be repeatable across situations and time. Assessment of consistency is sometimes overlooked in studies of personality traits (e.g. Minvielle et al., 2002; Dochtermann and Jenkins, 2007 and Hollander et al., 2008) as it is almost invariably assumed that individual consistency exists for the traits in question, and that the challenge for scientists lies in discovering trade-offs or causal links between them and/or with other traits. However, research on the species best studied in terms of personality, human beings, demonstrates that while some behavioural traits remain stable over an individual's adult lifetime in a manner consistent with personality, others vary with mood, hormonal cycles, age and other factors not yet identified. Some such changes might be adaptive in line with developmental stage of an organism or environmental context (Sinn et al., 2008a; Roussel et al., 2009), or their individual experience (Bell and Sih, 2007a), while other changes might vary more unpredictably or be epiphenomena of other processes. This emphasises the necessity to measure behavioural traits repeatedly and over various time scales (Sinn et al., 2008a).

In this work, I adopt the view that in order to qualify as a personality trait a certain pattern of behaviour needs to be exhibited in a consistent way over different situations and over time, but that this consistent behaviour may vary between individuals (Briffa et al., 2008; Schuett et al., 2010). Based on this notion of consistency we introduce a novel statistical model and develop a new approach for assessing intra-individual consistency. We used our new model to investigate to which extent individual bees responded consistently to flowers of novel colours over various time scales. We discuss the implications of the findings from these experiments for regarding neophilia/neophobia as a personality trait.

Another hypothesis worth investigating is the potential effect of an individual's abilities on its personality (see chapter 4). I showed in chapter 4 that individual bumblebees vary consistently in their ability to learn to discriminate between pairs of stimuli. Because learning often involves new stimuli (in the case of bumblebees, novel flowers might come into bloom or restrictions to previously available food source might constrain the bees to look for novel patches/locations), neophobia may play a great role in its initial stages:

learning that a new flower species delivers nectar cannot occur if the bee constantly avoids it. One could therefore predict that individuals which are less neophobic would be faster learners than individuals which are more neophobic. Indeed, this has been shown to be the case in starlings (Boogert et al., 2006): starlings which were fastest to feed in a novel environment were also fastest in solving a foraging task. I therefore combined the neophilia-neophobia experiment with a discrimination learning paradigm to investigate a potential link between individual neophilia-neophobia and ability to discriminate between colours.

5.2 Material and Methods

5.2.1 Experiment 1: Choice of colours for the consistency tests

Since I am interested in the bee's response to novel colours, I have to ensure that the bees are able to distinguish between the colours used. Indeed, a lack of response to a novel colour could simply mean that the bees do not perceive the colour as differing from the previous one. To this end, I selected colours that, following the hexagon model of bee colour vision (Chittka, 1992; Fig. 5.1), should be easily distinguishable for bees. I used red, cream and pink artificial 'flowers' of the same shape as those used for pre-training. To bees, these flower types appeared as UV, blue-green and, UV-blue respectively and were roughly equidistant in a bee's colour space (Fig. 5.1). I then ascertained experimentally that bees could easily discriminate the colours.

Five colonies (henceforth referred to as colonies A to E) of *Bombus terrestris* containing approximately 40 workers each were obtained from Syngenta Bioline (Weert, Netherlands) between September 2008 and April 2009. Queens of this species mate only once in their life; therefore all the foragers within a colony are full sisters (Schmid-Hempel and Schmid-Hempel, 2000).

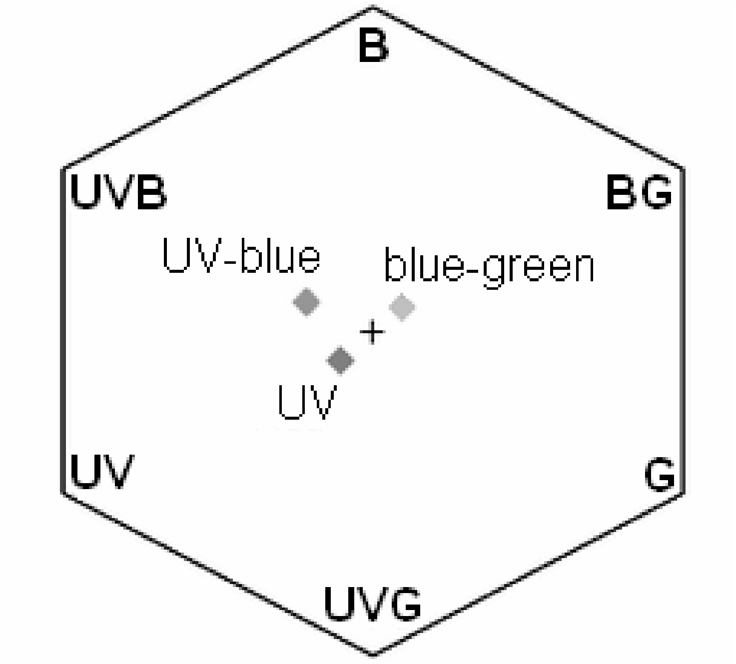


Figure 5.1. *Loci of the artificial flower colours in the colour hexagon. Loci are calculated according to the relative stimulation of the three receptor types (UV, blue, green) elicited by the stimulus (Chittka, 1992). The colour hexagon coordinates were obtained using spectrophotometer readings from 300nm to 700nm (i.e. including the ultraviolet range). The angular position (measured from the centre) in this colour space indicates bee-subjective hue, which is in turn determined by the relative excitations of bees' UV, blue and green receptors. A colour locus in the lower left portion of the colour hexagon indicates a 'bee-UV' colour, a colour locus in the 'up' direction denotes 'bee-blue' and so forth. Distances between colour loci indicate discriminability; the total distance between the centre and any of the corners of the colour hexagon equals unity, and distances above 0.1 are typically well distinguishable. Euclidian distances between the colours used here are 0.23 between UV and UV-blue, 0.24 between UV-blue and blue-green and 0.19 between UV and blue-green, and are therefore predicted to be well distinguishable by bees. B = blue, BG = blue–green, G = green, UVG = UV-green, UV = UV and UVB = UV-blue.*

Upon delivery, colonies were transferred into bipartite wooden nest boxes (28×16×11 cm). All tested bees were individually tagged with coloured dots or Opalith number tags

(Christian Graze KG, Germany). The nest box in which the colony was housed was connected through a plastic tube to a foraging flight arena (120×100×35 cm) covered with a UV-transparent Plexiglas lid. Bees could be allowed one at a time into this arena using a system of shutters built in the connecting tube. The room in which the colonies were kept had an average ambient temperature of 21ºC. Controlled illumination for laboratory experiments was provided by high-frequency fluorescent lighting (TMS 24F lamps with 4.3 kHz ballasts; Philips, The Netherlands) fitted with Activa daylight tubes (Osram, Germany) to simulate natural daylight above the bee flicker fusion frequency (Dyer and Chittka, 2004).

Preparation and pre-training

Colour-naïve foragers (i.e. bees that had never encountered any food-providing coloured object) were initially allowed to forage from translucent gravity feeders (Von Frisch, 1967, p19, Fig. 18) which provided unlimited supplies of 50% (w/w) sucrose solution for two days; the same concentration was used in all experiments described below. Subsequently, individual foragers were 'pre-trained' in the arena (Fig. 5.2) to use an array of eight translucent artificial 'flowers' (Plexiglas squares of 24x24mm, 4mm thick and with a well for sucrose solution in its centre, mounted on 4.3cm high glass 'stalks'). In what follows I refer to the time the bee spent foraging in the arena between two unloading trips to the nest as a 'foraging bout'. Here and in all experiments below, the position of each flower on a 6 x 5 grid (with 14cm between positions) was randomised by using a computer random generator. We used 15 different random spatial patterns, varying the pattern between bees and between two foraging bouts (this shuffling of the position of the flower was necessary because the bees would otherwise have learned to associate location with reward). The artificial flowers were cleaned with soap and water between foraging bouts so as to ensure there were no scent marks left from previous visits (Saleh et al., 2007). In order to encourage the bees to visit all eight flowers in the arena we adjusted the amount of sucrose solution available in each flower so that the total volume in all eight flowers matched the stomach capacity of each individual bee. This was achieved by decreasing or increasing the volume of sucrose available in each flower over the three pre-training foraging bouts, until the bees visited all eight flowers. The translucent flowers are achromatic and pre-training to achromatic stimuli has been shown not to affect colour preference during subsequent exposures to coloured stimuli (Giurfa et al., 1995; Raine et al., 2006a). For this reason and because the first three foraging bouts were simply training to the set up (and adjustment of the quantity of sucrose per flower), these foraging bouts were not included in the data analysis.

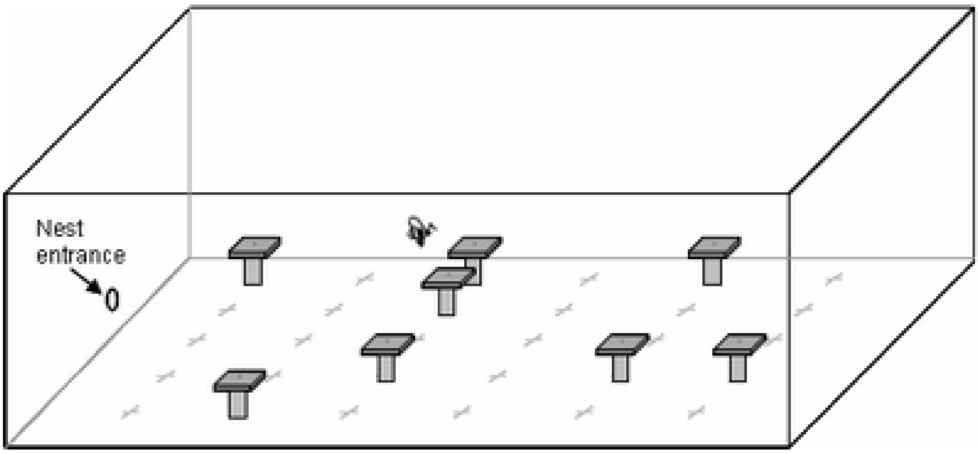


Figure 5.2. *Experimental set up. The bees are released one at a time in the arena containing the artificial flowers distributed in a random pattern. The sides of the arena are made of wood whereas the top lid is UV transmitting Plexiglas. The position of the flowers is changed after every foraging bout.*

Training and test

To assess the bees' ability to discriminate between the colours used, 10 foragers from colonies A, B. C, and 6 from colony D were tested. The experiment consisted of a training phase of three foraging bouts followed by a test. Individuals were first exposed to eight randomly positioned flowers of the colour A ('known' colour) for three consecutive foraging bouts. During the colour discrimination test, the arena contained 16 randomly distributed flowers, eight of which were of the same colour as in the immediately preceding foraging bout ('known colour') and eight of which were of the novel colour. These tests were unrewarded; all flowers contained 10μl of water to mimic the visual appearance of sucrose solution. The number of flowers of each type visited was recorded as correct (landing on the 'known' colour) or incorrect (landing on the 'novel' colour) and the number of correct and incorrect choices out of the 10 first flowers chosen was used as a measure of the ability of the bees to discriminate between colours. Different individuals were used for the colour discrimination tests (experiment 1, section 5.2.1) and the experiments 2 and 3.

5.2.2 Experiment 2: individual consistency in response to novel colours

The same colonies as the previous section were used to provide the foragers for this experiment and the foragers' preparation and pretraining was identical to the ones from the previous section.

Short term consistency test

The main variable measured throughout this experiment was the feeding latency of the bee - that is, the time elapsed between flight initiation and first probing (proboscis extension) of the well of a flower. Bees were exposed to three colours (which, to bees, appeared UV, blue-green and UV-blue, see Fig. 5.1) for three consecutive foraging bouts each (i.e. three foraging bouts on colour A, then three foraging bouts on colour B and finally three foraging bouts on colour C). Because the bees were colour-naive, each colour appeared to the bee as ‘novel’ at the beginning of the first foraging bout. Over three foraging bouts, bees learned to associate the colour with a reward (see experiment 1, section 5.3.1) and so the colour could be regarded as familiar at the end of these three bouts. At foraging bout number four, colour B appeared as ‘novel’. The bee then learned to associate it with reward during the course of foraging bout numbers 4-6. Colour C appeared as ‘novel’ in foraging bout number seven with gradual familiarisation until bout nine.

Just as in the pre-training phase, the arena contained eight rewarded flowers randomly positioned on the grid. I varied the order in which the colours were presented to control for order effects. Eighty-one foragers were tested from colonies A (N = 27), B (N = 27) and C (N = 27). For each colony, 15 bees were presented with the order UV – Blue-green - UV-blue, 6 were given the sequence Blue-green - UV - UV-blue and 6 were given UV-Blue – UV – Blue-green.

Video recordings of the beginning of each foraging bout (until the first probing of a flower) for 28 bees were used to split the feeding latency into two variables: the first approach time, which is the time between initiation of flight and approach (hovering within two centimetres) of the first flower by the bee; and cumulative investigation time, which is the time the bee spent hovering within a two centimetre radius of individual flowers before the first landing. Sometimes the bee would approach more than one flower before landing, so the sum of the time spent investigating each flower prior to the first landing was used to produce the cumulative investigation time. Since I was interested in the strength of the relationships between these variables, Spearman’s rank correlation coefficients were calculated between first approach time and feeding latency and between cumulative investigation time and feeding latency.

To test for a relationship of body size and response to novelty, I measured the maximum thorax width three times per individual and took the average as an estimate of the bee’s size (thorax width is the most common measure of body size for bumblebees; Goulson et al., 2002). I measured all tested 27 bees from colony A. I performed a Spearman’s rank correlation test using the maximum thorax width and the average novelty response as variables. A bee’s novelty response to a given colour was calculated by subtracting the feeding latency of the last foraging bout on this particular colour from the feeding latency of the first foraging bout of this colour. The novelty responses for the three colours are then averaged in order to provide us with a single measure of individual response to novel colours, which we refer to as the average novelty response (NR):

$$NR = \frac{\left[\left(L_{FB1} - L_{FB3}\right) + \left(L_{FB4} - L_{FB6}\right) + \left(L_{FB7} - L_{FB9}\right)\right]}{3}$$

where NR is the average novelty response (s), L is the landing latency (s) for a given foraging bout, and FBn is the foraging bout. By using the difference between the first and last foraging bout, we accounted for putative differences between bees in terms of overall flying speed.

In a subset of bees (colony A: N = 7; Colony C: N = 15 – total of 22 individuals) the identification tags were applied within 48 hours of emergence from the pupae, enabling us to determine their age at the time of experiment. To test for a potential correlation between the response to a novel stimulus and age, a Spearman’s rank correlation test was performed using the age (days since emergence from the pupa) as one variable and the average novelty response as the other.

Long term consistency test

This experiment aimed at assessing bees’ consistency over several days instead of a few hours. It followed the same template as the short term experiment with individual, colour-naïve bees being exposed to three colours for three foraging bouts each. However, in this experiment, only one colour per day was presented to the bee. Thus, on day 1, a bee would be tested for three consecutive foraging bouts with colour A. On day 2, the same bee would be tested for three consecutive foraging bouts with colour B and on day 3, she would be tested with colour C in the same way. As for the short term experiment, we varied the order of the colours and each bee was pre-trained with translucent flowers (meaning that she would do three foraging bouts with translucent flowers on day 1 before being exposed to colour A). On subsequent days, each set of three foraging bouts was preceded by one foraging bout with translucent flowers so as to ensure the bee resumed foraging. Twenty-five bees from colony E were used for this experiment.

Consistency Model

A frequent difficulty in demonstrating consistency of behavioural traits is to demonstrate consistency statistically, despite the inevitable variation in nearly all behaviours. Indeed, if under the given design regardless of the colour of the flower (which can influence the bee’s behaviour, see results section) the feeding latencies of some bee decreased steadily between the first and third foraging bout, then this might be explained by some characteristic of the bee which may be regarded as an aspect of her ‘personality’. Of course, such a regular pattern is likely to vary between individual bees (see Fig. 5.3). Thus it is necessary to identify those bees showing consistent behaviour in terms of a stable pattern of feeding latencies. The procedure I propose amounts to comparing fitted individual profiles of feeding latencies with the empirical data and classifying those bees for which the fitted and actual feeding latencies are in good agreement as being consistent.

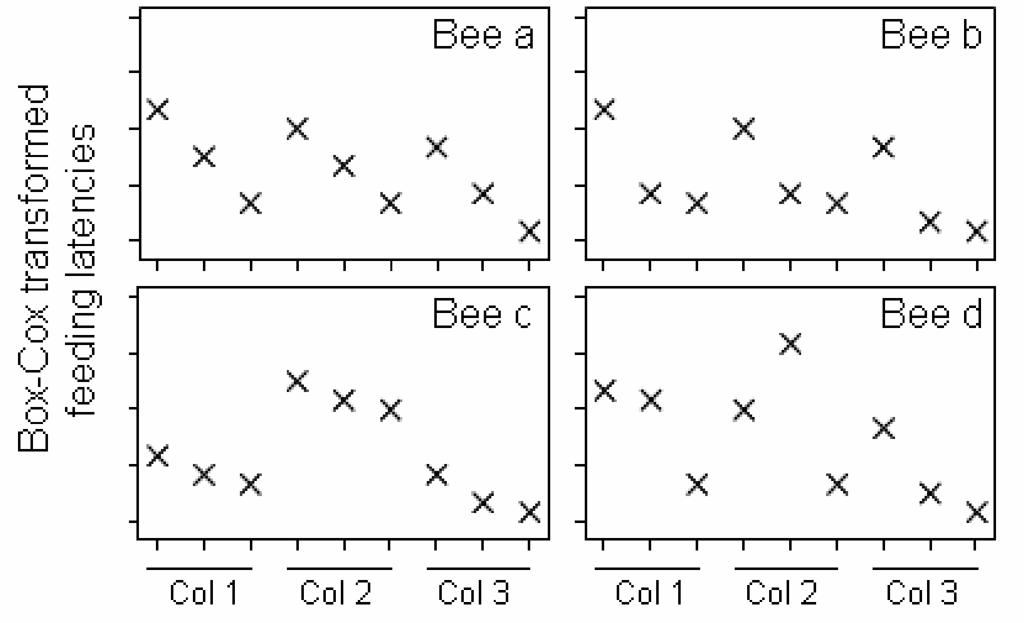


Figure 5.3. *Schematic representation of different patterns for four fictitious bees. 'col 1', 'col 2' and 'col 3' are the colours used for each set of three foraging bout (each cross represents the Box-Cox transformed feeding latency for one foraging bout). Bee a shows gradual decrease of latencies to the stimuli, while bee b shows a sharp decrease in her latency to feed from a 'new' stimulus after just one exposure. Bee c has a strong aversion for the second colour. Bee d shows no consistency as her response to the stimuli varies both within and between colours.*

To this end, an ANOVA-type general linear mixed effects model was first fitted to the data. This model takes into account the repeated measurements structure of the data and incorporates several factors likely to have an effect on the response (e.g. the colour of the flower). Moreover, the model captures the crucial aspect of consistent behaviour by allowing every bee to have a different pattern of feeding latencies which, for any given individual, is assumed to stay the same over the three sets of three foraging bouts. Secondly, a distance measure is used to determine, for each bee separately, how good the fitted and actual feeding latencies agree. By specifying a cut-off value it is then possible to classify all bees for which the discrepancy between the fitted profile and the original observations does not exceed the cut-off as being consistent. A disadvantage of this approach is that specifying the threshold necessarily involves some sort of judgment. One possibility to circumvent this problem is to look at the cumulative distribution function (CDF) of the distances which for every possible cut-off value x depicts the proportion of consistent bees. This is useful for exploring how the proportion of consistent bees varies with the threshold. Furthermore, results from different experiments can easily be compared by comparing the corresponding CDFs.

As feeding latencies are typically positively skewed, prior to fitting the model a transformation was employed to make the distribution of the data more normal. Here we use the well-known Box-Cox transformation (Box and Cox, 1964) which is a kind of power transformation and depends on a single parameter λ that can be estimated from the data. The basic nested mixed model for the transformed feeding latencies is then

$$Y = \mu + a_i + b_{j(i)} + c_{k(ji)} + \alpha_l + \beta_{m(ji)} + e$$

where Y represents the response and e is the error term. The Greek and Latin letters represent fixed and random effects respectively, which are to be interpreted as follows:

μ overall mean

a_i random effect of colony i

$b_{j(i)}$ random effect for bee j within colony i

$c_{k(ji)}$ bee specific random effect for the kth set of three foraging bouts per bee

α_l fixed overall effect of colour l

$\beta_{m(ji)}$ bee specific effect of foraging bout $m = 1,2,3$ within every set of three bouts within bee j in colony i. These three parameters per bee are the same for every set k of three bouts and define the bee's response pattern.

This model reflects that sets of three foraging bouts, where each set of three corresponds to a different colour, are nested within individual bees, which in turn are nested within colonies. The repeated measurements nature of the data is accounted for by allowing correlations between (a) the feeding latencies for the three foraging bouts per colour within each bee and (b) the feeding latencies across colours within each individual bee.

The basic model can be refined to separate, for every individual bee, the effect of the first foraging bout within a set of three, which corresponds to the onset of a new colour, from the combined effect of the other two bouts for the same colour. Similarly, it is possible to separate the effect of any of the colours from the combined effect of the other two colours. These modifications correspond to testing predefined orthogonal contrasts as part of the analysis of variance.

A Bonferroni adjustment of the 1% significance level is adopted in order to account for the fact that multiple tests are performed. In total, there are $k = 5$ tests and hence an effect will only be regarded as being significant if the P-value is smaller than $\alpha = 0.01 / k = 0.002$.

This approach is very conservative, but is intended to avoid drawing conclusions which may not stand up in replication studies.

Using Genstat Release 10.1, this model was fitted separately to the data from the short term and long term experiments. By fitting the model, for each bee a profile consisting of nine values is obtained. These are the values which give the best fit to the transformed feeding latencies in the three sets of three foraging bouts. In order to assess how closely the fitted profiles agree with the data, an appropriately standardized version of the usual Euclidean distance can be computed for each bee separately. More specifically, the distance measure we propose is defined as

$$d = \frac{\sqrt{\sum_{i=1}^{9}(Y_i - \hat{Y}_i)^2}}{IQR}$$

where Y_i and $\hat{Y}_i$ are respectively the transformed feeding latencies and fitted values for a single bee, and IQR is the interquartile range of the transformed feeding latencies over all bees. Dividing the Euclidean distance by the interquartile range is similar to standardizing the distance by means of the standard deviation. However, using the IQR makes the proposed distance measure more robust against extreme observations and hence appears to be preferable. This distance can be regarded as a consistency index (one per bee) with small values of d indicating greater consistency. As explained above, it is a measure of the fit between the predictions of the model and the (Box-Cox transformed) data and therefore measures the repeatability of a bee’s behaviour across colours and foraging bouts.

Additionally, we tested for a potential relationship between individual consistency and the level of the response to novelty. Indeed, in the same way as the level of expression of a personality trait might vary, the level of consistency of a trait might vary between individuals of a same population: for example Dingemanse et al. (2009) found that very aggressive mice are very consistent whereas less aggressive mice are more flexible and more responsive to the environmental conditions. We therefore calculated the Spearman rank correlation coefficient between the average novelty response (see above) and the consistency index for the short term experiment.

5.2.3 Experiment 3: Neophilia-neophobia and learning

All the bees used in this experiment came from the same colonies A (N = 11), B (N = 10) and C (N = 10) as for the experiment 2 in section 5.2.2 and therefore the rearing conditions and flight arena were identical to the ones described in this section. Preparation and pretraining was done as for section 5.2.2 and the first three (training) foraging bouts were not included in the data analysis. Following the training phase, Bees were exposed to two colours: first bee-UV and then bee-blue-green (see Fig. 5.1) for three consecutive foraging bouts each (i.e. three foraging bouts on bee-UV, then three foraging bouts on bee-blue-green). The feeding latency of the bee was recorded for each foraging bout for each bee.

After the third trial for blue-green, individual bees were presented with a colour discrimination task. Eight blue-green flowers (familiar) were filled with saturated quinine (hemisulfate salt; VWR, UK) solution whereas eight UV-blue flowers (unfamiliar) were rewarded with 50% (w/w) sucrose solution. This task is similar to a reverse learning task in that the previously rewarding colour becomes punishing. The behaviour of each bee was then recorded: landing on a UV-blue flower (unfamiliar-rewarded) was recorded as a ‘correct’ choice whereas landing on a blue-green flower (familiar-punishing) was recorded as incorrect.

The Bee consistency model used in section 5.2.2 could not be used here as there were only two colours tested instead of three. Instead, I calculated Spearman's correlation coefficients between each measure and the average novelty response for the two colours (NR):

$$NR = \frac{\left[\left(L_{FB1} - L_{FB3}\right) + \left(L_{FB4} - L_{FB6}\right)\right]}{2}$$

where NR is the average novelty response (s), L is the landing latency (s) for a given foraging bout, and FBn is the foraging bout.

5.3 Results

5.3.1 Experiment 1: Choice of colours for the consistency tests

We found that bees are able to discriminate between the three colours we used in our experiments. Only 5% of the bees tested made more than 30% incorrect choices when having to discriminate between UV and blue-green (average percentage of incorrect choices and standard deviation: 12%±13%) and the same was true for when bees had to discriminate between blue-green and UV-blue (average percentage of incorrect choices and standard deviation: 8%±13%). All the bees tested for discrimination abilities between UV and UV-blue made less than 20% incorrect choices (average percentage of incorrect choices and standard deviation: 7%±10%). We found that the observed number of correct choices for all colour discrimination tests are significantly different from chance (Binomial test: UV vs Blue-green: $p<0.0001$; blue-green vs UV-blue: $P<0.0001$ and UV vs UV-blue: $P <0.0001$). This experiment demonstrated that bees are able to discriminate well between the selected colours after only three foraging bouts.

5.3.2 Experiment 2: individual consistency in response to novel colours

Short term consistency test

Foragers were found to have greater feeding latencies when the colour of the flowers was unfamiliar than when it was a colour that they had previously experienced (see Fig. 5.4). This appeared to be the case independently of the colour considered or of the position of the colour in the sequence and is confirmed by statistical analysis (see below).

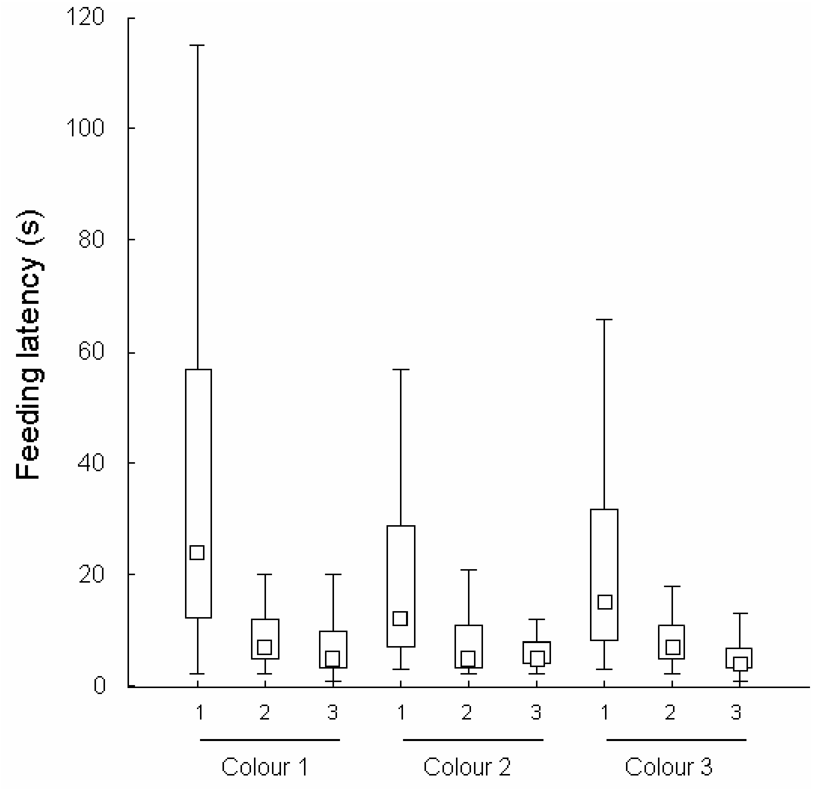


Figure 5.4. *Box-plot diagram of the feeding latency of all workers, all colonies, all colour-orders pooled together. Small squares represent medians, large rectangles are inter-quartile ranges and whiskers indicate the ranges of largest non-outlier observations. Numbers on the x axis correspond to the foraging bout of the experiment. The colour to which the bee was exposed depended on the order of colours she was been subjected to. The median, interquartile range and largest non-outlier range of the feeding latency were all larger for the first exposure to each colour than for the two subsequent exposures, suggesting that the bees responded to novelty by delaying feeding from the unfamiliar colour.*

There was no significant correlation between the bees' sizes and their average response to novelty (Spearman's rank correlation: $r_s = 0.09$, $N = 27$, $P = 0.63$). The same was true for the age of the bees and their average response to novelty (Spearman rank correlation: $r_s = 0.15$, $N = 22$, $P = 0.50$).

Video recordings demonstrate that the observed latency in landing on a novel stimulus is due to the bees' reluctance to land on an unknown flower colour, and not caused by the bee persisting in searching for the previously rewarding stimulus (which would be indicative of dietary conservatism). This is demonstrated by breaking up the feeding latencies into first approach time (time spent flying in the arena before approaching the first flower) and cumulative investigating time (hovering close to the new stimulus). I found a significant correlation between the feeding latency (all colours, all colonies pooled) and the first approach time (Spearman's rank correlation: $r_s = 0.28$, $N = 28$, $P < 0.001$) and between the feeding latency and the cumulative investigating time (Spearman's rank correlation: $r_s = 0.77$, $N = 28$, $P < 0.001$). Because the cumulative investigating time (mean: 9s and standard deviation: 21.7s) was much more strongly correlated with the feeding latency (mean: 23s and standard deviation: 66.0s) than the first approach time (mean 4s and standard deviation: 10.7s), we conclude that most of the observed variation in the response to the new colour is explained by the amount of time the bees spend investigating (hovering close to) the new colour. The first approach time explains much less of the feeding latency. If the bee was merely ignoring the new stimulus while actively searching for the known stimulus, then we would expect her to spend most of her feeding latency flying in the arena instead of hovering close to the new stimulus. We would expect the first approach time to explain much more of the feeding latency than the cumulative investigation time and we observed the opposite phenomenon. This suggests that feeding latency is an appropriate measure of a bees' response to a novel stimulus.

Results from the ANOVA analysis:

The maximum likelihood estimate of the parameter λ of the Box-Cox transformation for the feeding latencies was found to be equal to -0.4. Table 5.1 shows the analysis of variance for the transformed data. This accounts for 80.6% of the variability in the transformed latencies as measured by the coefficient of determination R^2. Residual plots indicate that the distribution of the residuals is close to normal.

Source	*d.f.*	Sum of squares	Mean square	*F*	*P*
Colony level	2	2.42	1.21	4.00	0.02
Bees within colonies level	78	23.61	0.30	3.08	<0.001
Sets within bees within colonies level					
Colour	2	3.60	1.80	18.32	<0.001
Sets within bees	160	15.72	0.10	1.45	0.003
Units level					
Bouts within sets within bees	162	46.21	0.29	4.20	<0.001
Residual	324	22.01	0.07		
Total	728	113.56			

Table 5.1: Analysis of variance for the short-term experiment

By adopting this standard, there is clear evidence for inter-individual variability in the feeding latencies between bees (see Table 5.1). The table also shows that there is a strong overall effect of colour (see Table 5.1). In addition and independently of the effect due to colour there is a strong intra-individual effect of the position of the foraging bout within the three replications of the same colour (see Table 5.1).

A refined analysis, in which a pre-planned comparison for distinguishing between the effect of the first bout and the combined effect of the remaining two bouts for any set of three bouts is included, splits the sum of squares and the degrees of freedom for bouts in table 5.1 into two independent components without changing any of the other rows of the table. The corresponding test reveals that the already reported effect of the position of the foraging bout is due to the difference in the feeding latencies for the first and the other bouts (ANOVA: $F_{81,324} = 7.14$, $P < 0.001$), that is the effect is due to the onset of a new colour, irrespective of what that colour is. The *P*-values for the other tests in Table 5.1 are also comparatively small. Although the corresponding effects are not regarded as being significant by these standard, these P-values confirm that colonies and sets within bees should be included as blocking factors in the analysis.

Consistency Model:

By fitting the ANOVA model, individual profiles of nine fitted values were obtained for each bee (Fig. 5.5 and Appendix Fig. 1a, 1b and 2). For every individual bee, this profile was superimposed on her Box-Cox-transformed feeding latencies and the consistency distance was calculated. For the 81 bees, the consistency distance varied between 0.13 and 2.13 with a mean of 0.78 and standard deviation 0.41.

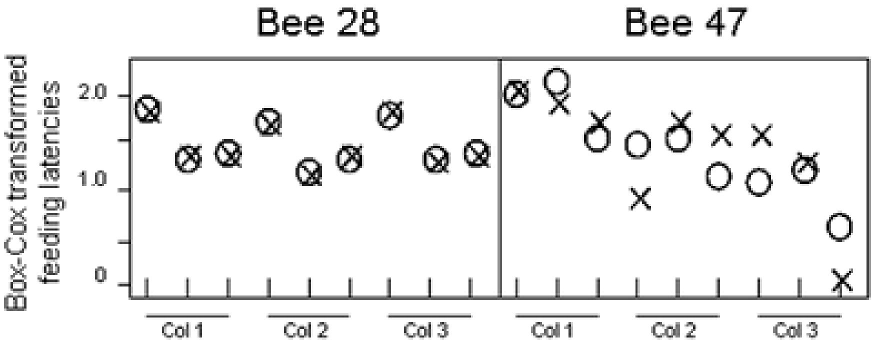


Figure 5.5. *Examples of individual profiles generated by the consistent bee model for two representative bees. Open circles are fitted values from the model and crosses are actual Box-Cox transformed feeding latencies. 'col 1', 'col 2' and 'col 3' are the colours used for each set of three foraging bouts (each cross represents the Box-Cox transformed feeding latency for one foraging bout). Bee 28 is a good example of a very consistent bee. She has a consistency distance of 0.22, reflecting a very good match between fitted and Box-Cox transformed feeding latencies. By contrast, bee 47 has a consistency distance of 2.1, reflecting a very poor match between fitted and Box-Cox transformed feeding latencies.*

The solid line in Fig. 5.6 represents the cumulative distribution function for the distances. For every possible cut-off value x on the horizontal axis it gives the proportion of bees in the experiment for which the discrepancy between the fitted profile and the data is smaller than or equal to the threshold x and which hence would be regarded as behaving consistently. An advantage of using the CDF is that the sensitivity of the classification can be easily explored.

For example, adopting a threshold of x = 0.65 for the distances the proportion of consistent bees as given by Fig. 5.6 is equal to 44% or 36 bees. For x = 0.60 and x = 0.70 the proportions from the figure are 36% and 49% corresponding to respectively 29 and 40 bees. Since the total number of 81 bees in the experiments is known, no information is lost by using the CDF and every proportion can be converted back to the underlying number of bees.

There was no significant correlation (rs = 0.151, N = 81, *P* = 0.179) between the consistency and response to novelty of individual bees.

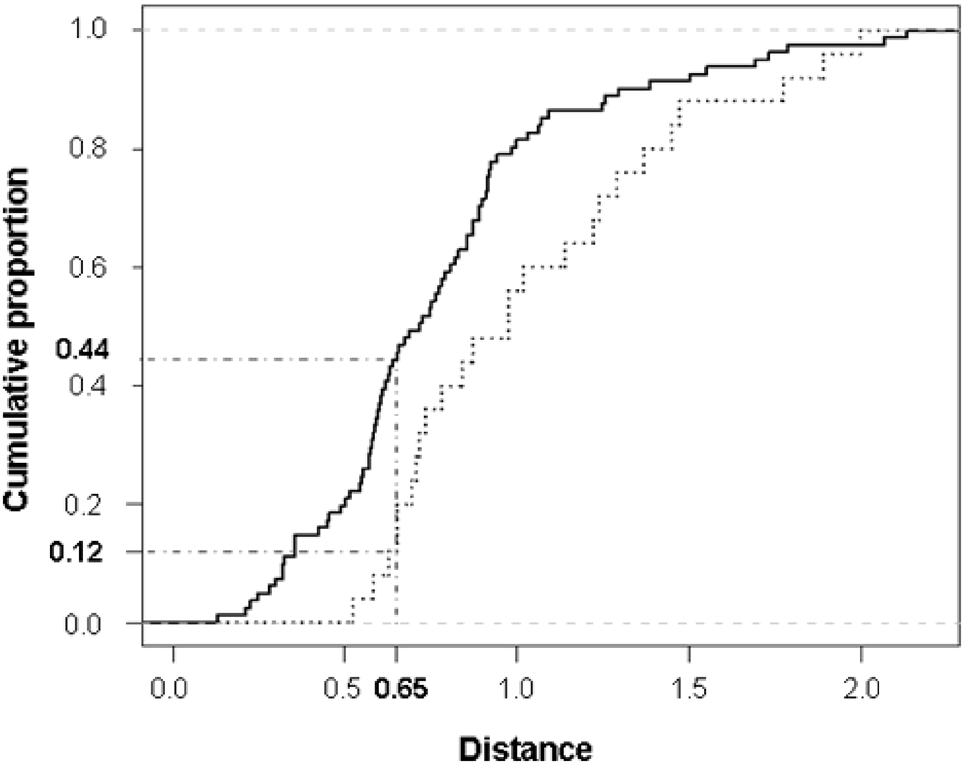


Figure 5.6. *Cumulative distribution function for the distances between Box-Cox transformed feeding latencies and ANOVA-fitted model values. The solid line represents the CDF for the short term experiment (within a day) whereas the dotted line represents the CDF for the long term experiment (between days). Note that while the CDF for the short term experiment is based on 81 bees, the underlying number of bees for the CDF in the long term experiment is 25. For every possible cut-off value x on the horizontal axis the CDF line gives the proportion of bees in the experiment for which the discrepancy between the fitted profile and the data is smaller than or equal to the threshold x and which hence would be regarded as behaving consistently. As an example, a threshold of x = 0.65 has been drawn, showing that there are 44% of consistent bees in the short term experiment and 12% in the long term experiment for this value of threshold. If bees are considered to behave consistently when they have a distance value of 0.65 or below, then 44% or 36 bees (out of 81) are regarded as consistent in the short term experiment and 12% or 3 out of 25 bees are consistent in the long term experiment.*

Long term consistency test

The analysis of the long term experiment paralleled that for the short term experiment except that no test for the colony effect could be performed since all bees were from the same colony. Here the maximum likelihood estimate of the parameter λ for the Box-Cox transformation was equal to -0.5. Table 5.2 presents the analysis of variance of the transformed feeding latencies. The analysis accounted for 75.7% of the total variation in the transformed latencies and the distribution of the residuals as judged by residual plots was again close to normal. As before, a Bonferroni adjustment of the 1% significance level is carried out; the number of tests is equal to $k = 4$ with a corresponding $\alpha = 0.01 / k = 0.0025$. Over the longer time scale inter-individual differences between bees cannot be detected (see Table 5.2). There is however strong evidence for differences between sets of foraging bouts (see Table 5.2) and an intra-individual effect of the position of the foraging bout within the sets of three bouts under any given colour (see Table 5.2). As in the short term experiment, the refined analysis shows that this position effect is due to the onset of a new colour (see Table 5.2).

Source	*d.f.*	Sum of squares	Mean square	*F*	*P*
Bees level	24	3.23	0.13	1.23	0.26
Sets within bees within colonies level					
Colour	2	0.28	0.14	1.26	0.29
Sets within bees	48	5.25	0.11	2.18	<0.001
Units level					
Bouts within sets within bees	50	6.85	0.14	2.74	<0.001
Residual	100	5.00	0.005		
Total	224	20.61			

Table 5.2: Analysis of variance for the long-term experiment

Since sets of foraging bouts were performed on different days, the significant effect due to sets indicates that between-day variation is probably the most important factor governing the bees' foraging behaviour. Nonetheless, the effect due to novelty of a colour still prevails as indicated by the significant result for bouts within sets and the significant position effect for the first bout within a set.

In order to assess consistency, individual profiles of fitted values and corresponding distances were obtained as in the short term experiment. Here the distances varied between 0.52 and 2.00 with a mean of 1.05 and a standard deviation of 0.42. The corresponding cumulative distribution function is shown by the dotted line in Fig. 5.6. As before, by using the CDF the effect of different threshold values x can be explored. For example, for a threshold of $x = 0.65$ the proportion of consistent bees is equal to 12% or 3 out of 25 bees. Similarly, for $x = 0.60$ and $x = 0.70$ the corresponding proportions are equal to 8% and 24% which correspond to respectively 2 and 6 bees.

Exact binomial tests for the threshold values $x = 0.60$, $x = 0.65$ and $x = 0.70$ of the null hypothesis that the proportion of consistent bees does not exceed 5% give P-values which are respectively equal to $P = 0.358$, $P = 0.127$ and $P = 0.001$. In the short term experiment, exact binomial tests for each of the three thresholds reject the null hypothesis that the proportion of consistent bees is smaller than or equal to 20% with a P-value of $P < 0.001$. Thus while for these values of x the proportions of consistent bees were clearly greater than 20% in the short term experiment, over longer terms the proportion can only be shown to be above 5% for $x = 0.70$.

By comparing the CDFs in Fig. 5.6 it is obvious that the proportion of consistent bees in the short term experiment is greater than the corresponding proportion in the long term experiment regardless of the choice of threshold. In case one raised the question of whether greater variability between the three colonies in the short-term consistency test compared to the long term consistency test might have affected our findings, I reanalyzed these data colony by colony and compared the results with those from the long-term consistency test; see Fig. 5.7. Visual inspection of the individual colonies' curves in the short term consistency tests reveals the same pattern (and the same difference with the long term consistency test) as for the pooled colonies; Fig 5.7). Moreover, for each colony from the short term experiment we tested if the proportion of consistent bees (when using, as above, a threshold value of $x = 0.65$ for classifying a bee as consistent) was larger than 10%. For each of the three colonies we obtained *P*-values of $P < 0.004$ when testing the null hypothesis that the proportion was smaller than or equal to 10%. In the long-term consistency test there was no significant evidence that the proportion of consistent bees was at least 5%. This demonstrates that our results also hold for each colony in the short term experiment separately and are not due to greater genetic variability in the combined sample

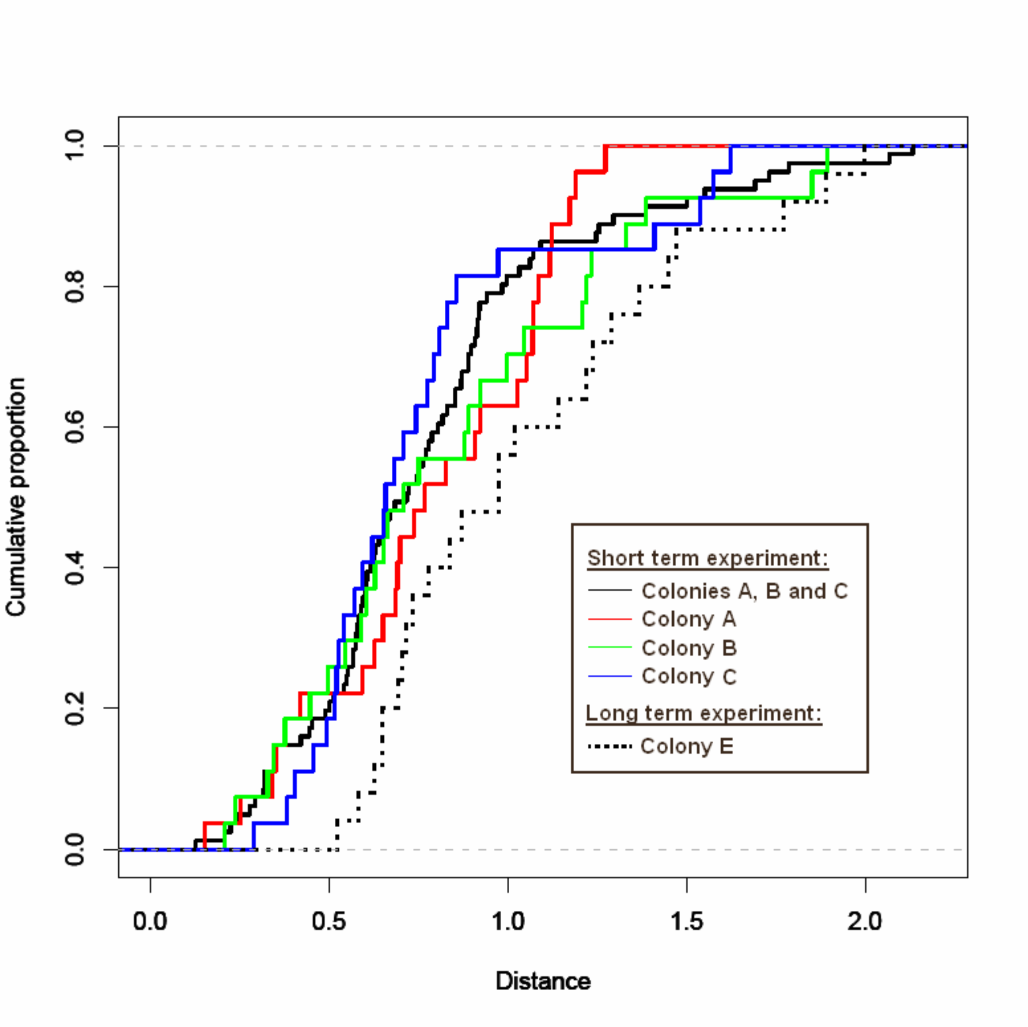


Figure 5.7. *Cumulative distribution function for the distances between Box-Cox transformed feeding latencies and ANOVA-fitted model values for the three colonies of the short term experiment and the single colony of the long term experiment. The solid lines represent the CDFs for each colony of the short term experiment (within a day) whereas the dotted line represents the CDF for the long term experiment (between days). For each of the three colonies in the short term experiment we fitted exactly the same model as in the long term experiment. Based on these separate analyses we plotted the cumulative proportions of bees for each threshold value for each colony in the short term experiment as in Fig. 5.6 and compared the resulting curve with the one for the colony in the long term experiment. All the curves for the individual colonies in the short term experiment are shifted to lower distance values than the data from the long term experiment, indicating greater consistency in every single colony used in the short term experiment compared to the long term experiment. This indicates that our findings are not caused by greater genetic heterogeneity in the pooled sample.*

In conclusion, the extent to which bees behave consistently within a day is much greater than it is when tested over several days. This finding can be corroborated by testing if the proportions for a given threshold x under the two conditions are equal. A non-parametric test that can be used for this purpose is Fisher's exact test. For example, for the threshold $x = 0.65$ the proportions of consistent bees in the short term and long term experiments are respectively equal to 36 out of 81 and 3 out of 25 for which Fisher's exact test gives a *P*-value of $P = 0.004$ providing clear evidence that the proportions are different. The same conclusion is reached when the equality of the proportions derived from other threshold values x is tested. For example, for $x = 0.60$ and $x = 0.70$ the *P*-values are equal to $P = 0.010$ and $P = 0.037$.

The reported *P*-values increase with x and for larger values of the threshold it is not always possible to demonstrate that there is a statistically significant difference between the corresponding proportions of consistent bees. Comparisons of proportions derived from a threshold $x > 0.70$ do not seem to be very meaningful, however, since in view of the narrow range of the scale on which the distances are measured basing the classification of bees on such a large threshold would appear to be too liberal. This assessment also seems to be supported by visual inspection of the profiles and the corresponding distances in Fig. 1a and 1b of the appendix.

5.3.3 Experiment 3: Neophilia-neophobia and learning

There was no significant correlation between the average novelty response and the number of correct choices for choices 31-50 (table 5.3). This means that there was no detectable association between the way a bee responded to novel chromatic stimuli and her ability to discriminate between two colours. Similarly, there was no significant correlation (table 5.3) between the average novelty response and the number of choices the bees needed to reach an 80% correct choices criterion.

	N	*Spearman's rho*	*P*
NR - bin1	31	-0.59	<0.001
NR - 1st correct choice	31	0.42	0.02
NR - bin2	16	0.12	0.66
NR - 80%criteria	31	0.11	0.54

Table 5.3: Spearman's rho correlation coefficients and P-values for several variables. 'NR': average novelty response (s); 'bin1': number of correct choices for the first 20 choices; 'bin2': number of correct choices for choices 31-50; '80% criteria': number of choices until a bee made eight correct choices out of her last 10 choices ; '1st correct choice': number of choices until the first correct choice is made

However, there was a significant negative correlation (table 5.3) between the average novelty response and the number of correct choices made during the first 20 choices. This suggests that the bees which most delayed landing on a new stimulus initially made fewer correct choices. Supporting this, there was a significant positive correlation (table 5.3) between the average novelty response and the number of choices made before making a correct choice.

5.4 Discussion

5.4.1 Individual consistency in response to novel colours

We showed that, as many species of vertebrates do (Heinrich, 1995; Mettke-Hofmann et al., 2002), bumblebees respond to novel objects by investigating them extensively before first accepting them as a food source. When confronted with a flower of a new colour, the vast majority of bees spent time hovering closely around it, presumably to enable visual exploration of the novel stimulus. This investigative behaviour was longest during the first encounter with the new stimulus and then drastically decreased during subsequent encounters. Such behaviour is similar to the response described for many species of vertebrates (Heinrich, 1995; Mettke-Hofmann et al., 2002) and is commonly quantified along a spectrum from neophilia (attraction for novel stimuli) to neophobia (repulsion for novel stimuli; Martin and Fitzgerald, 2005). Our study therefore confirms anecdotal reports of ‘novelty response’ (Heinrich, 1976) or neophobia (Forrest & Thomson 2008) in bumblebees. Additionally, our finding that the flower colour in itself had an effect on the bee’s response to a novel flower is consistent with earlier studies which showed that bees have innate colour preferences (Lunau et al., 1996).

Sampling new food sources can be risky. While doing so, foragers might spend valuable time on unprofitable flower species (e.g. orchids mimicking nectar producing flowers; Jersakova et al., 2006) or risk being attacked by ambush predators lurking on flowers (such as crab spiders; Ings & Chittka 2008). Following this view, ‘neophilic’ bees might take greater risk in term of predation and might jeopardise their foraging efficiency more than ‘neophobic’ bees. Nevertheless, the gains from sampling new food sources are potentially high. As Chittka et al. (1999), Mettke-Hofmann et al. (2002) and Martin and Fitzgerald (2005) pointed out, exploiting known resources is only advantageous if the foraging environment changes little over time. Foraging environments, however, appears to be ever-changing, across and within days (Goulson, 2003; Heinrich, 2004). Therefore, sampling new food sources will often be rewarding in the long term. By keeping themselves up-to-date with the resources available in their environment, bumblebee’s workers run less risk of suffering from the depletion of their current food source. It is conceivable that temporally variable conditions in terms of predation threat and foraging conditions could maintain the variability in terms of the ‘neophilia-neophobia’ gradient observed here, or indeed explain a lack of selection towards behaviour consistency in this dimension. Additionally, variability in foragers’ response could be beneficial at the colony level (Müller and Chittka, 2008). A ‘bet hedging’ strategy has often been invoked in bees to account for intracolony variability in traits such as foraging speed-accuracy tradeoffs (Burns and Dyer, 2008) and could well explain some of the variability in response to novelty observed here.

Our results suggest that bumblebees’ behavioural responses towards novel objects are not consistent enough to formally qualify as a ‘personality trait’ in the common use of the term. Many bees showed reasonable consistency (repeatable response to novelty) over a few hours, but very few bees could potentially be described as consistent when the experiment was repeated over three days. Individual bumblebees therefore appear to be inconstant in their response to novelty over period of time longer than a few hours (which is consistent with Dukas’s findings; Dukas, 1994). The observed decrease in ‘consistent bees’ across time is unlikely to be explained by a developmental process (Sinn et al., 2008a), because the change in response to novel targets was not predictable from worker age. It can also not be explained by an adaptive response to variation in environmental context, for example changes in predation threat or flower profitability, because all these parameters are kept constant in our experiments. Thus our results are more comparable to the more unpredictable variation in mood variability in humans (although hormonal changes sometimes predict such variation) or to the recent study by Pronk et al. (2010) which came to very similar conclusions in the gloomy octopus, *Octopus tetricus*. Pronk et al. (2010) found that individual octopuses exhibited marked repeatability in response to various visual stimuli within a day but not between days, and termed this phenomenon ‘episodic personality'. Both our and the results of Pronk et al. (2010) results emphasise the necessity to investigate personality traits repeatedly, and ideally, over various times scales appropriate to the animals' life span. Single 'snapshots' of an individual's behaviour responses (or even repeated measurements over short time scales) might be misread to indicate individually predictable responses when indeed such responses might fluctuate over longer time scales. As in our study, such variation might not have easily identifiable environmental inducers, or internal contributing factors such as those correlating with age.

5.4.2 Neophilia-neophobia and learning

The results of this experiment clearly suggest that neophobia does have an impact on the number of correct choices made by an individual bee at the initial stage of a discrimination task, with neophobic individuals making fewer correct choices than less neophobic individuals. However, this effect disappeared rapidly with habituation to the novel stimulus (i.e. within the first 50 choices). These results therefore suggest that, except for the very early stages of the discrimination learning process (within the first 10 choices), there was no detectable association between the bees' response to novelty and ability to discriminate between stimuli.

As with any correlation, it is difficult to ascertain which variable is influencing the other. However, I am confident that here there is an effect of neophilia on the performance of the bee at the discrimination task and not *vice versa*. The reason is that the first neophilia-neophobia test (i.e. the first novel colour presented) did not require any learning from the bee compared to the pretraining. The only difference between the last pretraining foraging bout and the first neophilia-neophobia foraging bout was the colour of the flower presented changed from translucent flowers to coloured flowers). Moreover, let’s imagine that some bees appeared neophilic because they could not tell the two colours of the neophilia-neophobia test apart. One would expect such bees to perform poorly at the colour discrimination task, which is the effect opposite to the one observed here. It is therefore unlikely that learning ability had any bearings on the novelty response.

I believe that the likely influence of neophobia on learning initiation has implications for flower constancy and food-source switching in bees. There are at least two potential costs to foraging on several species of flowers or switching from one species to another: (i) searching costs, i.e. the bee needs to find the additional/new species and (ii) exploitation costs: the bee needs to learn to exploit the new food source, which requires for example to learn to handle the new flower species and discriminate it from other species (with potential memory constraints; Worden et al., 2005). Even if all bees had equal learning abilities (but see chapter 4), individual variation in neophobia would still constrain the likelihood for individuals to switch food sources (Forrest and Thomson, 2008) or forage on more than one flower species. Neophobic bees would tend to forage solely on the species they know (as described by Hill et al., 1997) whereas less neophobic bees would be more ‘adventurous’ and be more likely to broaden their food-source range and learn to exploit other flower species.

Further work could investigate potential associations between learning and behavioural traits other than neophilia/neophobia. More stable individual behavioural traits (neophilia/neophobia in bumblebees appears to be an episodic personality trait, see section 5.4.1) such as predation-risk-taking would be good candidates and might reveal complex interactions between an individual’s personality and cognitive abilities in bees.

Chapter 6: Foraging behaviour and response to predator threat: personality traits in the bumblebee *Bombus terrestris*?

6.1 Introduction

The way an individual responds to predation threat is crucial for its survival and hence its fitness. For this reason, an individual's propensity to take risks has been a choice trait for personality studies (e.g. Johnson and Sih, 2007; Smith and Blumstein, 2010; Hollander et al., 2008; Gabriel and Black, 2010), sometimes resulting in counterintuitive findings (as in guppies, *Poecilia reticulata*, where males that took more risks survived for longer; Smith and Blumstein, 2010). This trait is commonly referred to as shyness/boldness or risk taking (for reasons discussed in the introduction, section 1.4, the term risk-taking will be used here instead of shyness/boldness and it will be use to mean risks related to predation only) and is often found to be part of a broader behavioural syndrome (e.g. Johnson and Sih, 2007; Hollander et al., 2008).

The fact that individuals vary in the amount of risk they may take is likely to reflect individual trade-offs. For example, an effective way of avoiding predation, i.e. frequent hiding, may result in an individual catching fewer prey items whereas an individual which spends less time hiding might be more successful at catching prey (as in the spider *Agelenopsis aperta*; Riechert and Hedrick, 1990). In this way, an individual's response to threat might depend on many factors which include the individual's physical condition and past experience (Nicol et al., 2011). Returning to the previous example, the potential costs of predation might be balanced by potential costs to reproduction in a starved individual compared to a well fed one (McNamara and Houston, 1994).

The effect of predation on workers from social species (such as bumblebees) has implications for the colony in terms of fitness (e.g. loss of productivity and resources have to be invested in replacing individuals). One can therefore expect to find predation-avoidance strategies in bumblebees. Some of the best studied predators of bumblebees are the crab spiders (Thomisidae, Ings and Chittka, 2009; Dukas, 2001; Dukas and Morse, 2003; Théry and Casas, 2002), which are sit-and-wait predators that ambush pollinators on flowers (Ings and Chittka, 2009). Ings and Chittka (2009) showed that avoidance of crab spiders by bumblebees is predominantly a learned response (through unsuccessful predation attempts; Ings and Chittka, 2008).

Ings and Chittka (2008) showed that bumblebees take more time to inspect flowers when the crab spiders are cryptic than when they are conspicuous. However, nothing is known about the responses to predation threat at the level of the individual. Do individual bees consistently differ in their response to predation threat? And if they do, are these differences dependent on intrinsic factors (e.g. genetic factors, size and age)?

6.2 Material and Methods

Four colonies of *Bombus terrestris* containing approximately 40 workers each were obtained from Syngenta Bioline (Weert, Netherlands). Queens of this species mate only once in their life; therefore all the foragers within a colony are full sisters (Schmid-Hempel and Schmid-Hempel, 2000). Upon delivery, colonies were transferred into bipartite wooden nest boxes (28×16×11 cm). All tested bees were individually tagged with Opalith number tags (Christian Graze KG, Germany). The nest box in which the colony was housed was connected through a plastic tube to a foraging flight arena (120×100×35 cm) covered with a

UV-transparent Plexiglas lid. Bees could be allowed one at a time into this arena using a system of shutters built in the connecting tube. The room in which the colonies were kept had an average ambient temperature of 21ºC. Controlled illumination for laboratory experiments was provided by high-frequency fluorescent lighting (TMS 24F lamps with 4.3 kHz ballasts; Philips, The Netherlands) fitted with Activa daylight tubes (Osram, Germany) to simulate natural daylight above the bee flicker fusion frequency (Dyer and Chittka, 2004). Foragers were initially allowed to collect 50% (w/w) sucrose solution from translucent gravity feeders (Von Frisch, 1967, p19, Fig. 18) which provided unlimited supplies in the flight arena for two days.

Eight artificial flowers (henceforth ‘flowers’) were vertically presented on the wall of the arena opposite the nest entrance. Each flower consisted of a small yellow plastic tube protruding slightly from the wall, which was covered in laminated green paper (Frisk Coverseal Film Rolls; Artcoe, Manchester, UK and khaki, Maya coloured card; Clairefontaine, Ottmarsheim, France). It was framed by two vertical arms covered in yellow sponge material. Each arm was linked through the wall to a forceps-like contraption which, when pressed, squeezed the two sponge pads together (Fig. 6.1). A forager could be captured and held between the two sponge pads, thus mimicking a failed predatory event by a crab spider (Ings and Chittka, 2009). As all flowers were identical, there was no cue indicating the location of the ‘predator’. This way, any potential inter-individual differences in discriminatory or learning abilities were excluded as potential factors contributing to the behavioural response. Additionally, since bees could not learn to detect the simulated predators, they could not learn to avoid them, ensuring that all bees experienced the same number of simulated predation attempts.

The flowers were regularly refilled with 50% sucrose solution throughout the foraging bout, by inserting the tip of a micropipette through an aperture in the back of wall (the quantity of sucrose solution added was adjusted to each bee’s crop content, see below). Both refilling the flowers and squeezing the sponge pads together could be done from outside the arena, therefore preventing any undesired disturbance to the bee’s foraging behaviour.

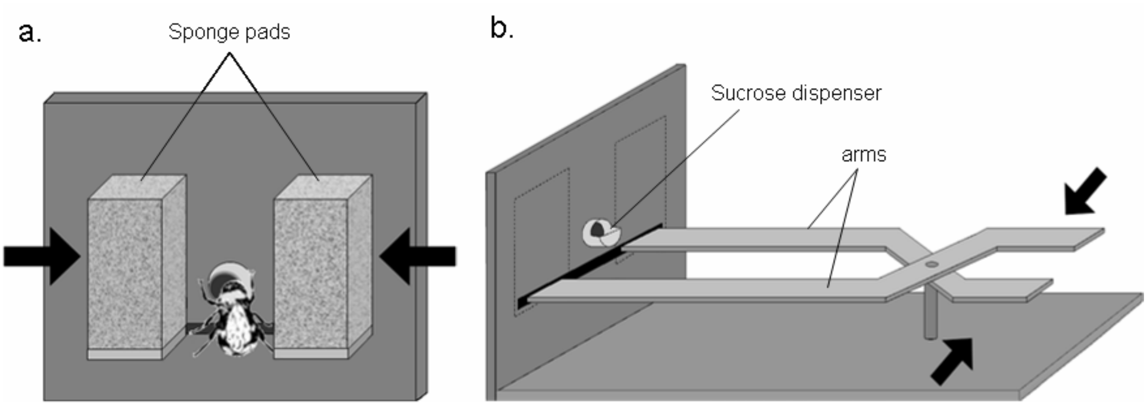


Figure 6.1: Artificial spider apparatus. a. Shows a bee’s view of the flower with the two movable sponge pads and the sucrose dispenser. b. Shows a view from the back of the arena with the two arms which the experimenter can squeeze to simulate the predation attempt and the aperture allowing to refill the sucrose dispenser.

Foragers were collectively trained to use the flowers for two to three hours. Following this collective training, a focal bee would be isolated to forage alone in the arena. The quantity of sucrose solution available in each flower was adjusted to the bee’s crop content so that she would visit at least eight flowers within a single foraging bout. The behaviour of the bee was then recorded using a video camera (Canon Legria FS20) during five consecutive foraging bouts (no predation simulations). On the sixth foraging bout, upon her second or third landing on a flower, the bee would be ‘caught’ by the artificial spider for two seconds and then released. This simulated an unsuccessful predatory attempt, equivalent in nature to a situation where a crab spider captures a bee which manages to break free after a struggle. The same procedure was repeated for foraging bouts 7, 8, 9 and 10 (five simulated predation attempts in total, one per foraging bout). I could therefore compare the foraging behaviour of the bee during the first five foraging bouts, when the bee was predator-naïve, to the last five foraging bouts when the bee would be subjected to a predation simulation at the beginning of each foraging bout. 25 bees were tested in total, three from colony A, nine from colony B, four from colony C and nine from colony D.

6.2.1 Analyses

The distributions of all the variables were positively skewed. For this reason, the median, instead of the mean, was chosen as the average (the median is resistant to extreme observations and therefore is a better descriptor of the data) and either parametric tests with transformed data (e.g. Box-Cox transformation) or non-parametric tests (e.g. Spearman’s rank correlation coefficient) were used. All statistical tests were performed using SPSS 16.0 (2007, SPSS Inc) and the Box-Cox transformation was performed using R 1.12.2 (2010, The R Foundation for Statistical Computing).

6.2.2 Interflower flight time

The interflower flight time was the amount of time (to the nearest 0.1s) a bee spent between leaving the flower she was feeding from and landing on the next flower (post-predation flight time excluded and repeated visits excluded, see below).

I aimed to ascertain whether interflower flight time fulfils the requirements for a personality trait, namely individual difference and consistency. In order to test for individual differences, a Kruskal Wallis test was performed using the median interflower flight time for each foraging bout for each bee (each bee was representing a different ‘sample’). To assess individual consistency, Kendall’s W test was computed, also using the median interflower flight time. Kendall’s W test, or ‘concordance test’ is a non-parametric test which quantifies the agreement between ranks of two or more variables and has been used in other studies to assess the consistency of behavioural traits (e.g. Briffa et al., 2008). Kendall's *W* ranges from 0 (no agreement, i.e. no consistency) to 1 (complete agreement, i.e. perfect consistency: individuals keep the same rank across foraging bouts).

In addition to assessing the potential of interflower flight time as a personality trait, the putative effects of the factors foraging bout, size and colony on interflower flight time were investigated. A GLM repeated measure model was computed, with individual bees as subjects, foraging bout as a within-subject factor (10 levels), size as a covariate and colony as a between-subject factor. As the data are known to be positively skewed, they were transformed using the well-known Box-Cox transformation (Box and Cox, 1964) which is a kind of power transformation and depends on a single parameter λ that can be estimated from the data (here, $\lambda = -0.74$).

Finally, a Wilcoxon test was used to test for the hypothesis that bees would adjust their interflower flight time in response to predation threat (N = 5 pairs of data: the median interflower flight time, all bees pooled, of a non-predation-threat foraging bout was paired with the median interflower flight time for its corresponding predation-threat foraging bout). Similarly, the same test was computed at the individual level (N = 5 pairs of data: for a given individual, the median interflower flight time of a non-predation-threat foraging bout was paired with the median interflower flight time for its corresponding predation-threat foraging bout).

6.2.3 Post-predation flight time

The post-predation flight time was the amount of time (to the nearest 0.1s) a bee spent between the moment she left the flower on which she had experienced the predator simulation and the moment she landed on the next flower. The same analyses as for interflower flight time were performed. The Kruskal Wallis test used the five measures of post-predation flight time available per bee and so did Kendall’s W test. Bee D5 was excluded from the analyses as she stopped foraging immediately after each predation threat (so no post-predation flight time could be measured). A similar GLM repeated measures model was computed with factors identical to the previous analysis (except for the within-subject factor foraging bouts which had only 5 levels instead of 10). The data were transformed using the Box-Cox transformation (λ = -0.13). A Spearman’s rank correlation coefficient was also calculated for the bees’ size and the difference between median interflower flight time and median post-predation flight time as variables.

6.2.4 Component behaviours of post-predation flight time and interflower flight time

For a subset of bees (N = 12), further variables were extracted from the videos. Within post-predation flight time, four behaviours were recorded (to the nearest 0.1 s) immediately after the predation simulation: feeding, still/defensive, inspecting and fly-away. Still/defensive was characterised by the bee remaining motionless after the predation simulation (sometimes lying on its back with protruding sting). Inspecting corresponded to slow hover-flight within four centimetres of the flower towards which the bee’s head was oriented. Fly-away is fast flight further than 4cm away from a flower (or any flight where the head was not oriented towards the flowers). In order to determine what changes in behaviour resulted from the predator encounter, the same behaviours needed to be recorded for a landing for which there was no predation simulation. Landing, feeding, inspecting and fly-away behaviours were therefore recorded for the landing immediately preceding the predation simulation (there were no still/defensive behaviours as there was no simulated predation event). As this analysis used only a small number of bees, correlation coefficients with other factors (such as size and age) were not calculated (in order to detect a correlation with N = 12 at $\alpha = 0.05$ and with a power of 80%, one would have required a size effect of 0.72 at least; SISA).

6.2.5 Are interflower flight time and post-predation flight time different?

Direct observation of the bees during the experiment suggested that immediately after a simulated predation attempt, bees tended to take much longer to land on the next flower than for previous and subsequent flower visits. To confirm this observation, Kolmogorov-Smirnov tests were used to test whether the distribution of post-predation flight time and interflower flight time differed. The Kolmogorov-Smirnov test is a non-parametric test which can be used to compare the distribution of two samples (Ho: the two samples are drawn from the same distribution).

The distribution of post-predation flight times comprised the five post-predation flight time values for each of the 24 bees, (N = 120), and the distribution of interflower flight times comprised all the interflower flight times available for all the bees (total N = 2251). Additionally, a Spearman’s rank correlation coefficient was calculated to check for any correlation between the median interflower flight time (one value per bee) and the median post-predation flight time (one value per bee).

6.2.6 Size

The bee’s body size was estimated using the maximum thorax width, which is the most common estimate of body size for bumblebees (Goulson et al., 2002; Spaethe and Chittka, 2003). The maximum thorax width was measured three times per individual and the average of these three measures was used in the analyses (22 bees measured).

6.2.7 Age

In a subset of bees (N = 15) the identification tags were applied within 48 hours of emergence from the pupae, so the age at the time of experiment could be determined. As the number of bees for which age was available was relatively small, age was not included as a covariate in the GLM analyses. Instead, Spearman’s rank correlation coefficients were calculated for age and median interflower flight time, age and median post-predation flight time, age and median feeding duration, age and repeated visits and age and size.

6.2.8 Feeding duration

The time spent by the focus bee feeding on each flower was recorded to the nearest 0.1s using the event-recording software ETHOM (Shih & Mok, 2000) during video analysis. As for interflower flight time and post-predation flight time, individual differences (Kruskal-Wallis test) and consistency (Kendall’s W) were investigated. Wilcoxon tests were performed to assess whether individual bees modified their feeding duration (decreasing it or increasing it) using the median feeding durations for each foraging bout (five pairs of values). To investigate whether the bees which have longer interflower flight time are bees which are slow in other behaviours, a Spearman’s rank correlation coefficient for feeding duration and median interflower flight time was calculated. Finally, a Spearman's correlation coefficient was calculated for median feeding duration and size.

6.2.9 Repeated visits

It was observed that some bees tended to land repeatedly on the flower they had just fed from. To determine whether this behaviour could be considered as a personality trait, the median numbers of repeated visits per individual per foraging bout were subjected to Kruskal-Wallis and Kendall's W tests. As for feeding duration and interflower flight time, Wilcoxon's tests were performed for individual bees, using pairs of median feeding durations for corresponding foraging bouts. Spearman's correlation coefficients were calculated for repeated visits and median feeding duration and repeated visits and size.

6.3 Results

6.3.1 Are individual bees consistent with respect to interflower flight time?

Individual bees significantly differed from each other (Kruskal Wallis test: $\chi^2 = 1694.6$, d.f. = 24, $P < 0.001$; see Fig. 6.2 for individual median landing latencies) and were significantly consistent in their median interflower flight time (Kendall's W test: W = 0.78, d.f. = 24, P <0.001; similar results were obtained when foraging bouts without or with predation simulation only were considered separately). Interflower flight time therefore appears to fulfil the requirements for a personality trait (i.e. individual difference and consistency; Dingemanse et al., 2009).

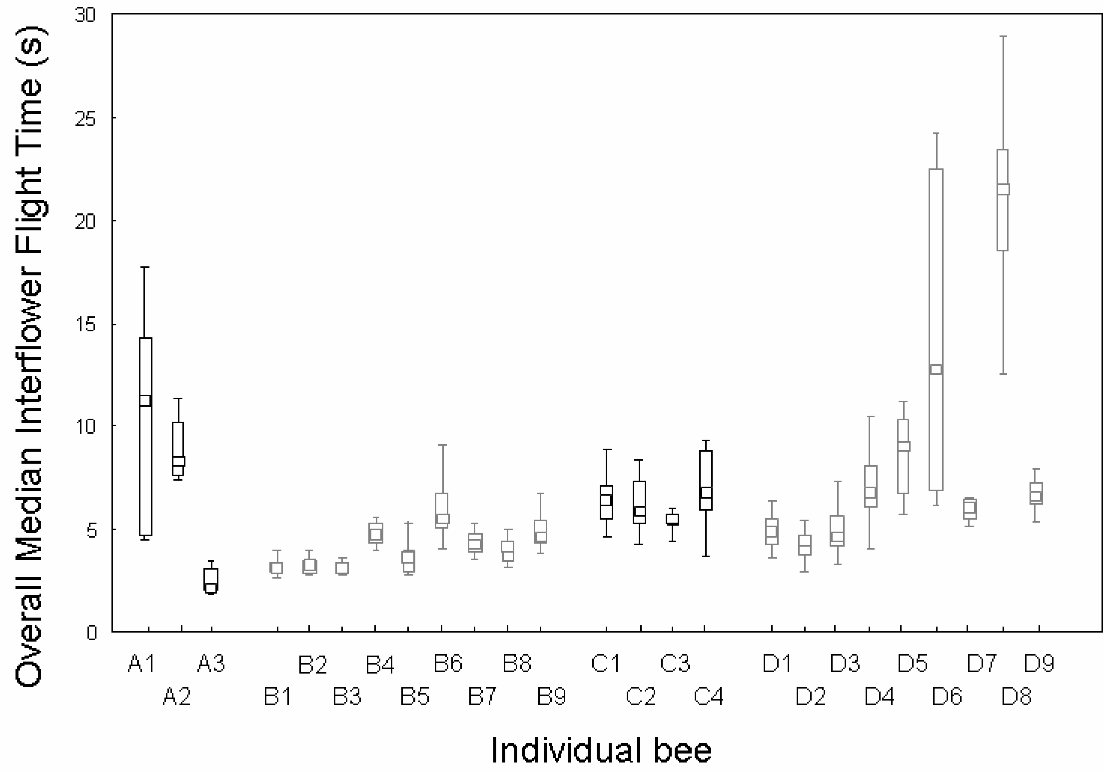


Figure 6.2: Box-plot representing the values of the interflower flight time for each individual. Squares are the median, rectangles are the inter-quartile ranges and whiskers are the 5th and 95h percentiles. The letters in the bees' names represent the colony from which they originate (colony A, B, C or D).

6.3.2 Is interflower flight time affected by factors other than the individuality of the bees?

There was a significant effect of foraging bout within bees (ANOVA: $F_{4.03,68.6} = 3.22$, $P = 0.017$) and a significant effect of size depending on foraging bout (interaction: $F_{4.03,68.6} = 3.08$, $P = 0.021$), within bees. The significant within-subject effect of size, depending on foraging bout, is characterized by a negative correlations between interflower flight time and size for the first five foraging bouts (Fig. 6.3).

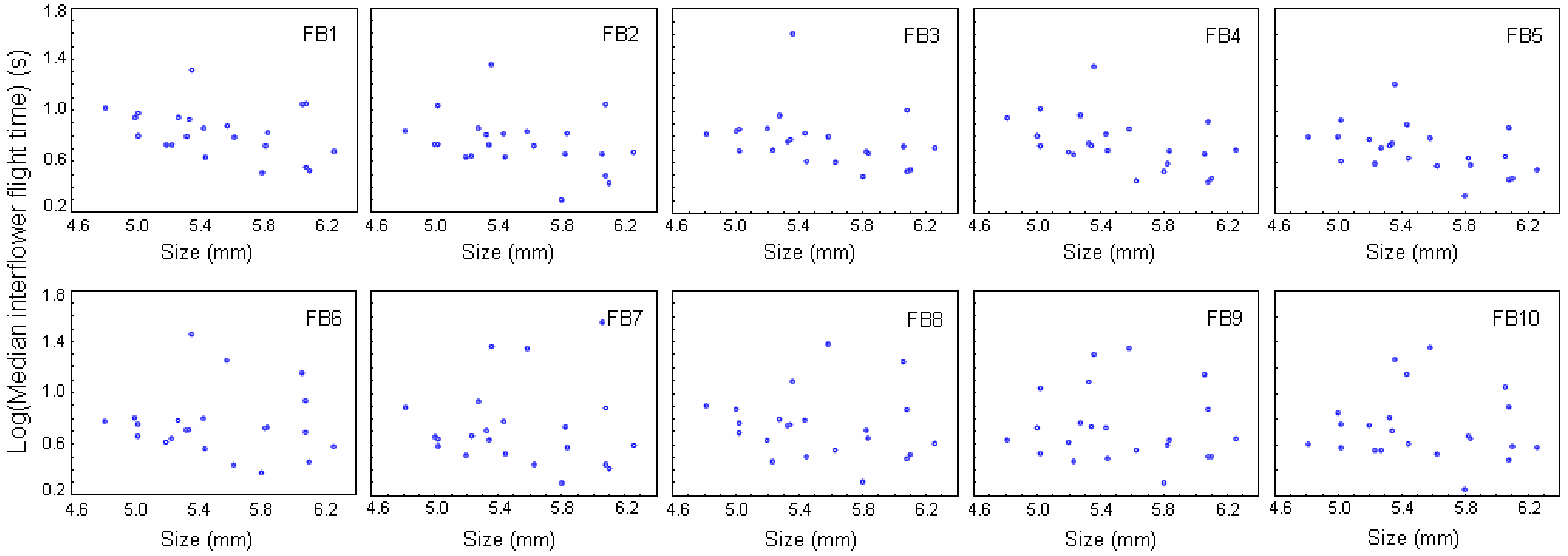


Figure 6.3: Pairwise correlations between the median interflower flight time and the size of the bees for each foraging bouts (FB). Corresponding correlation coefficients (Spearman's rho) are presented in the table 6.1.

		FB1	FB2	FB3	FB4	FB5	FB6	FB7	FB8	FB9	FB10
size	Spearman'	-0.37196	-0.33955	-0.46893	-0.49774	-0.52572	-0.17571	-0.21051	-0.27062	-0.10659	-0.10568
(N=22)	P value	0.088269	0.122097	0.027703	0.018413	0.011975	0.434131	0.34704	0.223177	0.636851	0.63974

Table 6.1: Spearman' rank correlation coefficient for median interflower flight time and size for each foraging bout. The Spearman's rank correlation coefficients for the first five foraging bouts were substantially higher than for the last five foraging bouts.

There was no significant effect of colony within or between subjects (within subjects: $F_{12.11,68.6} = 1.15$, $P = 0.337$, between subjects: $F_{3,17} = 2.28$, $P = 0.115$) and there was no effect of size between subjects ($F_{1,17} = 0.142$, $P = 0.711$). The discrepancy between within-subjects and between subjects effects for size could be due to the fact that some bees decreased their median interflower flight time after predation threat whereas others increased their median interflower flight time (see below for more details).

6.3.3 Do bees modify their interflower flight time in response to predation threat?

There was no significant difference in median interflower flight time before and after predation threat (Wilcoxon test: $Z = -1.461$, $N = 5$, $P = 0.144$). However, this could be explained by individual bees responding in opposite ways to the predation threat (some with decreasing median interflower flight time and others with increasing median interflower flight time).

At the individual level, the median interflower flight time significantly differed before and after predation simulation for eight bees. Amongst these eight bees, five decreased their interflower flight time (bees B6, B8, C2, D1, and D3) and three increased their interflower flight time after the predation simulation (bees A1, B2 and D6; see Fig. 6.4a, b and c).

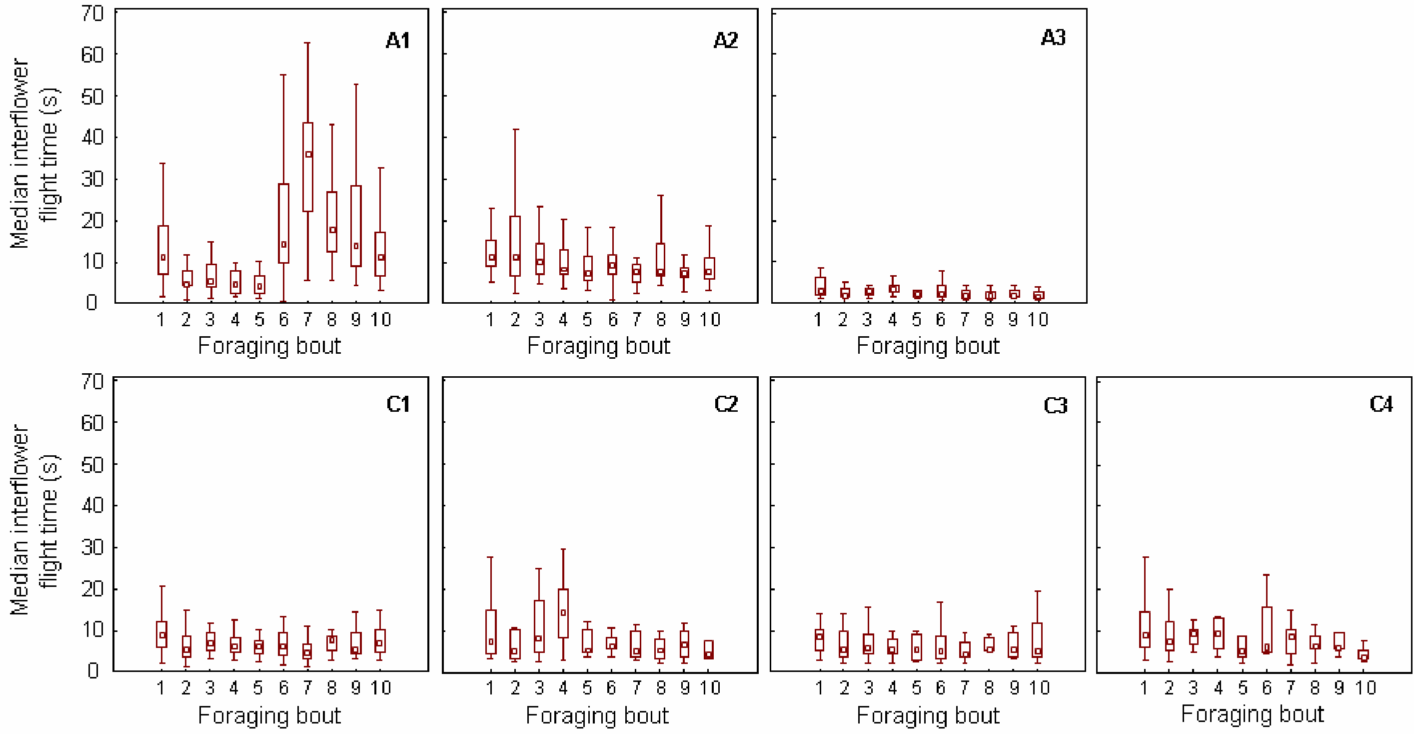


Figure 6.4a: Box-plot representing the median interflower flight time (small squares), interquartile range (rectangles) and 5th and 95h percentiles (whiskers) for each of the ten foraging bouts (numbered 1-10) for each individual bee (one graph per bee, the bee's ID number is the top right corner: the letter stands for the colony to which the bee belonged). Foraging bout 1-5 were devoided of any predation simulation whereas there was one predation simulation for each foraging bout 6-10. The bee C2 was found to significantly decrease its median interflower flight time when the first five and last five foraging bouts were compared (Wilcoxon test: Z=2.02, N=5, p=0.04) and A1 was found to significantly increase its median interflower flight time (Wilcoxon test: Z=2.02, N=5, p=0.04).

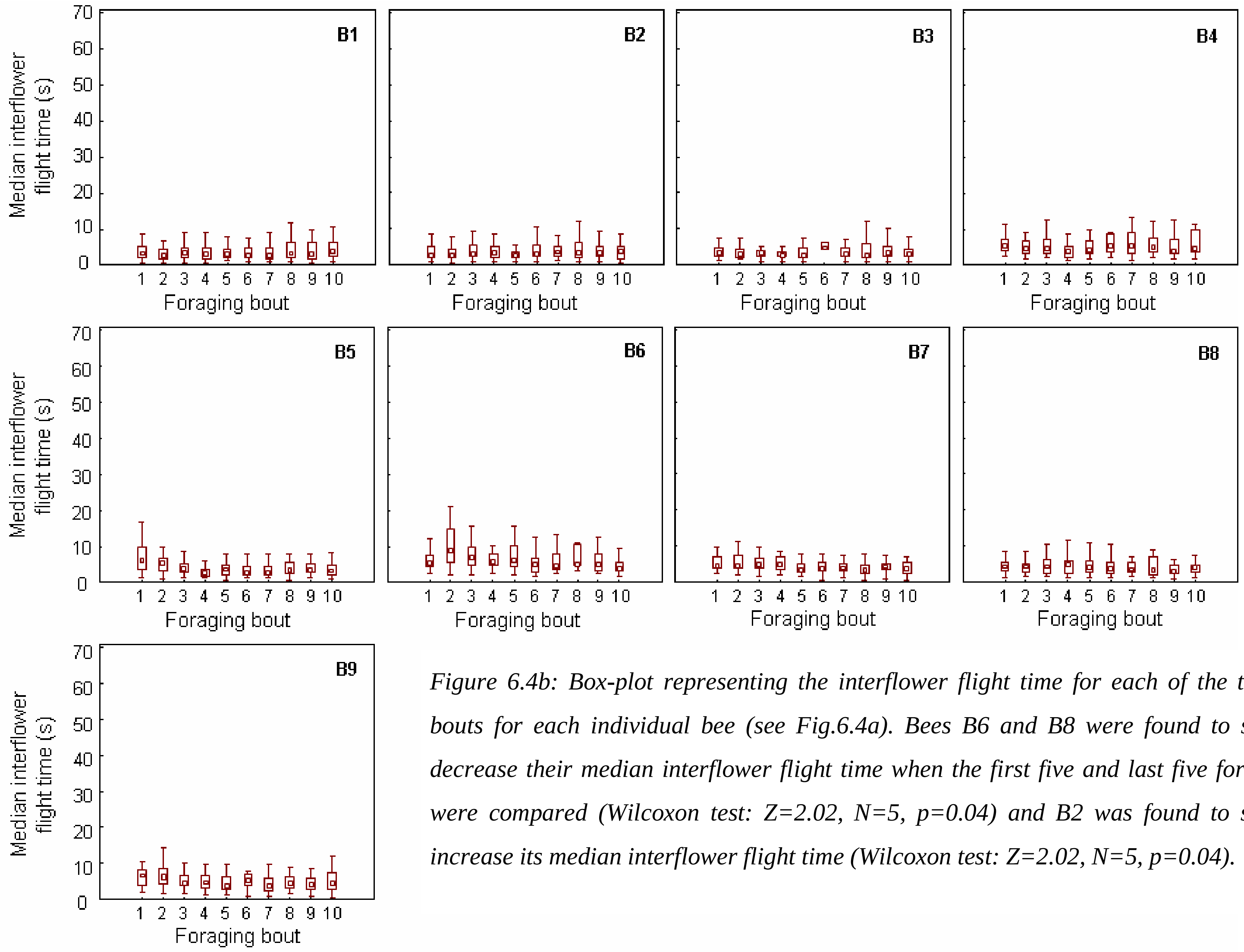


Figure 6.4b: Box-plot representing the interflower flight time for each of the ten foraging bouts for each individual bee (see Fig.6.4a). Bees B6 and B8 were found to significantly decrease their median interflower flight time when the first five and last five foraging bouts were compared (Wilcoxon test: Z=2.02, N=5, p=0.04) and B2 was found to significantly increase its median interflower flight time (Wilcoxon test: Z=2.02, N=5, p=0.04).

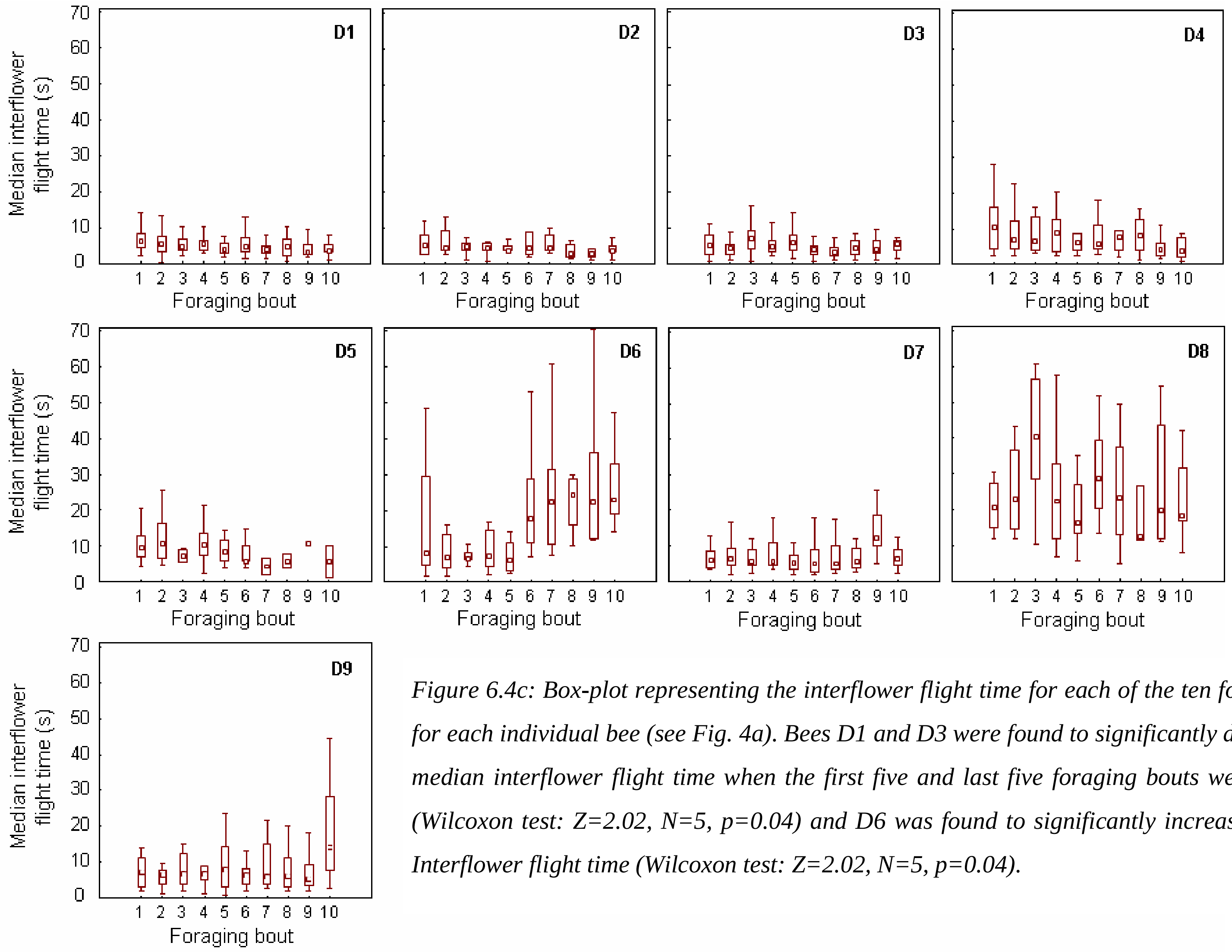


Figure 6.4c: Box-plot representing the interflower flight time for each of the ten foraging bouts for each individual bee (see Fig. 4a). Bees D1 and D3 were found to significantly decrease their median interflower flight time when the first five and last five foraging bouts were compared (Wilcoxon test: Z=2.02, N=5, p=0.04) and D6 was found to significantly increase its Median Interflower flight time (Wilcoxon test: Z=2.02, N=5, p=0.04).

6.3.4 Are individual bees consistent with respect to post-predation flight time?

Individual bees significantly differed from each other (Kruskal Wallis test: χ^2 = 62.5, d.f. = 23, *P* < 0.001, see Fig. 6.5) and were significantly consistent in their post-predation flight time (Kendall's W test: W = 0.51, d.f. = 23, *P* < 0.001). Post-predation flight time therefore appears to fulfil the requirements for a personality trait.

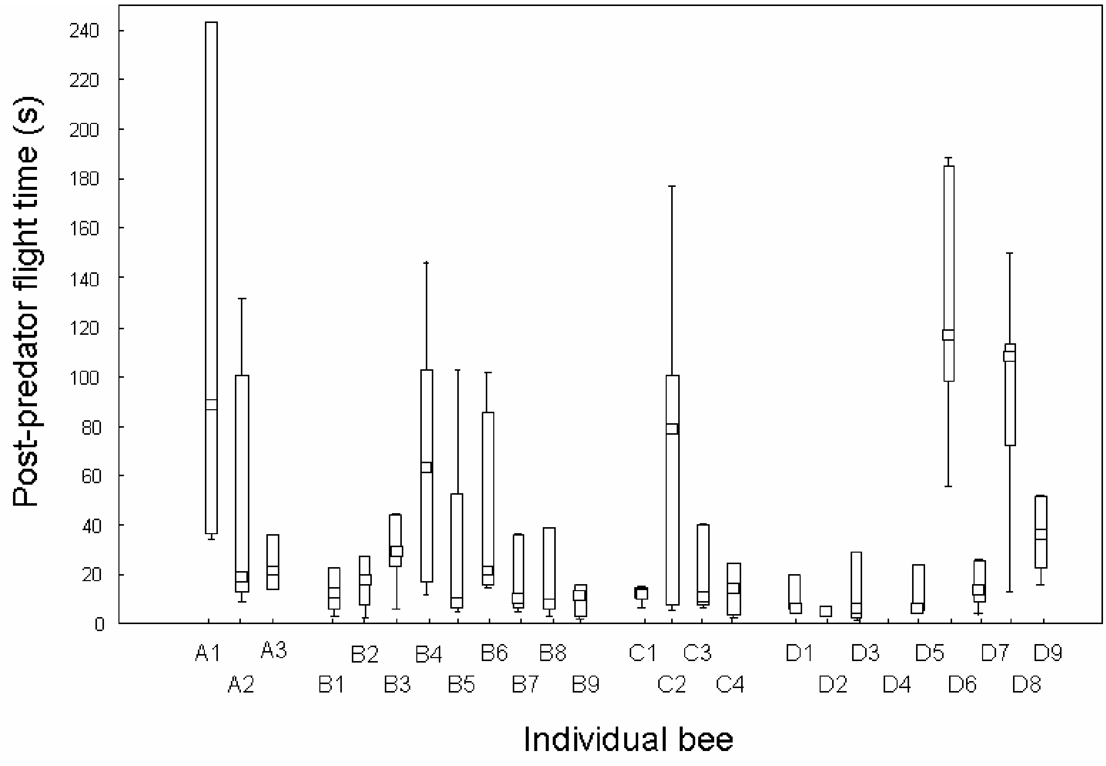


Figure 6.5: Box-plot representing the values of the post-predation flight time for each individual (squares are the median, rectangles are the inter-quartile ranges and whiskers are 5th and 95h percentiles.

6.3.5 Is post-predation flight time affected by factors other than the individuality of the bees?

The number of repeated measures (five per subject) and the number of subjects (N = 24) were relatively low. This resulted in a very low detecting power for the within subject analysis (maximum observed power for all factors = 46%), drastically reducing the probability to detect any potential effect of within-subjects factors. The between-subjects analysis showed a significant effect of size ($F_{1,16}$ = 5.55, P = 0.03) but no significant effects of colony ($F_{3,16}$ = 2.01, P = 0.15).

There was no significant correlation between size and the median post-predation flight time (Fig. 6.6). However, bees with a thoracic width below 5.3mm tended to have lower median post-predation flight time compared to larger bees; in other words, these bees were almost unresponsive (four out of the six smallest bees had a difference of less than 2s between their median post-predation flight time and median interflower flight time).

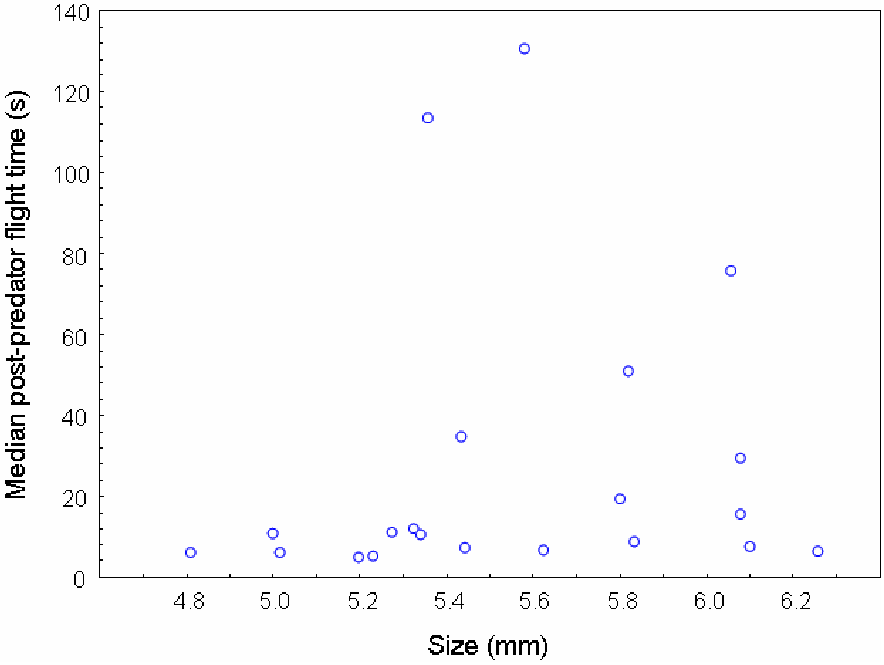


Figure 6.6: Non significant relationship between the median post-predation flight time and size (Spearman's rank correlation coefficient: r_s = 0.37, N = 21, P = 0.10).

6.3.6 Are interflower flight time and post-predation flight time significantly different?

If interflower flight time and post-predation flight time are two different behaviours (despite being both partly dependent on an individual's flight speed), then I would expect interflower flight time and post-predation flight time to have different distributions and to be uncorrelated. The Kolmogorov-Smirnov test showed that interflower flight time and post-predation flight time do not come from the same distribution (Z = 5.29, N = 2371, $P < 0.0001$). However, there was a borderline, non-significant, correlation between post-predation flight time and median interflower flight time (Fig. 6.7).

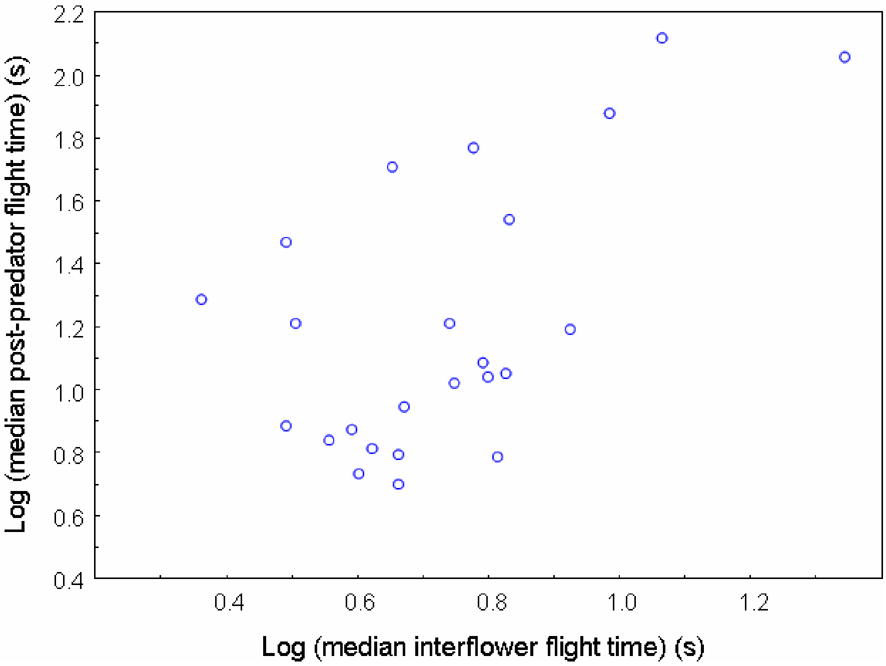


Figure 6.7: Correlation between the median post-predation flight time and the bees' interflower flight time (Spearman's rho: $r_s = 0.39$, *N = 24, P = 0.06).*

6.3.7 Age

There were no significant correlations between age and median interflower flight time (Spearman's rank correlation coefficient $r_s = 0.11$, N = 15, $P = 0.71$), age and median post-predation flight time ($r_s = 0.42$, N = 14, $P = 0.134$), age and median feeding duration (: $r_s = 0.16$, N = 15, $P = 0.56$), age and repeated visits ($r_s = 0.03$, N = 15, $P = 0.91$) and age and size ($r_s = 0.26$, N = 14, $P = 0.37$).

6.3.8 Feeding duration

Individual bees significantly differed (Kruskal-Wallis test: $\chi^2 = 153.5$, d.f = 24, $P < 0.001$) and were significantly consistent (Kendall's W test: W = 0.61, d.f. = 24, $P < 0.001$). Only 5 bees out of 25 significantly modified their feeding durations in the last five foraging bouts (all $Z < -2$, $P < 0.05$) and four out of these five bees increased their feeding duration. There was no significant correlation between the median feeding duration and size (Spearman's rho: $r_s = -0.06$, N = 22, $P = 0.77$). However, there was a significant correlation between median feeding duration and median interflower flight time (Spearman's rho: $r_s = 0.62$, N = 25, $P < 0.001$).

6.3.9 Repeated visits

Individuals bees significantly differed (Kruskal-Wallis test: $\chi^2 = 97.49$, d.f. = 23, $P < 0.001$) and were significantly consistent (Kendall's W test: W = 97.5, d.f. = 23, $P < 0.001$). Only 3 bees out of 25 significantly modified their number of repeated visits and all 3 decreased it (Wilcoxon test: all $Z < 2.02$, N = 5 pairs, all $P < 0.043$). There was a significant correlation between repeated visits and size (Spearman's rho: $r_s = 0.44$, N = 21, $P = 0.045$).

There was a borderline correlation between median feeding duration and repeated visits (Spearman’ rho: $r_s = -0.39$, N = 24, $P = 0.057$).

6.4 Discussion

When foraging, individual bumblebees differed from one another in a consistent manner over the duration of the experiment. Such consistent individual variation in behaviour has been described as animal personality traits in other animal species (e.g. Bell, 2007a; Dingemanse et al., 2010a). I identified two components of a foraging personality trait: interflower flight time and feeding duration. A bee’s interflower flight times were linked to her size, with larger bees tending to have shorter flight durations than smaller bees (although this trend decreased with experience).

Size did not appear to have any significant effect on feeding duration. However, feeding duration and the number of repeated visits made by a bee might vary inversely and the number of repeated visits made by a bee was positively correlated to its size. Additionally, feeding duration and interflower flight time were positively correlated. These findings suggest that there is a trend for larger bees to feed by making short but repeated visits to the same flower until it is emptied with short flying bouts between different flowers, whereas smaller bees tend to empty a flower in fewer, longer feeding bouts with longer flying bouts between different flowers. As there was no significant effect of colony (and all workers within a colony are sisters), I am unable to comment on a potential influence of genetic factors on foraging behaviour.

When faced with a predation threat, not only did 11 out of 25 bees significantly modify at least one behavioural trait for the remainder of the experiment, but also, all bees but one presented a short-term response (substantial increase in interflower flight time for the landing immediately following the predation simulation). However, only a few bees appeared to modify their foraging behaviour (i.e. interflower flight time, feeding duration and repeated visits) in a way which could be intuitively interpreted as part of a predation-avoidance strategy. For example, one would expect that the presence of a concealed predator would result in the bees spending more time inspecting each flower before landing (as in Ings et al., 2008), which would result in an increase in their interflower flight time. However, this was rarely observed. Out of the eight individuals who did significantly modify their interflower flight time when predation was simulated, only three increased their interflower flight time. The remaining five actually decreased their interflower flight time. A similar, counter-intuitive, pattern was found for the feeding duration: amongst the five individuals which significantly modified their behaviour, four increased their feeding duration, which would potentially result in increased exposure to an ambushed predator. In the case of at least three of the five bees (B6, C2 and D1, see Fig. 6.4a, b and c) which appeared to decrease their interflower flight time, this trend could be explained by habituation: the decrease in interflower flight time did not coincide with the onset of predation simulation, but rather seems to be a gradual process which started before the predation simulation. The bees must have simply become more skilful at landing on the flowers with experience. However, this explanation does not hold for the two remaining bees (B8 and D3, see Fig. 6.4b and c), as the decrease in interflower flight time appears to coincide with the onset of predation simulation.

As far as 'feeding duration' was concerned, the significant increase observed in some individuals could be accounted for in another way. Due to the position of the camera recording the bee's behaviour, it was impossible to determine whether the proboscis of the bee was extended or not. The bee was assumed to be feeding when she was positioned over the sucrose dispenser. However, it is possible that the bee might not have been imbibing sucrose solution. Instead, she might have been scanning her immediate surrounding for visual and olfactory cues of a predator, thus explaining the increased ‘feeding’ duration (this is supported by the fact that the quantity of sucrose solution available in each flower remained constant throughout the experiment, so some of the ‘feeding’ time must have been spent in other ways).

If individual interflower flight time and feeding duration sometimes appeared to change in counterintuitive ways, the post-predation flight time after each predation simulation however appeared to match my expectations. As mentioned above, all 25 bees but one responded to the predation simulation by taking more time to land on a flower immediately after it. This increase in interflower flight time is explained by three factors: (1) there was a slight increase in the time bees spent inspecting the flowers before landing, but more importantly, (2) there was a substantial increase in the time bees spent flying away from the flowers and (3) bees displayed a new behaviour, the still/defensive behaviour. Unlike what was claimed in Ings and Chittka (2008)’s experiment, most of the bees did not maintain higher landing latencies throughout the foraging bouts. This could be due to the fact that the predators in the present case could not be associated with any cues which could be learned by the bees. The absence of cues could have made it unnecessary for the bee to invest time in inspecting further the flowers prior to landing. If this is true, then it would be consistent with Ings and Chittka (2008)’s conclusion that the increase in inspection time arises from increased investment in discrimination between predatory and non-predatory cues. Additionally, Ings and Chittka (2008)’s results might be partially explained by the fact that they did not separate post-predation flight time from interflower flight time and that they used the mean, rather than the median, which is much more influenced by extreme values. Extreme 'interflower flight times', such as the post-predation flight time, could have inflated the average interflower flight time in Ings et al. (2008) compared to our findings.

In Ings and Chittka (2008)'s experiments with cryptic and non-cryptic spiders, the size of the bee was not found to have any significant effect on inspection time (a component of interflower flight time). Here, I found that there was no correlation between the size of the bees and their post-predation flight time; however, the smallest bees appeared to be much less responsive than larger bees. In other words, large bees slowed down proportionally more when presented with a predation threat than smaller bees. Goulson et al. (2002) and Spaethe and Weidenmüller (2002) propose several hypotheses to explain differences in foraging speed due to the bee's size. However, most cannot apply to situations when there is a risk of predation. Two which can be applied even appear to contradict the present findings. Spaethe and Weidenmüller (2002) proposed that small bees might be put at a disadvantage compared to large bees, given that they might suffer from lower visual discrimination abilities (Spaethe and Chittka, 2003; Spaethe and Weidenmüller, 2002). This could account for some of the variation in efficiency between large and small bees in other experimental and natural settings, but it is unlikely to explain the variation in interflower flight time observed here as there was no discriminatory task for the subjects to solve (there were no cues associated with the simulated predation events) and small bees actually took less time to land after a predation threat than larger bees. Goulson et al. (2002) argued that foragers tend to be large because large bees are at lower risks from predation by crab spiders than small bees. If differential predation risk indeed modulates foraging speed depending on size, then this could explain the finding here that small bees have longer interflower flight times than large bees but it would certainly contradict the present finding that small bees have shorter post-predation flight times than larger bees. How might one explain this phenomenon? This effect could be attributed to a difference in the operation of the artificial spiders. Here, the ‘spiders’ were operated manually, whereas they were operated mechanically in Ings and Chittka (2008)’s experiments. It is possible that larger bees could have experienced greater pressure as the maximum compressibility of the foam was reached and this would be consistent with the observation that small bees had shorter landing latencies immediately after the predation simulation. Moreover, the conclusions which can be drawn from this experiment are limited by the lack of a control for time as a potential confounding factor. Indeed, the conclusions could be strengthened or light might be shone on some hard-to-explain phenomena if the behaviour of some bees which were never exposed to predation simulation had been recorded for 10 foraging bouts in the same conditions (control). The behaviour of the control group could then have been compared to the behaviour of the bees exposed to predation threat.

The behavioural response observed immediately after the predation threat was very similar to fear responses observed in vertebrates (e.g. Koolhaas, 1999; Lang et al., 2000) and spiders; Riechert and Hedrick, 1990; Riechert and Hedrick, 1993): the bees were responding to a threat by fleeing (flying away from the flowers or avoiding foraging as for bee D5), fighting (defensive behaviour or, as in bee A2, attack of the spider apparatus) or freezing (‘still’ behaviour). The unusual behaviour of bees D5 and A2 are unlikely to be mere idiosyncrasy (which is different from personality, see introduction, section 1.1) as other bees presented intermediate phenotypes (e.g. bee A3, B1 and B3 attacked the artificial spider once each and bee B9 landed on the nest entrance prior to resuming foraging after the simulated predation threat). Such ‘unusual’ bees are more likely to represent extremes of other behavioural axes. In accordance with this view, 13 out of 24 bees appeared to show persistent and significant modifications of their foraging behaviour following simulated predation (responsive bees), meaning that the remaining bees did not modify their behaviour, or did so in ways which could not be detected with the methods used here (less/unresponsive bees). When behavioural change did occur, it must be reflecting a change in the bee's perception of the level of threat in her environment, and therefore possibly represent an attempt at minimizing the predation risk. The modifications of the bees' internal state in anticipation of a negative stimulus could be paralleled to anxiety-like state, which is a state reflecting anticipation of a fear-triggering stimulus found in vertebrates (Lang et al., 2000) and honeybees (Bateson et al., 2011).

Hollander et al. (2008) found that in great tits (*Parus major*) individuals differed in their strategies against predators, depending on their personality type. Here, the personality spectrum identified had slow foragers (long landing latencies and long feeding durations) and fast foragers (short landing latencies and short feeding durations) for extremes. These personality types responded differently to simulated predation: slow foragers also tended to be slow at resuming foraging after a predation simulation whereas fast foragers resumed foraging quicker. Foragers therefore exhibited variation in their anti-predator strategies depending on their foraging personality (which is linked to the bees' size: large bees tending to be fast foragers and small bees tending to be slow foragers).

The present findings of slow to fast personality types corroborates previous work by Chittka et al. (2003), where bees could be classified on a fast-and-sloppy to slow-and-accurate continuum. In addition, the present study demonstrates that the 'foraging speed' of the bee is consistent through time (over the few hours) and situation (no predation threat versus predation threat) and that it correlated to some intrinsic properties of the bee (i.e.

size) and to the bees' response to predation threat. As for many other personality traits, foraging behaviours might not necessarily be fixed and a bee's personality might change with time (see chapter 5) and experience (e.g. bees A1 and D6 became substantially 'slower' after experiencing a predation threat).

Why do I observe this positive correlation between foraging speed and predation response? As discussed in Chittka et al. (2009) and Ings and Chittka (2008), the increase in time spent inspecting flowers resulted in a more accurate detection (and avoidance) of the threat. Slowing down foraging speed is therefore a way by which bees can decrease the predation risk they take. Could it be that small bees are at greater risk of falling victim to crab spiders and thus spend more time inspecting flowers than larger bees (Goulson et al., 2002)? This seems unlikely given that large bees were proportionally more responsive to predation threat than small bees.

In conclusion, bumblebees vary individually and consistently in their foraging behaviour and in their response to predation threat (which includes their propensity to take risk), in qualitative (types of behaviour exhibited) and quantitative (frequency/intensity of occurrence of the behaviour) terms, and this individual variation in behaviour is likely to reflect complex individual tradeoffs which include size and sensory, motor and cognitive abilities and experience.

Chapter 7: General discussion and conclusion

The overall aim of this thesis has been to investigate the existence of personality traits in bumblebees comparable to those found in non-pollinators and non-eusocial animals and to test for a potential link between individual behaviour and cognitive abilities. The data presented here suggest that bumblebees indeed display personality traits similar to those found in other animals and that an individual's personality could interact with its abilities, affecting further the individual's behaviour.

7.1 Can bumblebee personality be compared to personality in other animals?

Individual bumblebees were found to vary consistently in their ability to discriminate between two different stimuli and this individual effect was maintained across modalities and dimensions. Learning ability is not, strictly speaking, a personality trait (see section 1.1) but it is relatively easy to conceive that, in both human and non-human animals, consistent individual variation in learning abilities could result in consistent individual variation in behaviour and hence, personality (Nettle, 2006a; Chiappe and MacDonald, 2005). I did find that there is a relationship between individual bees' novelty response and their ability to discriminate between stimuli, i.e. that there was a negative correlation between the bees' response to novelty (i.e. degree of neophobia) and their ability to discriminate between stimuli. This finding is comparable to findings in starlings (Boogert et al., 2006) and rats (e.g. Hernádi et al., 1997). It would be premature to claim that the basis for vertebrate and insect personality is the same. Insect and vertebrate personality might be a case of convergence (and so could be the product of different mechanisms), but it would certainly be worthwhile to investigate it further.

I showed that there are many similarities between the personality traits I found in bumblebees and the personality traits found in other animals. As many species of vertebrates do (Heinrich, 1995; Mettke-Hofmann et al., 2002), bumblebees responded to novel objects by investigating them extensively before first accepting them as a food source and individuals could be positioned on a neophilia-neophobia continuum (Müller et al., 2010 and chapter 5). Similarly, the bees' response to a predation threat (chapter 6) was very reminiscent of risk-prone and risk aversive strategies as well as of the fear responses observed in vertebrates (e.g. Koolhaas, 1999 and Lang et al., 2000), i.e. the characteristic flee, fight or freeze responses. As discussed in section 1.1, I believe that this study provides sufficient support for the use of the terms neophobia-neophilia and risk-prone/risk-averse to describe particular behaviours of bumblebees in so much as they appear analogous. Again, I should stress that I do not imply that there is continuity between the bumblebee's and human's behaviour, but rather that these personality traits represent convergences between insect and human behaviour. One needs to keep in mind that nothing is known of the mechanisms resulting in the observed behavioural traits in bumblebees and that given their mode of reproduction (i.e. rearing sisters instead of direct offspring) the selective pressures applying to individuals of the bumblebee species are likely to differ from the pressures applying to any other animals in which personality was studied.

The present work also showed that, for the one trait measured over several days, i.e. novelty response (see chapter 5), individual bees were consistent within a day but changed their response between days. A similar phenomenon was found in the gloomy octopus *Octopus tetricus* by Pronk et al. (2010). Pronk et al. (2010) judged that such rapid changes in behaviour were too unstable for the observed behavioural traits to qualify as true personality traits. Instead, Pronk et al. (2010) named this changing personality ‘episodic personality’. By definition, personality traits are consistent across time (see section 1.1): when assessing the consistency of behavioural traits through time, the choice of an appropriate time scale is left to the experimenter (some studies of ‘personality traits’ are even based on a single measure per individual e.g. Dochtermann and Jenkins, 2007; Hollander et al., 2008; Minvielle et al., 2002; Bolivar and Flaherty, 2004). It is therefore possible that episodic personalities are more widespread than it appears because the time-scales selected by experimenters were too short to highlight them. In humans, where personality has been studied extensively, individuals are known to change personality through time (e.g. Asendorpf and van Aken, 1991). Changes in personality were also found to occur in the dumpling squid, *Euprymna tasmanica* (Sinn et al., 2008a), where the behaviour of some individual changed when they reached sexual maturity. It is possible that the observed variation across days in novelty response (chapter 5) could in fact reflect adaptive behavioural plasticity. Individuals in both Pronk et al. (2010) and the response to novelty experiment in chapter 5 could have responded to an unidentified environmental or internal cue (e.g. hormonal factor) and adapted their behaviours accordingly on a daily basis. As emphasised by Sinn et al. (2008a), there is a great need for more studies on the variation in personality throughout an individual’s lifetime. Life-long studies would be very helpful in defining a timeframe for the study of personality and therefore in determining whether episodic personality is a special case of personality and what role episodic personality is likely to play in an individual’s life-history.

7.2 Bumblebee personality and task division

In social insects, individuals specialise in a certain task, sometimes for their entire life. Bumblebee workers are totipotent (i.e. workers can perform a variety of tasks such as brood care, nectar foraging, incubating or fanning; Weidenmüller, 2004; Cartar, 1992): there is no strong evidence for age polyethism in this species (Jansen et al., 2009) and different patrilines cannot account for the task specialisation observed (*Bombus terrestris* queens are singly mated; Schmid-Hempel and Schmid-Hempel, 2000). How then do workers end up specialising in certain tasks? As discussed in section 3.1, specialization might be explained by developmental and nutrition factors as well as individual variation in response threshold to an environmental stimulus (Weidenmüller, 2004). In addition, recent works have shown that individual experience (Ravary et al., 2007; Chittka and Müller, 2009) and physical changes (such as mandible and wing wear; Foster and Cartar 2010; Schofield et al. 2011) during an individual's life-time can also affect an individuals' behaviour and task specialization.

In the present work, I showed that individuals differed in their ability to discriminate between stimuli in the environment. This ability is very likely to affect an individual's foraging performance (Raine and Chittka, 2008). We also know that in ants, individuals have been shown to specialise in a task at which they were successful and to switch tasks when they were unsuccessful (Ravary et al., 2007), and we know that a honeybee's ability to detect sucrose in solution (i.e. sucrose sensitivity) is related to the task she will specialise in (e.g. bees very sensitive to sucrose tended to be pollen foragers instead of nectar foragers; Page and Scheiner, 2006). It is therefore likely that the ability of an individual bee to discriminate between potential food sources could have an impact on whether it will specialise in a foraging task. Similarly, the fact that individuals appear to vary in their behaviour with regards to novel objects and predation threat is likely to affect task specialisation. Predation-risk-averse individuals, for example, might give up foraging and specialise in within-nest tasks after an encounter with a predator (as did some of the bees in this study see chapter 6). The extent to which personality might affect task specialisation, however, needs to be investigated since we know that the behavioural trait ‘response to novelty’ also varies with time within individuals. One could imagine that different individuals will be differently successful depending on their behavioural response and environmental conditions at the time of foraging, which might result in complex interactions between episodic personality and task specialisation. It will be interesting to explore experimentally whether there is a link between an individual’s learning ability or its level of predation-risk-aversion or neophobia and its likelihood to become a forager. Such an ability-based division of labour relies on the reasonable assumption that labour division must be efficient. As discussed in section 3.1.1, empirical assessment for this assumption is scarce and recent work on *Temnothorax albipennis* ants (Dornhaus, 2008) indeed does not support this view; one should keep in mind that the presence of division of labour does not necessarily imply that division of labour is adaptive (Jeanson et al., 2008).

If an individual’s personality is indeed found to affect its task specialisation, then it will no doubt have interesting consequences at the colony level. Wray et al. (2011) describes what they call ‘collective personalities’ in honeybees’ colonies. In other words, there appears to be consistent variation between colonies in a range of collective behaviours and Wray et al. (2011) suggest that these consistent variations have an impact on the colonies’ fitness (and the same was true for differences in learning abilities, see Raine et al., 2006b). One could speculate that the proportions of particular personality types (and the variety of individual abilities) within the colony would affect the ‘collective personality’ of the colony and hence the (inclusive) fitness of the individuals composing it.

7.3 Conclusion and future directions

At the beginning of this study, I set myself the following aims: to assess the existence of personality traits in *Bombus terrestris* and, if personality traits were found, to investigate a possible link between personality and individual ability. I believe that this work established that *Bombus terrestris* workers have personalities, even if these personalities might be found on relatively short time-scales. Individual bumblebees appeared to respond to novelty and predation threat in ways that were very similar to vertebrates and such responses were found to be predictable for a particular individual (given its personality type) within a day (though it was found to change across days). This work also discussed the relevance of these findings for task specialisation in eusocial insects and shed light on what appears to be a promising area of research: the (episodic) personality of bumblebee workers is likely to have consequences not only at the individual and colony level, but may also have cascading effects (through their role as pollinators) on complex ecological communities.

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As the proverb goes, it takes a whole village to raise a child. In my case, it took a whole department and more to enable me to complete this work.

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