Modulators of foraging behavior by nectar-feeding bats (*Glossophaginae*)

Behavioral plasticity, resource defense,
social group composition and daily energy expenditure

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Contents

Summary ..... **vii**

Zusammenfassung ..... **ix**

CHAPTER 1: General Introduction **1**

1.1. The origins of animal personality research 1

1.2. Evolution of consistent individual differences in behavior 2

1.3. Consequences of intra-specific behavioral variation 3

1.4. Quantifying within- and between-individual variation 4

1.5. Glossophagine bats 5

1.6. Scope of the thesis 6

References 7

CHAPTER 2: Flexibility and behavioral plasticity are independent traits in the nectar-feeding bat Glossophaga commissarisi **11**

2.1. Introduction 12

2.2. Methods 14

2.2.1. Subjects and study site 14

2.2.2. Experimental setup 15

2.2.3. General experimental procedure 16

2.2.4. Measuring of sampling rates 16

2.2.5. Contextual plasticity of sampling behavior 17

2.2.6. Behavioral Flexibility 18

2.2.7. Statistical Analysis 18

2.3. Results 19

2.3.1. Individual differences in contextual plasticity 19

2.3.2. Individual differences in behavioral flexibility 21

2.3.3. No correlation between contextual plasticity and flexibility 22

2.4. Discussion 23

References 27

CHAPTER 3: Increasing foraging costs cause a decrease in individual daily energy expenditure in the nectar-feeding bat Glossophaga commissarisi **31**

3.1. Introduction 32

3.2. Methods 34

3.2.1. General description of the experiment 34

3.2.2. Foraging activity and estimates of individual daily energy expenditure (DEE) 35

3.2.3. Sampling rate of unrewarding flowers 36

3.2.4. Statistical analysis 36
3.3. Results 37
3.4. Discussion 42
3.4.1. Influence of foraging costs on DEE 42
3.4.2. Individual differences in DEE 45
References 46
CHAPTER 4: Resource defense in the nectar-feeding bat Glossophaga soricina 49
4.1. Introduction 50
4.2. Methods 52
4.2.1. Subjects and housing 52
4.2.2. Experimental setup 52
4.1.1. Experimental procedure 52
4.1.2. Chasing behavior 54
4.1.3. Statistical Analysis 55
4.2. Results 56
4.3. Discussion 63
References 66
CHAPTER 5: Changes in social group composition do not disrupt individual behavioral differences in the nectar-feeding bat Glossophaga soricina 69
5.1. Introduction 70
5.2. Methods 72
5.2.1. Subjects and housing 72
5.2.2. Experimental setup 73
5.2.3. General procedure 73
5.2.4. Behavioral traits 75
5.2.5. Foraging traits 77
5.2.6. Statistical Analysis 77
5.3. RESULTS 79
5.3.1. Effects of resource distribution 79
5.3.2. Repeatability of behavioral traits 79
5.3.3. Influence of social environment on individual differences in multiple behavioral traits 81
5.3.4. Influence of individual differences in behavior on foraging performance 83
5.4. Discussion 86
References 90
CHAPTER 6: Conclusions and future directions 93
References 95
Appendix 97

A 1. Chapter 2: Supplementary material 97
A 1.1. Individual differences in acquisition rates 97
A 1.2. Change of individual sampling rates throughout the night (Chapter 2) 97
A 2. Chapter 4: Supplementary material 98
A 2.1. Scratch marks on wings of Glossophaga soricina 98
A 2.2. Proportion of visits at flowers depending on social status 99
A 2.3. Distribution of visits across flowers in the male-only group 100
A 2.4. Pattern in automatically collected data indicating chasing 100
A 3. Chapter 5: Supplementary material 101
A 3.1. Repeatability estimates of foraging activity and agonistic behavior depending on the experimental regime 101
A 3.2. Repeatability estimates of behavioral traits 101
A.3.3. Correlation of individual activity between the clumped and distributed resource condition 102
A.3.4. Correlation of individual aggressiveness between the clumped and distributed resource condition 103
List of contributions 104
Acknowledgements 105

SUMMARY

Similar to the distinctive behavioral characteristics that shape human personality, animals also show individual differences in their behavior that are consistent over time and across contexts. Animal personality research aims to answer the questions of why and how these consistent individual differences in behavior evolve within a population of a given species, how these differences persist and what their ecological relevance is. In order to further explore these questions, I investigated different aspects of foraging behavior by neotropical, nectar-feeding bats of the genus *Glossophaga* not only to investigate consistent individual differences in their behavior but also to test assumptions and predictions proposed in the field of animal personality research. By using an experimental setup consisting of artificial, computer-controlled flowers, it was possible to conduct all experiments in a controlled but naturalistic environment.

However, individuals not only differ consistently in their individual behavioral characteristics but they can also differ in how plastically they adapt their behavior to changes in the environment. A common assumption is that behavioral plasticity is a general trait in which individuals differ because some animals might be generally more responsive to environmental stimuli than others. In the second chapter I tested this prediction by quantifying two types of plasticity within the same individuals. During foraging *Glossophaga commissarisi* constantly have to decide between exploiting known resources and sampling of new, potential more profitable flowers. During the first series of experiments one type of behavioral plasticity, namely contextual plasticity, was quantified by measuring to which extent individuals adjust their sampling rate in response to decreasing food availability. During the second series of experiments, a reversal learning paradigm was used to assess individual differences in behavioral flexibility, another type of behavioral plasticity. I could show that, contrary to expectations, contextual plasticity and behavioral flexibility were independent traits in these bats. This result challenges the common assumption of behavioral plasticity being a single trait and illustrates the need of further studies that measure more than one type of plasticity within the same individuals.

Furthermore, increasing evidence suggests individual differences in behavior covary with physiological and life-history traits. In particular the covariation of individual differences in metabolic rates and behavior has been investigated in various species. However, most studies have focused on individual differences in basal or resting metabolic rates, although only the measure of daily energy expenditure includes energy spent on actual behavior. In the third Chapter, the relationship between individual behavior and metabolic rates was investigated in *Glossophaga commissarisi* and the results show that individuals not only differed in their daily energy expenditure but also in how much their daily energy expenditure decreased in response to increasing foraging costs. Additionally, it could be

shown that individuals with the highest increase in foraging activity in response to increasing foraging costs also had a higher daily energy expenditure and invested more in the exploration of flowers. These results confirm that also in *Glossophaga commissarisi* consistent individual differences in behavior correlated with differences in metabolic traits and that individuals differed indeed in their daily energy expenditure.

The fourth chapter focused on aggressive resource defense in *Glossophaga soricina*. Contrary to nectarivorous birds, aggressive resource defense has rarely been studied in flower bats. Although, free living nectarivorous bats have been observed to occasionally defend profitable flowers aggressively, not much is known about the social structure and the extent to which resource defense influences the nectar intake of individuals. In addition to the further investigation of resource defense behavior in these bats, changes in the resource distribution during the experiment also provided the possibility to test a prediction from theoretical considerations of interference competition. It is suggested that aggressive interactions increase the more resources are spatially concentrated. After developing a method to fully automatically quantify aggressive interactions during foraging, I was able to show that in mixed-sex groups only males successfully monopolized flowers. However, contrary to subordinate males which experienced a severe reduction in their nectar intake, females seem to be unaffected by aggressive interactions. Furthermore, in accordance with the theoretical prediction, the amount of aggressive interactions was significantly higher when resources were spatially concentrated. These results show, for the first time, sex-dependent differences in the resource defense behavior in a nectarivorous bat.

However, not only ecological factors like resource availability, but also social factors can influence behavior. The social niche construction hypothesis predicts that repeated social interactions and competition avoidance can lead to consistent individual differences in behavior, thereby proposing a mechanism that can explain how consistent individual differences emerge and persist despite of the unpredictability of social interactions. The experiments presented in the last chapter were designed to assess the influence of social factors on the expression of individual behavior during foraging. Therefore, the influence of social group composition on the expression of multiple behavioral traits was quantified. The results show that the influence of the social environment on individual behavioral trait expression during foraging was minimal. However, some aspects of individual foraging performance were influenced by the behavioral composition of the group.

Altogether, during the experiments of this study it was not only possible to assess individual differences in the foraging behavior of glossophagine bats but also to investigate the role of different mechanisms in shaping these behavioral differences.

Keywords: consistent individual differences, foraging behavior, behavioral plasticity, resource defense, daily energy expenditure, Glossophaginae

ZUSAMMENFASSUNG

Nicht nur Menschen unterscheiden sich in ihrer durch individuelle Verhaltensmerkmale geprägten Persönlichkeit, sondern auch Tiere zeigen stabile individuelle Unterschiede. Die Erforschung der Ausprägung dieser Persönlichkeitsmerkmale in verschiedenen Tierarten bietet die Möglichkeit noch ungeklärte Fragen zu untersuchen. Unter anderem, warum und wie sich diese stabilen Verhaltensunterschiede in einer bestimmten Tierart entwickelt haben, wie diese Unterschiede innerhalb einer Population erhalten bleiben und welche ökologische Relevanz sie haben. Um der Beantwortung dieser Fragen etwas näher zu kommen, habe ich in dieser Arbeit verschiedene Aspekte des Nahrungssuchverhaltens von nektartrinkenden, neotropischen Fledermäusen der Gattung *Glossophaga* untersucht und individuelle Unterschiede im Verhalten quantifiziert. Die Verwendung von künstlichen, computergesteuerten Blüten ermöglichte es die Verhaltensexperimente in einer kontrollierten und dennoch der Situation während der natürlichen Nahrungssuche ähnlichen Umgebung durchzuführen.

Eine Vielzahl von empirischen Studien hat gezeigt, dass Individuen einer Art jedoch nicht nur in der Ausprägung von bestimmten Verhaltensmerkmalen variieren, sondern dass sie sich auch dahingehend unterscheiden können wie stark sie ihr Verhalten an Veränderungen in ihrer Umgebung anpassen. Allgemein wird angenommen, dass die Plastizität von Verhalten ein Merkmal ist in dem sich Individuen generell unterscheiden. Diese Annahme wird dadurch begründet, dass manche Tiere sensitiver auf Reize aus der Umwelt reagieren und daher ihr Verhalten allgemein stärker als andere Individuen an Veränderungen anpassen. Im zweiten Kapitel habe ich diese Annahme überprüft indem ich die Verhaltensplastizität in zwei verschiedenen Situationen in denselben Individuen gemessen habe. Während der Nahrungssuche müssen sich nektartrinkende Fledermäuse der Art *Glossophaga commissarisi* ständig zwischen der Ausbeute bekannter Blüten und der Exploration neuer und potentiell profitableren Optionen entscheiden. Im ersten Teil der Experimente wurde die individuelle Verhaltensplastizität bestimmt, indem gemessen wurde wie stark jedes Tier seine Explorationsrate an eine reduzierte Nahrungsverfügbarkeit anpasst. Im zweiten Teil der Experimente wurde gemessen wie schnell Tiere eine zuvor profitable Blüte verlassen, das heißt wie flexibel sie auf das versiegen einer etablierten Nahrungsquelle reagieren. Entgegen der Erwartungen korrelierte die individuelle Plastizität im Explorationsverhalten nicht mit der Flexibilität mit der die Tiere auf das Versiegen einer zuvor nektargebenden Blüte reagierten. Dieses Ergebnis stellt die Annahme in Fragen, dass manche Tiere generell sensitiver auf Reize aus der Umwelt reagieren und zeigt das verschiedene Arten von Verhaltensplastizität unabhängige individuelle Merkmale sein können.

Des Weiteren lassen empirische Daten aus vorherigen Studien darauf schließen, dass Tiere nicht nur stabile Unterschiede im Verhalten zeigen, sondern dass diese auch mit individuellen Unterschieden in physiologischen Merkmalen korrelieren. Besonderes der Zusammenhang zwischen individuellem Verhalten und Energieumsatz wurde in zahlreichen Arten untersucht. In einem Großteil der empirischen Studien wird jedoch nur die basale Stoffwechselrate berücksichtigt obwohl die tatsächlichen energetischen Kosten von Verhalten nur im täglichen Gesamtenergieverbrauch mit einberechnet werden. Im dritten Kapitel habe ich den Zusammenhang zwischen individuellem Verhalten und Energieumsatz in Glossophaga commissarisi untersucht. Dabei konnte ich zeigen, dass die Tiere sich nicht nur im täglichen Gesamtenergieumsatz unterscheiden, sondern auch wie stark dieser reduziert wird, wenn die Kosten für die Nahrungssuche ansteigen. Außerdem konnte gezeigt werden, dass Tiere welche ihre Aktivität am stärksten an die steigenden Kosten für die Nahrungssuche anpassen, einen höheren Energieumsatz haben und mehr in die Exploration von potentiell profitableren Blüten investieren. Diese Ergebnisse bestätigen, dass auch in Glossophaga soricina individuelle Verhaltensunterschiede mit Unterschieden in physiologischen Merkmalen einhergehen und dass auch im täglichen Gesamtenergieumsatz konsistente individuelle Unterschiede bestehen.

Im vierten Kapitel wurde die in nektartrinkenden Fledermäusen kaum erforschte aggressive Ressourcenverteidigung in *Glossophaga soricina* untersucht. Im Freiland wurden zwar gelegentlich nektartrinkende Fledermäuse dabei beobachtet wie sie Blüten verteidigen, es ist jedoch kaum etwas über die soziale Struktur bekannt und darüber wie stark diese aggressive Ressourcenverteidigung die individuelle Nektaraufnahme beeinflusst. Neben dem Ziel die sozialen Strukturen während der Ressourcenverteidigung besser zu verstehen, wurde außerdem die theoretische Vorhersage überprüft, dass die Aggression zwischen Individuen mit zunehmender Ressourcendichte ansteigt. Nach der Entwicklung einer Methode, welche es erlaubt aggressive Interaktionen während der Nahrungssuche voll automatisiert zu erfassen, konnte ich zeigen, dass nur Männchen in gemischt-geschlechtlichen Gruppen Blüten erfolgreich monopolisieren. Im Gegensatz zu unterlegenen Männchen, welche mit einer stark verminderten Nektaraufnahme konfrontiert waren, schienen die Weibchen von diesen aggressiven Interaktionen nicht beeinflusst zu werden. Die Ergebnisse zeigen außerdem, dass die Anzahl aggressiver Interaktionen tatsächlich zunimmt, wenn die Ressourcendichte ansteigt. Mit diesen Experimenten konnten zum ersten Mal geschlechtsspezifische Unterschiede während der aggressiven Ressourcenverteidigung in Blütenfledermäusen gezeigt werden.

Jedoch können neben ökologischen Faktoren wie zum Beispiel Veränderungen in der Nahrungsverfügbarkeit auch soziale Faktoren individuelles Verhalten beeinflussen. Durch soziale Interaktionen und um innerartliche Konkurrenz zu vermeiden, können soziale Nischen entstehen, welche zu der Entstehung von stabilen, individuellen Verhaltensunterschieden beitragen können. Das Ziel der Experimente des letzten Kapitels war es den Einfluss von sozialen Faktoren auf die Ausprägung individuellen Verhaltens während der Nahrungssuche zu erfassen. Hierfür wurde der Einfluss der sozialen Gruppenzusammensetzung auf mehrere individuelle Verhaltensmerkmale bestimmt. Die

Ergebnisse zeigen, dass in *Glossophaga soricina* die soziale Umgebung nur einen minimalen Einfluss auf die Ausprägung der individuellen Verhaltensmerkmale hat. Allerdings zeigt sich in einer explorativen Analyse, dass das durchschnittliche Verhalten der Gruppenmitglieder die individuelle Nahrungssucheffizienz beeinflussen kann.

Insgesamt wurden in dieser Studie nicht nur individuelle Unterschieden im Nahrungssuchverhalten nektar-trinkender Fledermäuse erfasst, sondern es war außerdem möglich die Rolle verschiedener Mechanismen bei der Entstehung von individuellen Unterschieden im Nahrungssuchverhalten von Blütenfledermäusen zu untersuchen.

Schlüsselwörter: Konsistente individuelle Unterschiede, Nahrungssuchverhalten, Verhaltensplastizität, Ressourcenverteidigung, täglicher Energieverbrauch, Glossophaginae

CHAPTER 1
General Introduction

1.1. The origins of animal personality research

In order to describe a familiar person, we do not have to rely exclusively on physical traits like hair color or body size; we can also describe this person's distinctive behavioral characteristics in order to complement the picture. We intuitively associate a familiar person with specific personality traits like "shy", "creative" or "outgoing". Personality can be broadly defined as the characteristics of an individual that describe and account for consistent patterns of feeling, thinking, and behaving (Pervin and John 1999). In human psychology, the study of personality has a long tradition and a major area in personality research is concerned with investigating the structure of personality. One successful strategy to explore the distinctive dimensions of human personality has been the lexicographic approach which is based on the assumption that the vocabulary commonly used to describe the distinctive behavioral attributes of an individual reflects all dimensions of personality. After researchers compiled an extensive collection of words extracted from questionnaires that asked people to describe the distinctive behavioral characteristics of a familiar person, a factor analysis was applied and it resulted in the widely acknowledged division of personality into five distinctive dimensions, the so-called "Big Five": Openness, conscientiousness, extraversion, agreeableness, and neuroticism (reviewed in (Digman 1990)). Interestingly, it has been shown that this structure of personality is highly consistent across cultures and therefore seems to have a solid biological basis (Yamagata et al. 2006).

However, people, particularly those who live or work in close contact with animals, have long known that distinctive individual behavioral characteristics are not confined solely to the human species. This observation led to an early interest in the investigation of personality structures in non-human animals, particularly livestock and pets. The individual behavioral characteristics of dogs have been of particular interest due to the multiple practical applications that arise from the characterization of their personality (Jones and Gosling 2005). For example, information about a dog's personality can be used to improve the matching of the right dog to a home or to try to predict which pups are suitable to be trained as guide dogs or police dogs.

Recently, the interest in so-called animal personality research has grown extensively, especially in the field of behavioral ecology. Similar to human personality research, one goal of investigating consistent individual differences in animal behavior is the exploration and description of the personality structure in the species studied. The results of this research in a large number of species show that

animals from a given population commonly differ consistently in distinctive behavioral traits like aggressiveness, boldness, exploration, sociability and activity (Sih et al. 2004, Réale et al. 2007). Furthermore, these behavioral traits can be correlated at the individual level. For example, highly aggressive individuals from a population of desert grass spiders (*Agelenopsis aperta*) are also bolder after a simulated attack and therefore emerge quicker from hiding (Riechert and Hedrick 1993). Furthermore, aggressiveness can also correlate with exploration as has been shown in great tits (Verbeek et al. 1996). These results from empirical studies in a vast range of species extending from comb-footed spiders (Pruitt et al. 2008) to chimpanzees (King and Figueredo 1997) suggest that consistent individual differences in behavior are not only a human feature but a ubiquitous phenomenon within the animal kingdom. Furthermore, animal personality research has shown that not only single behavioral traits but also correlated traits can be heritable (Stirling et al. 2002, van Oers et al. 2004, Sinn et al. 2006) and that individual differences in behavioral traits can be associated with differences in fitness (Smith and Blumstein 2008).

The existence of consistent individual differences implies that behavior is less flexible than previously thought and therefore individual behavioral plasticity is limited. However, in addition to differences in average behavioral traits, individuals can also differ in their behavioral plasticity (Dingemanse and Wolf 2013, Stamps 2015). Together, these observations lead to several outstanding questions: (1) Why do consistent individual differences in behavior evolve? (2) How are these consistent individual differences maintained within populations? (3) What are the consequences for ecology and evolution? Amongst others, the research field of animal personality tries to find answers to these questions.

1.2. Evolution of consistent individual differences in behavior

Several conceptual frameworks have been developed to explain why consistent individual differences in behavior evolve and how they are maintained within a population of a given species. The life-history approach proposes that individual differences in life-history traits favor the evolution of consistent individual differences in behavior (Wolf et al. 2007, Biro and Stamps 2008). Consistent intra-specific variation in life-history traits like fecundity, time of maturity and growth rate can emerge because of changing selection pressures during fluctuations in both environmental conditions and population density (Reznick et al. 2002). Results of various empirical studies support this prediction by showing that individual differences in life-history traits can indeed be associated with individual differences in behavior, especially with differences in boldness, aggressiveness and exploration (reviewed in (Biro and Stamps 2008)). Recently, this framework has been expanded by incorporating the pace-of-life syndrome theory which predicts that individuals that differ in their life-history-strategy should also consistently differ in physiological traits (e.g. hormone profile, immunity and metabolic rate) (Réale et al. 2010, Le Galliard et al. 2013). However, the direction of these correlations is less

clear and could change depending on the ecological context. Overall, the life-history approach provides a conceptual framework which can explain the evolution of consistent individual differences in behavior by connecting behavioral, physiological and life-history traits.

In addition to individual differences in life-history strategies, the game-theoretic approach proposes that competition for limited resources and negative-frequency dependent selection can also contribute to the evolution of consistent individual differences in behavior (Wolf and McNamara 2012). Intra-specific behavioral variation through negative-frequency dependent selection occurs if the payoff of alternative behavioral strategies decreases the more individuals chose the same strategy. For example, negative-frequency dependent payoffs favor the coexistence of producer-scrounger foraging tactics within populations (Barnard and Sibly 1981, Giraldeau and Caraco 2000). Furthermore, recently Wolf and colleagues presented a theoretical model that shows how negative-frequency dependent payoffs can lead to the emergence of responsive and unresponsive individuals within a population (Wolf et al. 2008). The social niche construction hypothesis generally predicts that in order to reduce social conflict individuals chose alternative behavioral strategies which are maintained via frequency-dependent selection (Bergmüller and Taborsky 2010).

However, negative-frequency dependent selection can only explain intra-specific variation in behavior but it is not sufficient to explain why individuals should behave in a consistent way. Several mechanisms have been proposed that could promote behavioral consistency. For example, positive feedbacks between individual state and behavior could lead to consistent individual differences in behavior (Sih et al. 2015). State variables can encompass a wide variety of factors ranging from individual energy reserves to the recent experience of losing a fight. Learning can be another potential important mechanism that reinforces behavioral consistency because training can lower the costs or increase the benefit of a certain behavior (Wolf et al. 2008). Furthermore, in a changing social environment it might be beneficial to avoid changing the behavioral strategy because switching can lead to conflict and thereby might be costly (Bergmüller and Taborsky 2010).

Together, intra-specific variation in life-history traits and/or negative-frequency-dependent selection of alternative behavioral tactics are powerful conceptual frameworks which generate predictions that can be empirically tested in order to better understand the evolution of animal personality.

1.3. Consequences of intra-specific behavioral variation

Consistent individual differences in behavior within a population of a given species have various implications for many fields, ranging from ecology and evolution to conservation (reviewed in (Wolf and Weissing 2012)). For example, in contrast to the relatively slow process of adapting through mutations, intra-specific behavioral variation can increase the speed in which a population adapts to environmental change because some individuals might already express behavior that will be

advantageous in the new condition. (Barrett and Schluter 2008)). Furthermore, personality differences can also influence the speciation process in the presence of gene-flow in both directions, positive and negatively. Speciation in the presence of gene-flow is driven by disruptive selection and is more likely to occur when the spectrum of resource use is broad (Dieckmann and Doebeli 1999). On one hand, consistent individual differences in behavior can promote specialization and diversification of resource use; on the other hand, individual behavioral variation can also weaken the influence of selection pressures on the population through increasing specialization and the resulting decrease in competition (Rueffler et al. 2006). Furthermore, personality differences can lead to the emergence of socially responsive and unresponsive individuals which in turn can influence the social structure and dynamic of a group (Wolf and Krause 2014). For example, the presence of socially responsive individuals that adapt their behavior to their interaction partners can increase group coordination.

Personality differences in animals can also impact issues in conservation. For example, consistent individual differences in behavior might influence the success of human-introduced, invasive species (Chapple et al. 2012). For example, the dispersal distance of the invasive mosquitofish is related to sociability and less social individuals are more likely to disperse farther indicating a personality-biased dispersal and invasion (Cote et al. 2010). Furthermore, assessing the personality of zoo animals can be implemented into population planning in order to increase animal welfare and to improve guest experience (Watters and Powell 2012). Together these examples show the various implications of consistent individual differences in behavior.

1.4. Quantifying within- and between-individual variation

Generally, animal personality research investigates individual differences in behavior that are consistent over time and/or across contexts. In recent years, several statistical approaches have been established as common analytical tools. Although the exact specifications of statistical tests are mentioned in the methods section of the chapters to follow, the following paragraph describes shortly some of the general principles underlying them.

In order to assess if individuals of a given species differ consistently in a certain behavioral trait, it is essential to quantify the behavior of interest repeatedly in multiple individuals and repeatability is a common measure that quantifies the proportion of the total variance that is explained by differences between individuals. It provides a standardized estimate of the consistency of individual behavior that can be compared across studies (Nakagawa and Schielzeth 2010). In order to calculate the repeatability of a behavioral trait, it is necessary to estimate the different variance components. Mixed-effects models with individuals included as random effects are especially suitable to partition the variance into within- and between-individual variation (Dingemanse and Dochtermann 2013).

However, individuals differ not only in their average behavior but they can also differ in how plastically they adapt their behavior to changes in the environment. Dingemanse and colleagues recently

proposed the behavioral reaction norm approach as a tool to quantify individual differences in behavioral plasticity (Dingemanse et al. 2010). For this, it is necessary to measure individual behavior along the environmental gradient of interest, for example along changes in resource availability or temperature. Individual behavioral plasticity can then be estimated by using random regression models that fit a regression line for each individual along the environmental gradient. The intercept of the individual regression line is the measure of an individual's average behavior and the steepness of the slope is an estimate for behavioral plasticity.

These three statistical approaches, the calculation of repeatability, the partition of variance with mixed effects models and the behavioral reaction norm approach to quantify behavioral plasticity are powerful statistical tools that can be used to analyze a large range of questions concerning the topic of animal personality.

1.5. Glossophagine bats

Throughout this thesis I conducted experiments with two neotropical bat species, Commissarisi's long-tongued bat *Glossophaga commissarisi* (Gardner) and Pallas' long-tongued bat *Glossophaga soricina* (Pallas). Both species are specialized to feed on nectar but their diet also includes fruits and insects (Gardner 1977). Nectarivorous bats are known to have an extremely high mass specific daily energy expenditure due to the limited amount of nectar produced by individual flowers (Voigt et al. 2006) and the high energetic costs of hovering flight while feeding on nectar (Voigt and Winter 1999). In order to cover their energetic demand, nectar-feeding bats have to make several hundred flower visits per night. Additionally, their fat storage capabilities are low (Kelm et al. 2011). Therefore, foraging efficiency and foraging behavior in general are likely to be especially important traits in flower bats (Von Helversen and Winter 2005). In addition to other adaptations, nectarivorous bats have developed several cognitive adaptations to their ecological niche. For example, it has been shown that *G. soricina* is able to estimate small time intervals, and since nectar is a renewable resource, this ability is useful to optimize the time intervals between revisits of flowers (Tölch 2006). Furthermore, in contrast to some plants which bloom for short periods of time with a large number of flowers, most bat-pollinated plants flower continuously for relatively long periods of time by only opening a few flowers per night (Von Helversen and Winter 2005). Therefore, remembering profitable locations in order to increase foraging efficiency is advantageous for these bats and laboratory experiments have shown that *G. soricina* indeed has an exceptional spatial working memory (Winter and Stich 2005).

1.6. Scope of the thesis

Until recently foraging behavior has been studied mainly by investigating the behavior of an average individual in the context of optimal foraging theory. With the exception of alternative foraging strategies for example producer versus scrounger tactics, consistent individual differences in foraging behavior have been rarely taken into account. One goal of this study was to measure consistent individual differences in multiple behavioral traits during foraging in glossophagine bats by conducting experiments with artificial, computer-controlled flowers in a semi-natural environment. However, the experiments were also designed to investigate theoretical predictions and assumptions stated in the field of animal personality research.

One common assumption is that behavioral plasticity is a single trait in which individuals differ because some animals might be generally more responsive to environmental stimuli than others. In the second chapter I tested this prediction by quantifying behavioral plasticity in two different situations but within the same individuals of the species *G. commissarisi*. I measured individual differences in the extent to which bats adapt their exploration during foraging to changes in resource availability and how flexibly they react to the depletion of a previously rewarding option. If behavioral plasticity is indeed a single trait, highly plastic individuals in one situations should be also more plastic in the second situation.

Furthermore, the life-history framework proposes that consistent individual differences in behavior are associated with individual differences in physiological traits like for example metabolic rates because individuals differ in their life-history strategies. In order to evaluate this prediction in *G. commissarisi*, during the experiments presented in chapter 3, I first quantified the individual daily energy expenditure and assessed how it changes due to increasing foraging costs. Then, I explored how individual daily energy expenditure is linked with two behavioral traits, exploration and foraging activity.

Besides extrinsic factors like resource availability or foraging costs, social factors can also influence individual differences in behavior, for example through social niche construction. The experiment presented in the fourth chapter was designed to assess the influence of the social environment on multiple behavioral traits and on the foraging performance in *G. soricina*.

In the fifth chapter I investigate aggressive resource defense behaviour, which has been rarely studied in nectar-feeding bats. Thereby I assessed the social structure and the influence of resource monopolization on individual nectar consumption in *G. soricina*.

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CHAPTER 2
Flexibility and contextual plasticity are independent traits in the nectar-feeding bat *Glossophaga commissarisi*

SUMMARY

Individuals often not only differ in their average behavior but also in how plastic these behaviors can be in response to changes in the environment. In behavioral ecology, contextual plasticity is commonly assessed by measuring the extent to which individuals adapt their behavior along an environmental gradient. However, flexibility, another type of behavioral plasticity, is typically assessed in behavioral neuroscience by quantifying how fast individuals modify their behavior in response to changes in previously established stimulus-response associations. Results of previous studies suggest that highly flexible individuals are more responsive to changes in the environment. This observation led to the hypothesis that behavioral plasticity could be a general trait in which individuals differ and therefore different types of plasticity should be correlated. The goal of this chapter was to explicitly test this prediction in the nectar-feeding bat species *Glossophaga commissarisi* by measuring behavioral flexibility and contextual plasticity within the same individuals. First, individual differences in contextual plasticity were assessed by measuring individual sampling rates. Sampling is part of the exploration/exploitation trade off that these bats face during foraging. They constantly have to decide between exploiting known resources and sampling of new flowers. Contextual plasticity was quantified by measuring to which extent individuals adjust their sampling rate to changes along a gradient of resource availability. Second, a reversal learning paradigm was used to assess individual differences in behavioral flexibility. Thereby bats were confronted with a sudden change of food location and their perseverance to the previously rewarding location was measured. The results show that bats generally increased their amount of sampling in response to decreasing resource availability. Furthermore, individuals differed in both types of plasticity but contrary to expectations these two types of plasticity were not correlated. This result challenges the assumption that behavioral plasticity is a single trait and illustrates the need of further studies that measure more than one type of plasticity within the same individuals.

2.1. Introduction

Individuals of the same species when exposed to the same set of environmental stimuli often differ consistently in their behavioral responses. Individual differences in various behavioral traits have been described in a vast variety of species ranging from insects to mammals (Sih et al. 2004, Réale et al. 2007). Although behavior is thought to be highly flexible, the presence of consistent individual differences in behavior implies that an individual does not express the full range of behaviors present in the general population (Dingemanse et al. 2010a) and therefore might not always behave optimal (Sih et al. 2012). However, despite these limitations, animals can adapt their behavior plastically in response to changes in the environment and in addition to differences in the average behavior, individuals can also differ in their behavioral plasticity (Dingemanse and Wolf 2013).

In the field of behavioral ecology, the behavioral reaction norm approach is commonly applied to investigate individual differences in behavioral plasticity (Dingemanse et al. 2010a). Thereby, the behavior of interest is measured several times along an environmental gradient of for example changing resource availability, temperature or even time. In the simplest case, it is assumed that there is a linear relationship between the individual behavior and the environmental gradient. The elevation of the individual regression line is a measure for the average behavior of the respective individual whereas the slope represents an estimate of the behavioral plasticity. This type of behavioral plasticity is commonly referred to as contextual plasticity (Stamps and Groothuis 2010). Individual differences in contextual plasticity have been found in several species (Dingemanse and Wolf 2013). For example, Ural owls not only differ consistently in their average level of aggressive nest defense but also in the plasticity to adapt their aggressiveness to changes in food availability (Kontiainen et al. 2009) and another example shows that individual great tits differ in how much they adjust their vigilance during foraging to perceived predation risks (Mathot et al. 2011).

In behavioral neuroscience, another type of behavioral plasticity has been extensively studied and is commonly referred to as behavioral flexibility (Coppens et al. 2010). Instead of measuring the immediate behavioral response along an environmental gradient, as it is done to quantify contextual plasticity, behavioral flexibility is in general a measure of how fast an individual modifies its behavior in response to changes in previously established stimulus-response associations (Izquierdo and Jentsch 2012). One possibility to quantify individual differences in behavioral flexibility is the reversal learning paradigm. In the simplest version individuals are confronted with two options that differ in quality, one option is associated with a positive reinforcement whereas the other option remains unrewarding. After individuals have learned to discriminate between these two options and reach a stable discrimination performance, the previously rewarding option suddenly becomes unrewarding and vice versa. Behavioral flexibility is therefore a measure of how fast individuals update their established stimulus-response association and switch to the newly rewarding option.

Animals that show high behavioral flexibility seem to be more responsive to external stimuli compared to inflexible individuals which are more prone to develop routine-like behavior (Coppens et al. 2010). For example, in an experiment male mice were trained to choose the right arm in a Y-maze in order to receive a reward. After the mice had reached a stable performance, a small piece of tape was put into the maze. In the subsequent trials low aggressive individuals which are also more flexible payed much more attention to this disturbance and they started to explore the maze again. More aggressive and inflexible individuals did not react to the piece of tape and they reached the reward as fast as before (Benus et al. 1987, Benus et al. 1990). The same pattern has been also observed in piglets. Individuals that showed high behavioral flexibility in a reversal paradigm conducted with a T-maze where more distracted by a small intra-maze change than inflexible piglets (Bolhuis et al. 2004).

These observations led to the hypothesis that highly flexible individuals not only are more responsive to external stimuli, but might also adapt their behavior to a greater extent to changes in an environmental gradient. This line of reasoning suggests that behavioral flexibility and contextual plasticity should be correlated within individuals (Coppens et al. 2010, Mery and Burns 2010, Sih and Del Giudice 2012, Stamps 2015). However, empirical studies that investigate the potential link between different types of behavioral plasticity are extremely rare (Stamps 2015). The goal of this chapter was to explicitly test how flexibility and contextual plasticity are correlated across individuals. The experiments were conducted with nectarivorous bats of the species *Glossophaga commissarisi*.

During one single night, these bats perform several hundred visits to flowers with renewable nectar reservoirs in order to meet their high energetic demands (Helversen and Reyer 1984). Thereby, they constantly have to decide between exploiting known profitable flowers and investing in the exploration of possible better locations with flowers of unknown state. On the one hand sampling is costly because it consumes time and energy but on the other hand the benefit of finding a new resource can outweigh these costs. According to game-theoretic considerations, the pay-off of finding a new resource should decreases the more individuals invest in sampling and therefore should be negative-frequency dependent (Mathot et al. 2012). Negative-frequency dependent payoffs have been proposed to promote individual differences in behavior (Wolf and McNamara 2012) and individual differences in sampling behavior have already been found for example in great tits (Krebs et al. 1978) and in pigeons (Shettleworth et al. 1988). However, it is less clear how the quality of the currently exploited option influences how much individuals invest in sampling.

Basically, three scenarios of how individual sampling rates are influenced by the current resource quality are possible. First, sampling rates could be positively correlated with the current resource quality because sampling is costly and animals may need enough energy reserves to be able to invest in visiting options of unknown state (Dall and Johnstone 2002). Second, the sampling rate could be a fixed rate that is independent of the current available resource quality, as proposed and implemented by some reinforcement-based learning models (Vermorel and Mohri 2005, Buchkremer and Reinhold 2010), or third, it could negatively correlate with the current resource quality, as it was shown in pigeons

(Shettleworth et al. 1988). This last option implies that low resource quality leads to higher investment in sampling in order to increase the chance of finding possible better options.

Experiments were conducted by using a flight cage setup with computer-controlled artificial flowers that were either rewarding or non-rewarding. By changing the reward probability at the rewarding flowers, it was possible to create a gradient of resource availability and measure how this gradient influences the amount of sampling of unrewarding flowers. In order to assess individual contextual plasticity of sampling behavior, I quantified the amount of sampling of unrewarding flowers repeatedly along the gradient of flower quality. With this procedure, it was possible to fit individual behavioral reaction norms and subsequently assess individual differences in slopes as a measure of individual differences in contextual plasticity (Dingemanse et al. 2010b).

Additionally, I quantified another type of behavioral plasticity in the same individuals, namely behavioral flexibility. To that purpose, the rewarding flowers were switched with previously non-rewarding flowers during the experimental night. Thus, the animals were confronted with an unexpected depletion of a previously profitable option, which means the learned association of a specific location and reward suddenly changed. By measuring the perseverance of an individual to the previously rewarding flower I could quantify how fast individuals update their previously learned stimulus-response association as a measure of behavioral flexibility.

With these two experiments, I was able to measure two types of plasticity within the same individuals, behavioral flexibility and contextual plasticity and thus, it was possible to test the prediction that these two types of plasticity are correlated across individuals.

2.2. Methods

2.2.1. Subjects and study site

The experiments were conducted with 44 adult male bats of the species *Glossophaga commissarisi* Gardner at La Selva Biological Station, Province Heredia, Costa Rica. Bats were attracted to trapping locations by sugar water feeders scented with dimethyl disulphide (von Helversen et al. 2000). Bats were weighed and marked with radio frequency identification (RFID) collars. They were kept in a flight cage (4x6m) with mesh walls and thus under the climatic conditions of the surrounding rainforest until the experiment started. Bats spent at least four days and not longer than fifteen days in this keeping flight cage. During this time, they were fed sugar solution (30% sucrose w/w) with added 3.5g/100ml hummingbird food (NektarPlus, Nekton) and 3.5g/100ml milk powder (Nido 1+, Nestle) ad libitum. Furthermore, they were given local bee-collected pollen and a piece of banana every three days. Three days before the experiment, two artificial flowers were installed in this flight cage to accustom the bats to visit artificial flowers. Every visit at those flowers was rewarded with 50 µl sugar solution (30% sucrose w/w) and all bats visited these two flowers at least five times before taking part in the

experiment. Permission for experimentation was obtained from Sistema Nacional de Areas de Conservación (SINAC) at the Ministerio de Ambiente y Energía (MINAE) Costa Rica.

2.2.2. Experimental setup

Experimental flight cages (4x6m) contained a 2x2m rectangular frame (height 1.5m) on which twelve artificial flowers were mounted, three on each side with flower heads pointing outwards (Fig. 2.1). A single stepper-motor syringe pump delivered the nectar via tubes to each flower and electronic valves controlled the nectar flow (Winter and Stich 2005). Rewards always contained 40 µl of nectar consisting of 20% w/w sugar concentration (sucrose: fructose 1:2). Hovering visits by a bat were detected from the interruption of an infrared light beam at the flower opening and a circular antenna around the flower head received the RFID signal for identification. All visits to flowers were recorded including to non-rewarding flowers during every experimental night from 6pm to 6am. During the experiment, bats were weighed regularly. The reward schedule allowed for individual-specific configurations (PhenoSoft 16 Control, Phenosys GmbH, Berlin, Germany). Two of these flight cages were available for experimentation.

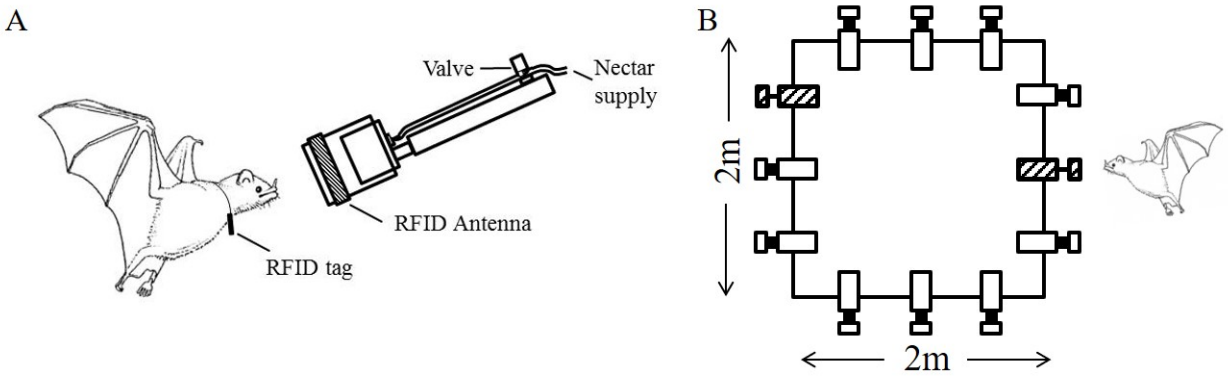

Figure 2.1. (A) Every individual was marked with a radio frequency identification (RFID) tag and could be identified at an artificial flower by detection of its RFID tag at the antenna. In case of reward delivery, the valve opened and nectar was delivered by a computer-controlled pump (not shown) into the flower head. Non-rewarding visits were recorded as well. (B) The experimental setup consisted of twelve artificial flowers that were arranged on a rectangular frame (2x2m, height 1,60m) in a flight cage. During each experimental night two rewarding flowers (hatched) were individually assigned to each bat. The remaining flowers never gave a reward to the respective individual (bat drawing by Holger Braun).

2.2.3. General experimental procedure

During experiments, twelve male bats were tested at the same time in one flight cage. Since it was not possible to catch forty-eight males, four individuals took part in the experiment twice in order to have the same number of individuals in each group. The data of these four individuals was only analyzed for their first participation. On any given night, each bat only received rewards from two out of the twelve artificial flowers and every bat had its own selection of rewarding flowers, in order to prevent social learning. However, since twelve bats used the flowers simultaneously, every flower was used by two different individuals. Pairs of individuals sharing one flower changed between the nights. The rewarding flowers of each bat were always on opposite sides of the frame and changed every night to the two other sides of the frame. New positions every night prevented habituation to specific locations. Rewards were delivered with three different probabilities (30%, 50% and 83%). These values were chosen because the step from 30% to 50% and from 50% to 83% represent the same increase in relative intensity on a psychophysical scale. Psychophysical intensity in a two-choice task is given by the difference of reward probabilities divided by their mean. The relative intensity determines how well an animal can discriminate two stimuli and equal relative intensities lead to similar discrimination performance (Nachev and Winter 2012). In this case (0.5-0.3)/0.5*(0.5+0.3) and (0.83-0.5)/0.5*(0.83+0.5) both equal 0.5. The sequence of rewards/non-rewards for each probability was generated pseudo-randomly using the sample function in R (R Core Team 2015). The first visit of an individual at its rewarding flower was always rewarded. All bats for each probability received the same sequence of rewards/non-rewards at each flower, in order to equalize experience.

2.2.4. Measuring of sampling rates

Sampling was defined as a visit to a non-rewarding flower. Since the positions of rewarding flowers changed every night, bats had to learn new positions daily. Sampling was quantified by using only the data after an individual had reached the asymptotic phase of its performance curve in order to exclude each night's initial learning phase. All visits made to non-rewarding flowers during the asymptotic phase were assumed to be made only for the purpose of collecting information about the current state of these flowers, i.e. sampling. For the analysis, visits of each individual were grouped in blocks of 20. The beginning of the asymptotic phase was determined by first computing sequential block averages of proportion of visits to rewarding flowers, and then determining when a bat had made more visits to rewarding flowers than the daily mean for two consecutive blocks (example given in Fig. 2.2).

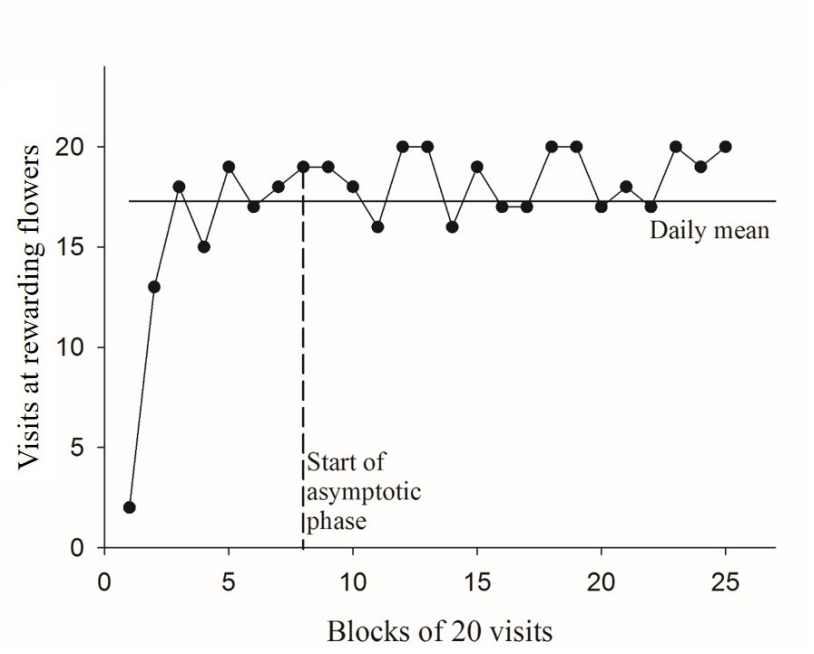

Figure 2.2. The procedure to quantify individual sampling rates is illustrated by showing the performance curve example of individual No. 6 during the fourth day at 50% reward probability. Every night the flower visits of an individual were grouped into blocks of twenty and the number of visits at rewarding flowers was determined. Sampling was only quantified during the asymptotic phase of the individual performance curve. To determine the beginning of the asymptotic phase, the mean number of visits per block at rewarding flowers was calculated for each day and individual. Two consecutive blocks with more visits at rewarding flowers than the daily mean marked the beginning of the asymptotic phase. The sampling rate was calculated as the proportion of visits at non-rewarding flowers during the asymptotic phase.

2.2.5. Contextual plasticity of sampling behavior

Since a sampling animal seeks new feeding opportunities, the frequency of sampling may change when overall food availability changes. To investigate how bats adapt their sampling rates to changes in food availability, I set three different probabilities of obtaining a reward in three different experimental runs. All individuals of one experimental group (12 bats) started with 50% reward probability for five days. After that six bats continued with a reward probability of 83% for four days and the other six bats of the same group continued with a reward probability of 30%. Thereafter, the condition was reversed for four days. The first day, when bats still familiarized themselves with experimental conditions, was not included in the analysis. For every individual, I obtained four measurements of nightly sampling rates for each reward probability (528 data points). Due to technical problems, some nights of some individuals had to be excluded (65 nights in total). Between experimental runs at different reward probabilities bats received for one night the same food as in the keeping flight cage.

2.2.6. Behavioral Flexibility

An animal's perseverance to keep visiting a previously rewarding flower was quantified as a negative measure of behavioral flexibility. For this, two rewarding flowers (reward probability 50%) stopped rewarding after 100 visits and two new flowers became rewarding. This number of visits before the switch was chosen because during the former experiment at 50% reward probability bats had reached asymptotic performance after less than 100 visits on 87% of individual nights. The visits to the previously rewarding flower during the next 100 visits after the switch were counted as a measure of perseverance. This procedure was repeated during four nights to obtain four measurements per individual. Additionally, I determined the proportion of visits to the rewarding flowers during the last 50 visits before the switch, since performance before the switch may influence the level of perseverance. Individual behavioral flexibility was determined during a four-day experiment (days 17 to 20 day of the experimental series).

2.2.7. Statistical Analysis

For each individual, the plasticity of sampling behavior along a gradient of three different reward probabilities was quantified by applying the behavioral reaction norm approach (Dingemanse et al. 2010b). With this approach generalized linear mixed models (GLMM) with random slopes and intercepts are used to fit regression lines for each individual. The slope of such a line is a measure of individual contextual plasticity.

Here I used Bayesian Markov chain Monte Carlo generalized linear-mixed models (MCMCglmm, (Hadfield 2010)) with the binomial error distribution of the multinomial2 family to fit random intercepts and slopes. Reward probability, weight and the interaction of flight cage (i.e. four experimental groups of 12 individuals each) and sequence of reward probabilities (2 groups) were included as independent variables. Only reward probability was mean-centered so that the intercept of the individual regression lines was determined at the middle of the environmental gradient. This is necessary for the calculation of the intercept-slope correlation. The multinomial dependent variable consisted of two columns, the number of visits at non-rewarding and rewarding flowers respectively. Individuals were included as random effect and the influence of reward probability was allowed to differ between individuals. As priors, I used an inverse-Wishart distribution for the residual variance and a parameter expanded prior for the random effect. From this model, a slope value for every individual was derived. However, the value on the scale of a binomial regression is not very intuitive, because the exponential of the slope value is the change in log odds of the individual probability to sample. To have a more intuitive measure of individual slopes, I calculated the sampling rate change as the difference between the predicted values of the sampling rates at 30% and 83% reward probability derived from the random regression model.

Individual differences and repeatability of sampling at each of the three reward probabilities was quantified by fitting three MCMCglmms to the respective data subsets with the same specifications as before but without reward probability as fixed effect. Since MCMCglmm uses additive over-dispersion the repeatability was calculated by dividing the between-individual variance through the total variance including the distribution specific variance of $\pi^2/3$ (Dean et al. 2011). I also calculated the correlation between the individual intercepts and slopes using the equation given by Dingemanse and Dochtermann (Dingemanse and Dochtermann 2013).

To quantify individual differences in flexibility (measured as number of visits to the previously rewarding flower) MCMCglmm was used as well but this time with a Poisson error distribution. I included experimental days as an independent variable to account for possible habituation to the experimental design. Individuals were included as random effects and I controlled for the influence of performance before the flower switch. Repeatability was calculated as for the individual differences in sampling rate but this time the distribution specific variance included was calculated with the equation $\ln(1/\exp(\beta_0)+1)$ ($\beta 0$ is the intercept on the link scale) as given by Nakagawa and Schielzeth 2010 (Nakagawa and Schielzeth 2010). Gelman diagnostics (<1.1), analysis of autocorrelation, effective sample size and visual inspection of trace plots were used to assess the models.

The correlation between individual behavioral plasticity (change of predicted sampling rate between 83% and 30% reward probability) and individual flexibility (mean number of visits at previously rewarding flowers) was assessed by two approaches. First, a linear model with normal error distribution was used with flexibility as dependent and plasticity as independent variable. In the second approach, I assigned ranks to individual values of flexibility and plasticity and calculated the Spearman rank correlation coefficient.

All statistical analyses were conducted using R version 2.15.1 (R Core Team 2015).

2.3. Results

2.3.1. Individual differences in contextual plasticity

During the three different reward probability conditions bats made on average (mean ± SD) 690 (±271), at 30%, 476 (±124) at 50% and 258 (±94), at 83% flower visits. In order to quantify individual differences in sampling rate I distinguished between visits to rewarding and non-rewarding flowers and calculated the proportion of visits at non-rewarding flowers. Individuals differed consistently in their sampling rate at each of the three reward probabilities (Fig. 2.3). The adjusted repeatability for each probability was r = 0.21 [95% CI 0.12, 0.35] at 30%, r = 0.15 [95% CI 0.08; 0.24] at 50% and r = 0.10 [95% CI 0.00; 0.22] at 83% respectively.

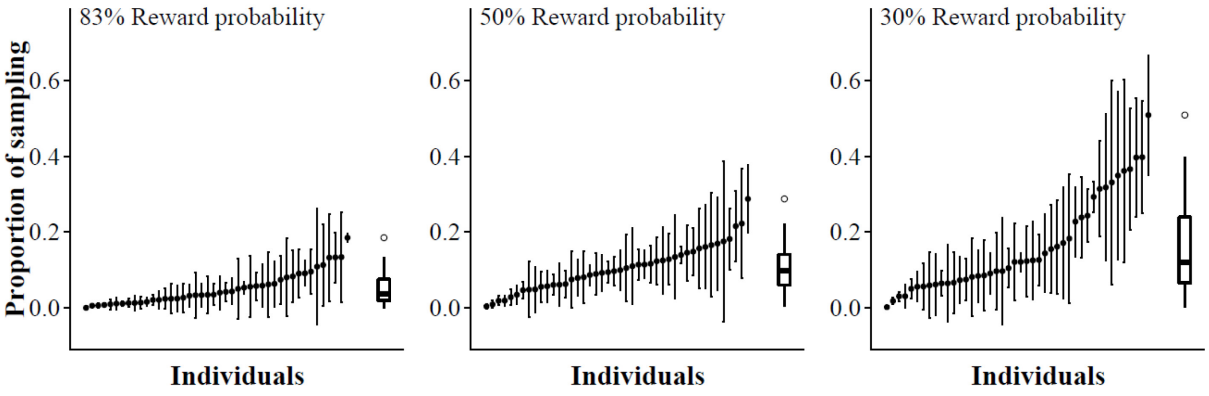

Figure 2.3. The mean individual sampling rate (± standard deviation) over four days sorted by rank at three different reward probabilities is shown. The sampling rate is defined as the proportion of visits at non-rewarding flowers after reaching the performance criterion (see Fig. 2.2). Additionally, the data are summarized by the boxplot representation on the right side of the graph. Individual rank can differ between panels.

Furthermore, reward probability was the only fixed effect that significantly influenced the proportion of visits to non-rewarding flowers (sampling rate) in the random regression model (Table 2.1). Weight, flight cage and experienced reward probability sequence, did not have any effect. The negative slope of -2.83 [95% CI: -3.69, -2.14] shows that sampling rate decreased when reward probability increased (Fig. 2.4 A). Thus, animals sampled most at the lowest food availability.

Table 2.1: Results of the random regression model (MCMCglmm) with binomial error distribution testing for the effects of independent variables (fixed effects) on sampling rates (dependent variable). Additionally, between- and within individual variance of sampling rates and variance of individual slopes was quantified. Numbers in parentheses show 95% credibility intervals.

Fixed effects	**Estimate**	**95% CI**
Intercept	-2.17	(-5.14, 0.70)
Reward probability(RP)	**-2.83**	**(-3.69, -2.14)**
RP sequence	0.70	(-0.44, 1.77)
Weight	-0.14	(-0.47, 0.21)
Flight cage 2	0.32	(-0.71, 1.51)
Flight cage 3	0.59	(-0.51, 1.66)
Flight cage 4	0.32	(-0.85, 1.50)
Variance components		
Between-individual	0.83	(0.40, 1.35)
Within-individual	1.28	(1.09, 1.49)
Between-individual slope variance	3.49	(0.72, 6.51)

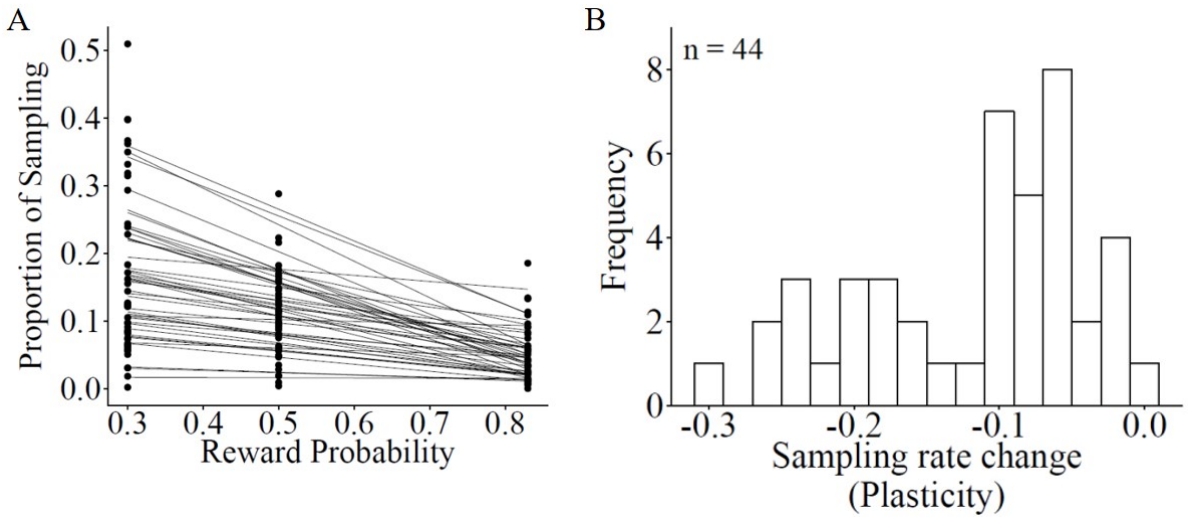

Figure 2.4. (A) For every individual, a behavioral reaction norm was fitted to its mean sampling rate (black circles) at every reward probability. Every line represents the regression line of the respective individual. (B) The histogram illustrates the distribution of slope values (sampling rate change) derived from individual regression lines. This sampling rate change was calculated for all 44 individuals as the difference of the predicted sampling rates at 30% and 83% reward probability. The greater the absolute value of this sampling rate change is (e.g. slope of the behavioral reaction norm), the higher the contextual plasticity of an individual is. (Individual lines were plotted using a linear model, for visualization only).

At the same time, the variance of the individual slopes ($\sigma 2 = 3.49$ [95% CI: 0.72, 6.51]) was significantly greater than zero (Fig. 2.4B). Individual slopes are a measure of contextual plasticity and a slope variance greater than zero shows that individuals differed in their response of adapting the sampling rate to differences in reward probability. The correlation of slope and intercept ($r2 = -0.47$ [95%CI: -0.76, 0.29]) was negative, indicating that individuals with high sampling rates during 30% reward probability showed the highest decrease with increasing reward probability, but this was not significant.

2.3.2. Individual differences in behavioral flexibility

Individuals' perseverance to visit non-rewarding flowers that had previously been rewarding was quantified as a measure for behavioral flexibility. Individuals differed significantly in this parameter. Flexibility differed threefold from the most flexible individual with the least perseverance (only 10 revisits out of 100 visits) to the least flexible individual the highest rate of perseverance (44 revisits out of 100 visits) and the differences between individuals were repeatable ($r = 0.25$ [95% CI 0.04, 0.44]) (Table 2.2, Fig. 2.5A).

Bats did not show signs of habituation, which would be indicated by a general increase in performance after the reversal over the course of this four-day experiment (number of day was not significant as a fixed effect, -0.06 [95% CI -0.13; 0.001]). However, it must be kept in mind that animals were not exposed to multiple serial reversals but instead only to a single reversal per experimental night.

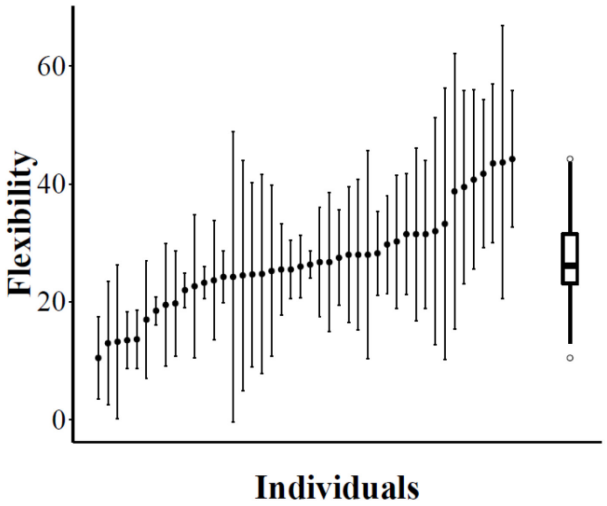

Figure 2.5. The mean flexibility (± standard deviation) over four days for every individual ordered by rank is shown. Flexibility was calculated as the number of visits at previously rewarding flowers during the first 100 visits after the rewarding flowers suddenly stopped giving rewards. The higher the number, the less flexible an individual is. The reward probability during this test was 50%. The data are summarized by the boxplot representation on the right side of the graph.

Table 2.2: The results of the general linear mixed model (MCMCglmm) with Poisson error distribution testing for the effects of independent variables (fixed effects) on the number of visits to previously rewarding flowers (perseverance). Additionally, between- and within individual variance of perseverance was quantified. Numbers in parentheses indicate 95% credibility intervals.

Fixed effect	**Estimate**	**95% CI**
Intercept	4.15	(3.28, 5.20)
Day	-0.06	(-0.13, 0.001)
Variance components		
Between-individual	0.06	(0.00, 0.12)
Within-individual	0.18	(0.11, 0.24)

2.3.3. No correlation between contextual plasticity and flexibility

Highly flexible individuals have been shown to track changes in the environment more closely. Therefore, the hypothesis has been proposed that behavioral flexibility and contextual plasticity are correlated across individuals. In order to test this hypothesis both types of plasticity, behavioral flexibility und contextual plasticity, have been quantified within the same individuals. During the first

set of experiments, contextual plasticity was quantified by measuring how much individuals increased their sampling rate as food became less abundant. During the second set of experiments, behavioral flexibility was assessed by quantifying the perseverance of individuals to previously rewarding flowers after they suddenly stopped giving rewards. Both types of behavioral plasticity measure the response of bats to changes in food availability. However, contrary to predictions, behavioral flexibility and contextual plasticity were not correlated in these bats. The spearman's rank correlation coefficient was $r^2 = 0.09$ ($p = 0.56$, $n = 44$) and also the linear model did not show any significant correlation ($r^2 = 0$, $p = 0.34$, $n = 44$).

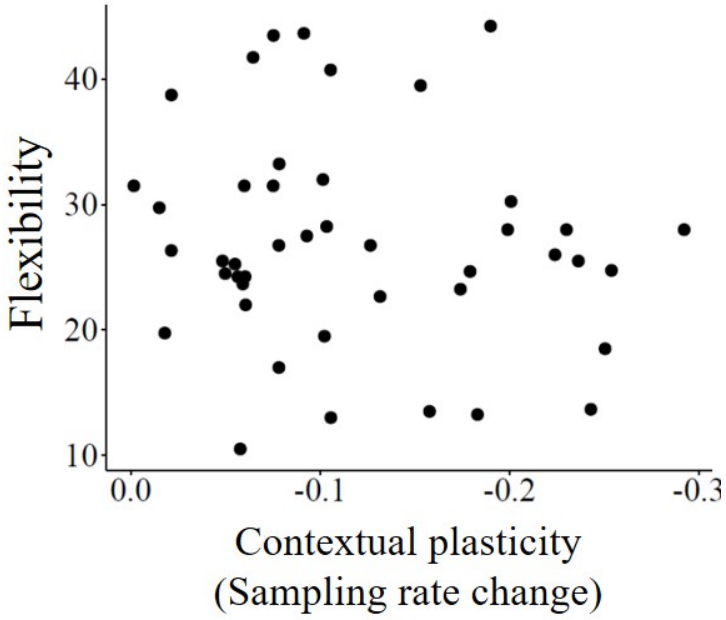

Figure 2.6. Contextual plasticity and behavioral flexibility are both measurements of how strongly individuals respond to changes in the environment. However, these two types of plasticity were not correlated between individuals (r^2 0.09, $p = 0.56$, $n = 44$). A binomial random regression model was used to quantify the extent to which individuals adapted their sampling rate to a gradient of resource availability and the predicted sampling rate change between 30% and 83% reward probability was used as a measure for contextual plasticity. Flexibility on the other hand was determined by the number of visits at the previously rewarding flowers after these flowers stopped giving a reward.

2.4. Discussion

It has been shown that individuals that express high levels of behavioral flexibility are also more responsive to environmental stimuli (Benus et al. 1987, Bolhuis et al. 2004). This observation has led to the hypothesis that highly flexible individuals might show also higher contextual plasticity and therefore a positive correlation between these two types of plasticity is expected (Coppens et al. 2010, Sih and Del Giudice 2012, Stamps 2015). In this chapter I tested this prediction with individuals of the

nectarivorous bat species *G. commissarisi*. In order to measure contextual plasticity, bats were confronted repeatedly with different flower qualities. The extent to which individuals adapted their sampling of unrewarding flowers to changes in flower quality was taken as a measure of individual contextual plasticity. Additionally, in a second series of experiments, individual behavioral flexibility was quantified in the same individuals. Thereby, bats were exposed to a food location reversal protocol where two previously rewarding food locations became dry while two alternate locations now provided food. The perseverance to leave the previously rewarding flower was used as an index of individual behavioral flexibility. The results show that individuals differed in both types of plasticity. However, contrary to expectations, contextual plasticity and behavioral flexibility were not correlated. This suggests that in *G. commissarisi* these two types of plasticity are independent traits possibly due to different underlying mechanisms.

Both types of plasticity measure the individual response to changes in environmental stimuli. However, a closer look at the choice situations reveals some differences. In both cases, bats were motivated by hunger to seek food during their active feeding phase and they foraged in a well-known environment with its 12 potentially rewarding feeders. During foraging bats were motivated to collect food from known feeding locations (exploitation) but they also had a behavioral tendency to search for and explore new feeding opportunities to track their current food potential (exploration). This was the case during both experiments. While measuring the reaction norm for contextual plasticity individual bats had already found their feeders that reliably provided food. Due to the probabilistic reward schedule, feeder visitation provided them with an experience of both, positive reinforcements when rewarded but also negative reinforcements when the reward was withheld. Taken together this provided some overall reinforcement value for the food locations and satisfied the bats' motivation to feed which in turn counterbalanced the motivation to explore.

During the first series of experiments, this level of satisfaction varied because the ratio of positive and negative reinforcements at active feeders differed due to the change in reward probability. In turn, the reinforcing effect of visiting active feeders differed and therefore also the counterbalancing effect of the feeding experience on the motivation to explore. Some individuals were more affected by the change of the reinforcement value of the rewarding flower and these bats showed a higher increase in their effort to explore potential better locations. Therefore, these individuals expressed higher contextual plasticity.

During the second series of experiments that measured behavioral flexibility, the situation was on the one hand very similar but contained an additional component. An individual had also found its feeders that reliably provided food. However, in this situation reward delivery suddenly stopped. Each subsequent visit now led to negative reinforcement. However, bats also still carried their fresh memories of successfully feeding at those locations just earlier. While in both experiments bats probably carried the same general tendency to explore their environment for food when they are hungry, their own recent experience of successfully feeding at a specific location also affected choice. In the flexibility

experiment, the tendency to retain a recently successful routine obviously differed between individuals. Some tended to stay with the former routine longer, while others adapted more quickly by leaving the previously rewarding flower in order to find new profitable options.

Thus, in the first experiments individuals differed in their balancing of exploitation versus exploration, and they also differed in how strongly the currently experienced reinforcement value at the feeders affected this balance. In the flexibility experiment on the other hand, individuals differed in how strongly a recently reinforced behavior pattern continued to control their choices or action selection. By this account, it seems plausible that contextual plasticity and flexibility as measured in this experiment are independent traits.

Behavioral flexibility in reversal learning paradigms has been studied extensively in behavioral neuroscience. This line of research has identified brain structures and two neurotransmitters, serotonin and dopamine, that are involved in regulating behavioral flexibility and therefore provides a potential proximate explanation for individual differences in flexibility (Coppens et al. 2010). The underlying physiological mechanism for individual differences in contextual plasticity is less clear. However, theoretical models propose some explanations for the emergence of individual differences in contextual plasticity. Wolf and colleagues suggest that individual differences in contextual plasticity arise if the payoffs are negatively-frequency dependent and that these individual differences can be maintained through positive-feedback mechanisms (Wolf et al. 2008). If only some *G. commissarisi* express high contextual plasticity, the potential benefit of being the first at a newly discovered resource is high. However, this payoff should decrease the more individuals follow the strategy of increasing their sampling rate in response to decreasing food availability. However, the more individuals increase their investment in sampling and leave the known profitable options, the higher the benefit of individuals that keep exploiting these known locations. This game-theoretic dynamic might be a driver for the emergence of individual differences in the contextual plasticity of sampling. Additional positive-feedback mechanisms, for example through learning, might be responsible for the consistency of these individual differences in contextual plasticity (Wolf et al. 2008). It has recently been shown that bats of the closely related species *Glossophaga soricina* can use socially transmitted information to reduce their search effort of finding rewarding flowers in a laboratory setting (Rose et al. 2015). These results might be the first indication that individuals that do not invest much in sampling of possible better feeding opportunities during lower food availability could use social information instead and follow more exploratory individuals to newly discovered locations.

Recently, individual differences in behavioral plasticity have received much attention. However, throughout the literature different types of behavioral plasticity are not clearly defined and labels are not coherent. For example, contextual plasticity has been also termed activational plasticity (Snell-Rood 2013) or responsiveness (Wolf et al. 2008) and behavioral flexibility is sometimes classified as developmental plasticity due to its dependence on past experiences (Stamps 2015). These circumstances

increase the challenge to summarize results from different empirical studies in order to draw general conclusions (Stamps 2015). The results of the present study show that different types of plasticity are not necessarily correlated and therefore they illustrate the importance of clear conceptual definitions and the need of a comprehensive framework of behavioral plasticity in order to investigate the links between different types.

Furthermore, contextual plasticity itself might not necessarily be a single repeatable trait (Dingemanse and Wolf 2013). An example that challenges this assumption can be found in a study of Mathot et al. in 2011 (Mathot et al. 2011). Contextual plasticity was measured twice within the same individuals by quantifying how much red knots adapted their vigilance and their escape flight duration to an increase in predation risk. Their results show that both behaviors changed along this environmental gradient but only plasticity in vigilance was different between individuals. This means that highly plastic individuals in adapting their vigilance were just as plastic as all individuals in adjusting their flight duration. Another example comes from a study with coral reef fish in which they measured contextual plasticity of three behavioral traits along a gradient of different temperature: activity, aggressiveness and boldness. Individuals differed in their contextual plasticity of activity but individuals did not differ in their contextual plasticity of aggressiveness and boldness. Therefore, as in the previous example, highly plastic individuals in terms of activity where just as plastic as their conspecifics in other behavioral traits (Biro et al. 2010). These examples show that even contextual plasticity itself can be inconsistent and might therefore not be a single trait. However, experiments measuring contextual plasticity of more than one trait within the same individual are rare and therefore, more empirical data is needed to further investigate the relationship between the contextual plasticity of different behavioral traits.

The results of this chapter also show that overall individual sampling rates were negatively correlated with flower quality which means that the bats sampled more the less nectar they received at the current available flowers. This result is in line with the study of Shettleworth et al. using pigeons (Shettleworth et al. 1988). Furthermore, it could be expected that the sampling rate is the highest at the beginning and then decreases throughout the night. However, in *G. commissarisi* the individual sampling rate did not differ significantly between the first and the second half of the night (Appendix A 1.2). Recently it has been proposed that consistent individual differences in behavior might be correlated with differences in cognitive styles (Sih and Del Giudice 2012). One important part in cognition is learning and therefore I assessed if individuals differed in how fast they learned the location of the rewarding flowers every night. However, individuals in this experiment did not differ consistently in their learning rate (Appendix A 1.1).

With the experiments presented in this chapter it was possible to test the prediction that contextual plasticity and behavioral flexibility are correlated across individuals because some individuals might be generally more responsive to environmental stimuli than others. However, the results show that

behavioral flexibility and contextual plasticity are independent traits in *G. commissarisi* and therefore contradict this prediction. However, more empirical data is needed in order to generalize this finding.

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CHAPTER 3
Increasing foraging costs cause a decrease in individual daily energy expenditure in the nectar-feeding bat *Glossophaga commissarisi*

SUMMARY

Daily energy expenditure (DEE) is an important ecological trait that summarizes all energetic costs of an individual. Extrinsic factors like increasing foraging costs could either decrease DEE because the same foraging effort leads to lower energetic intake or increase DEE because more energy is spent on foraging in order to receive the same energetic gain. So far, results from empirical studies are inconsistent and provide support for both hypotheses. Furthermore, DEE can also be influenced by the individual itself, and a substantial part of the variance in DEE can be explained by between-individual differences. The life-history approach proposes that consistent individual differences in behavior correlate with individual differences in metabolic rates as part of different life-history strategies. During the experiment described in the previous chapter, 44 male bats of the species *Glossophaga commissarisi* were confronted with changes in foraging costs due to the manipulation of reward probabilities at computer-controlled artificial flowers. The lower the probability of getting a reward, the more flower visits are necessary to receive the same amount of nectar. Because of the known assimilation efficiency of sugar and a pure nectar diet during the experiment, it was possible to estimate individual DEE indirectly by combining the energy gained from both nectar intake and mobilizing body reserves. The results show that in *G. commissarisi* the average DEE decreased with increasing foraging costs and the proportion of energy received from mobilizing body reserves was highest during the condition with the highest foraging costs. Furthermore, individuals differed consistently in their DEE and in how much their DEE changed in response to changes in reward probability. Consistent individual differences in foraging activity and the resulting differences in nectar intake mostly explained the observed individual differences in DEE. Furthermore, individuals that showed the highest increase in foraging activity in response to increasing foraging costs lost less weight and also invested more in the exploration of unrewarding flowers. Thus, as proposed by the life-history approach, these results show how individual differences in DEE can be linked to individual behavior in *G. commissarisi*.

3.1. Introduction

Daily energy expenditure (DEE) is a measure that summarizes all energetic costs of an individual, including energy spent on foraging, reproduction and other behaviors. DEE also includes the resting metabolic rate which is defined as the amount of energy an individual spends at rest in a thermoneutral environment: basically, the minimum cost of life (Hulbert and Else 2004). Besides potential intrinsic limitations on the amount of energy an individual is able to spend per day (Speakman 1999, Welcker et al. 2010), it has been shown that the daily energy expenditure of animals is highly influenced by extrinsic factors (Speakman et al. 2003). Amongst others, the change of foraging costs is an important factor that can influence DEE. However, foraging costs can theoretically influence individual DEE in two alternative ways. On the one hand, DEE could increase with decreasing foraging costs because in this case the same foraging effort leads to higher energy intake, enabling animals to spend more energy (Speakman et al. 2003). On the other hand, DEE could increase with increasing foraging costs because in order to keep the energy intake constant higher foraging efforts are necessary (Wiersma et al. 2005). Empirical support has been found for both hypotheses. For example, the DEE of female mice increased with decreasing foraging costs due to higher food intake (Schubert et al. 2008) and the same pattern was found in zebra finches (Deerenberg et al. 1998). However, in starlings the DEE increased with increasing foraging costs (Wiersma et al. 2005). Notably, during this study, variable instead of fixed reward schedules were used, which might simulate natural conditions more realistically. The different relationship between foraging costs and DEE depending on the reward schedule could indicate different underlying mechanisms depending on whether or not the delivery of rewards is predictable (Schubert et al. 2008). Furthermore, one study with free-living field voles showed that site quality which was assessed by quantifying food availability, influenced DEE in opposite ways depending on the time of the year (Speakman et al. 2003). Together, these findings suggest that the influence of foraging costs on DEE might be complex and context-dependent and additional empirical data can help to determine the ecological conditions that shape the directions of this relationship.

In addition to environmental influences like changes in foraging costs, several studies have shown that DEE is also influenced by individual factors. For example, female meadow voles (Berteaux et al. 1996) and wild chipmunks (Careau et al. 2013) differed consistently in their DEE, and around 30% of variation could be explained by between-individual differences, even after controlling for weight.

The interest in individual variation in metabolic rates has been growing recently because of the possible relationship with consistent individual differences in behavior, e.g. animal personality (Biro and Stamps 2010). Several behavioral traits have been shown to correlate with individual differences in metabolic rates in a variety of species (reviewed by (Careau and Garland Jr 2012)) and inspired by these observations, a theoretical framework has been proposed that links individual differences in behavioral and physiological traits with life-history trade-offs embedded in the pace-of-life syndrome theory (Wolf et al. 2007, Réale et al. 2010). The concept of the pace-of-life syndrome traditionally has been used to

describe differences between species or populations along the slow-fast life-history continuum but also within populations individuals might differ along this axis. High levels of aggressiveness, activity and boldness might be attributes of a "fast" lifestyle which is characterized by early reproduction and high reproductive success but lower survival rates. The reason for this could be that individuals with these behavioral characteristics might be more successful in foraging and resource defense but they might be also more likely to take higher risks, therefore lowering their chance of survival (Réale et al. 2010). Furthermore, individuals that show high levels of aggressiveness, boldness and activity are considered to have a proactive coping style (Careau et al. 2008, Careau and Garland Jr 2012) which means that these individuals are more likely to interact with their environment actively in order to cope with challenging situations as opposed to reactive individuals that tend to be more passive (for example 10 reactive rats show freezing behavior more often) (Koolhaas et al. 1999). The proactive coping style has been associated with several physiological traits like higher sympathetic reactivity to stressful situations, higher testosterone reactivity and higher heart rate (Deruiter et al. 1992, Korte et al. 1998, Koolhaas et al. 1999). Individuals with these behavioral and physiological characteristics are thought to have higher energetic demands in order to support their "fast" lifestyle and therefore might have higher metabolic rates. The trade-off between "fast" and "slow" lifestyles proposed by the life-history approach could explain how individual differences evolve and are maintained within a population.

In support of the proposed framework, there are several results from empirical studies that link individual differences in behavioral traits with individual differences in metabolic rates. For example, exploratory behavior in deer mice has been shown to be genetically linked to resting metabolic rates (Careau et al. 2011). Furthermore, individual differences in boldness in salmon and individual differences in aggressiveness in arctic char have been both shown to correlate positively with individual differences in standard metabolic rates (Cutts et al. 2001, Finstad et al. 2007). Additionally, these behavioral traits have also been linked to life-history traits, for example more exploratory and risk-prone superb fairy-wrens (*Malurus cyaneus*) have a lower probability of survival (Hall et al. 2015) and Smith and Blumstein concluded in their meta-analysis that boldness is generally related to higher reproductive success but also leads to lower survival (Smith and Blumstein 2008). However, as Réale and colleagues already concluded in their review, not all results from empirical studies support that general and intuitive theory and the direction of the correlations might differ depending on environmental factors (Réale et al. 2010). For example, although aggressive and less docile bighorn rams had lower survival rates as expected, boldness was associated with higher longevity (Réale et al. 2009).

So far, most studies investigated the link between individual differences in behavior and individual differences in resting or standard metabolic rates although, unlike DEE, these types of metabolic rates do not measure energy spent on actual behavior (Careau et al. 2015). Although it has been shown that individuals can differ consistently in their DEE (Berteaux et al. 1996, Careau et al. 2013), the potential

correlation of DEE with behavioral traits has received much less attention and is therefore much less established.

The neotropical, nectarivorous bat species *Glossophaga commissarisi* provides the unique possibility to estimate individual DEE indirectly. In captivity, the diet can be restricted temporarily to pure nectar and because of a known sugar assimilation efficiency of 99% (Winter 1998a), it is possible to calculate the energy an individual receives through food intake. In addition to the energy derived from food assimilation, animals can also spend energy derived from mobilizing body reserves. The average caloric value of body reserves (= 24h body mass change) of several nectar-feeding bats of the family Phyllostomidae has been quantified as 31 kJ/g before (Winter and Von Helversen 1998) and therefore, by measuring daily weight differences it is possible to calculate the amount of energy derived from mobilizing body reserves. By adding these two energetic components, energy derived from food assimilation and energy derived from the mobilization of body reserves, individual DEE can be estimated. With this procedure, it is possible to measure individual DEE repeatedly with a non-invasive method as opposed to injecting doubly-labelled water to estimate energy expenditure (Nagy 1983, Voigt et al. 2006).

Here, the individual DEE of 44 male bats was estimated repeatedly at three different resource qualities, simultaneously to other behavioral traits whose results are described in Chapter 1. The resource quality was manipulated by changing the reward probability at artificial computer-controlled flowers. As a consequence of decreasing reward probability foraging costs increase because at lower reward probabilities bats have to make more flower visits in order to receive the same amount of nectar. Rewards were delivered at artificial flowers with three different reward probabilities (30%, 50% and 18 83%) for four days each. With this experimental schedule, it was not only possible to quantify consistent individual differences in DEE but also individual differences in the plasticity of DEE in response to changes in foraging costs. Additionally, by quantifying individual foraging activity and the amount of flower exploration it was possible to explore the relationship between these two behavioral traits and the DEE of individuals.

3.2. Methods

3.2.1. General description of the experiment

Data analyzed in this chapter were collected during a previous experiment and therefore a more detailed description of study site, experimental setup and keeping conditions of bats can be found in the methods section of Chapter two.

Four groups of twelve males of the nectar-feeding bat species *Glossophaga commissarisi* were tested in flight cages at La Selva Biological Station, Province Heredia, Costa Rica. Since the walls of the flight

cage consisted of mesh, individuals were exposed to the climatic conditions of the surrounding rainforest. Since it was not possible to catch forty-eight males, four individuals took part in the experiment twice in order to have the same number of individuals in each group. The data of these four individuals were only analyzed for their first participation. In each flight cage twelve artificial flowers were arranged on a rectangular frame (for more details see Chapter 2, Figure 2.1).

On any given night (from 6 PM – 6 AM), each bat received rewards consisting of 40 µl nectar from only two out of the twelve artificial flowers. Every bat had its own set of rewarding flowers, in order to prevent social learning. However, since twelve bats used the flowers simultaneously, every flower was used by two different individuals. Pairs of individuals sharing one flower changed between the nights and also the position of rewarding flowers changed every night so as to prevent habituation to specific locations. In order to manipulate resource quality and consequently foraging costs, rewards were delivered with three different probabilities (30 %, 50 % and 83 %). All individuals of one experimental group (12 bats) started with 50 % reward probability for four days. After that six bats continued with a reward probability of 83 % for four days and the other six bats continued with a reward probability of 30 %. During the next four days, the condition was reversed between the two groups (from 30 % to 83 %, or vice versa). The reward probability was configured pseudo-randomly and all bats experienced the same sequence of rewards/non-rewards at a specific flower in order to equalize experience. The first visit of an individual at its rewarding flower was always rewarding.

3.2.2. Foraging activity and estimates of individual daily energy expenditure (DEE)

Foraging activity was defined as the total number of flower visits per individual and experimental night. In order to estimate DEE, an indirect method was used. In general, the amount of energy a bat spends per day consists of two components: energy derived from food assimilation and energy derived from mobilizing body reserves (Winter and Von Helversen 1998). During this experiment bats received food only in form of nectar rewards at artificial flowers and therefore the daily amount of nectar intake for each individual was known. The nectar consisted of 20 ± 0.2 % w/w sugar concentration measured with a digital refractometer (A. Krüss Optronic GmbH, Germany). 20 % w/w concentration of a mix of sucrose and fructose (1:2) corresponds to 216.2 g of sugar per liter (Wolf et al. 1984) and the caloric equivalents of sucrose and fructose are 16.8 kJ/g and 15.6 kJ/g respectively (Wieser 1986). Thus, one reward of 40 µl nectar was equivalent to 0.14 kJ. The assimilation efficiency of sugar in these bats is known to be 99% (Winter 1998b) and therefore it was possible to calculate the individual energetic intake.

In order to estimate the amount of energy mobilized from body reserves (= 24 h body mass change), every bat was weighed every day at approximately the same time, 8-10h after the experimental night ended (between 2 pm and 4 pm). Due to logistic limitations, it was not possible to weigh every bat

exactly at the same time. The energetic costs of resting have been estimated to be 0.25 W in *G. commissarisi* (Winter and Von Helversen 1998) and therefore bats spend around 1.8 kJ in two hours which is 4% of the average daily energy expenditure of free living *Glossophaga commissarisi* (Voigt et al. 2006). Thus, the inaccuracy introduced by slightly varying weighing times is relatively small.
For the purpose of weighing every individual was caught using a hand net consisting of a wire ring with a bag of mist net attached. The advantage of the mist net was that it is less conspicuous to the bats and thus it facilitated the capture without chasing the bats away from their roosting site which minimized the energy spent during chasing. The accuracy of the scales was ± 1 mg. Winter and von Helversen determined the average caloric equivalent of 1g of body reserves (= 24h body mass change) as 31 kJ/g in nectarivorous bats of the family *Phyllostomidae* (Winter and Von Helversen 1998) and this factor was used here to convert the daily weight difference into a caloric value. This caloric value could be either positive or negative depending on whether the individual lost or gained weight within the last 24h. By adding these two caloric values derived from food assimilation and mobilizing energy from body reserves, it was possible to obtain an estimate of the energy an individual spent per day. Since DEE is known to correlate positively with weight, the individual DEE was standardized to kJ/g.

3.2.3. Sampling rate of unrewarding flowers

Sampling, as a measure of exploration during foraging, was defined as a visit to a non-rewarding flower. Since the positions of rewarding flowers changed every night, bats had to learn new positions daily. Sampling was only quantified after an individual had reached the asymptotic phase of its performance curve in order to exclude each night's initial learning phase. I assumed that all visits made to non-rewarding flowers during the asymptotic phase were for the purpose of collecting information about the current state of these flowers, i.e. sampling. Since the total number of flower visits varied between nights and individuals, the proportion of sampling visits on the total number of visits was calculated. More details about the calculation of individual sampling rates can be found in the methods section of Chapter two.

3.2.4. Statistical analysis

Markov chain Monte Carlo generalized linear-mixed models (MCMCglmm, (Hadfield 2010)) with random slopes and intercepts were used to assess the influence of increasing foraging costs on both, DEE and foraging activity. Thereby, the individual plasticity of DEE and foraging activity could be quantified across the three different reward probabilities. The error distribution was assumed to be Gaussian in both models. Reward probability, the interaction of flight cage (i.e. four experimental

groups of 12 individuals each) and sequence of reward probabilities (2 groups), forearm length and experimental day were included as fixed effects. Only reward probability was mean-centered so that the intercept of the individual regression lines was determined at the middle of the environmental gradient. Individuals were included as a random effect and the influence of reward probability was allowed to differ between individuals. An inverse-Wishart distribution was used as a prior for the residual variance and for the random effects a parameter expanded prior was used. Analysis of autocorrelation, effective sample size and visual inspection of trace plots were used to assess the models. The slope of the individual regression lines, derived from these two models, is a measure of how much individual DEE and foraging activity change along the gradient of different reward probabilities and therefore these slope values are a measure of individual plasticity. Variance between individuals and the repeatability of DEE and foraging activity at each of the three reward probabilities (30%, 50% and 83%) was quantified by fitting three MCMCglmms to the respective data subsets with the same specifications as before but without fixed effects. Repeatability, the proportion of variance explained by differences between individuals, was calculated following Nakagawa and Schielzeth (Nakagawa and Schielzeth 2010).

A linear mixed model (LMM) with individual as random effect was used to assess if the proportion of daily energy that was derived from mobilizing body reserves changed depending on resource quality. Reward probability, experimental group and reward probability sequence were included as fixed effects. Thereafter, a post hoc pairwise comparison of the proportion of energy mobilized from body stores between different reward probabilities was conducted and significance values were adjusted using the Tukey's honest significance test provided by the lsmeans package in R (Lenth 2016).

Additionally, another linear mixed model was used to assess the change of nectar consumption depending on reward probability with experimental group and reward probability sequence included as fixed effects and individual included as random effect. In order to assess how individual mean foraging activity and mean sampling rate influenced individual nectar intake at each reward probability, linear models were fitted to each data subset respectively.

Linear models were also used to assess if individual plasticity in foraging activity predicted DEE during the lowest reward probability and if individual plasticity in foraging activity predicted individual plasticity in sampling behavior. Values for the plasticity of sampling behavior were derived from Chapter 2.

3.3. Results

Individuals differed consistently in their DEE during all three reward probabilities. The repeatability of the daily energy expenditure was R = 0.31 (95% CI: 0.22, 0.56) at 30% reward probability, R = 0.34 (95% CI: 0.18, 0.53) at 50% reward probability and R = 0.52 (95% CI: 0.34, 0.66) at 83% reward

probability. Furthermore, results of the random regression model show that the mean DEE increased significantly with increasing reward probability and therefore decreasing foraging costs (Table 3.1). DEE increased by 18% from 3.37 kJ/g (sd ± 0.63) during 30% reward probability to 4.09 kJ/g (sd ± 0.86) during 83% reward probability.

Table 3.1: The results of the two random regression models (MCMCglmm) with Gaussian error distribution testing for the effects of independent variables (fixed effects) on foraging activity and DEE respectively. Additionally, between- and within individual variance and variance of individual slopes were estimated. Numbers in parentheses indicate 95% credibility intervals and numbers in bold indicate significant values.

	Daily energy expenditure (DEE)		**Foraging activity**	
Fixed effect	**Estimate**	**95% CI**	**Estimate**	**95% CI**
Intercept	12.81	(4.92, 21.51)	1347.75	(33.75, 2700.97)
Reward probability(RP)	**1.38**	**(0.95, 1.76)**	**-902.13**	**(-1004.42, -795.64)**
RP sequence	-0.42	(-1.12, 0.19)	0.96	(-111.78, 114.44)
Forearm length	**-2.61**	**(-5.22, -0.24)**	-224.48	(-619.68, 172.00)
Flight cage 2	-0.39	(-1.01, 0.26)	-20.11	(-118.20, 86.06)
Flight cage 3	-0.26	(-0.91, 0.49)	32.23	(-71.33, 142.36)
Flight cage 4	-0.23	(-0.89, 0.48)	26.21	(-94.81, 126.76)
Day	**-0.03**	**(-0.05, -0.01)**	**-5.28**	**(-9.30, -0.43)**
RP sequence: Flight cage 2	0.78	(-0.06, 1.74)	86.41	(-54.33, 253.41)
RP sequence: Flight cage 3	0.82	(-0.23, 1.74)	34.47	(-96.79, 194.82)
RP sequence: Flight cage 4	-0.29	(-1.47, 0.77)	-79.35	(-266.28, 104.40)
Variance components				
Between-individual	0.36	(0.18, 0.55)	14199	(6481, 21726)
Within-individual	0.48	(0.41, 0.54)	24191	(21131, 27541)
Between-individual slope variance	0.91	(0.22, 1.77)	73986	(27851, 127137)

In addition to changes in reward probability, experimental day and forearm length significantly influenced individual DEE. With time, the daily energy expenditure decreased independently from the sequence of the reward probabilities (β = -0.03, 95% CI: -0.05, -0.01) and individuals with longer forearms had also lower DEE (β = -2.61, 95% CI: -5.22, -0.24) (Table 3.1). Furthermore, the between-individual slope variance was significantly greater than zero (variance = 0.91 (95% CI: 0.22, 1.77), which shows that the change of DEE with decreasing foraging costs differed between the individuals (Table 3.1, Figure 3.1).

The DEE of the individual that showed the highest increase in DEE with decreasing foraging costs ranged from 3.70 kJ/g during 30% to 5.66 kJ/g during 83% reward probability which represents an

increase of 53% whereas the DEE of other individuals remained close to constant (for example 2.71 kJ/g during 30% and 2.57kJ/g during 83% reward probability). The significant intercept-slope correlation (0.73, 95% CI: 0.26, 0.99) implies that individuals with higher daily energy expenditure during 30% reward probability also showed a higher increase of daily energy expenditure during 83% reward probability (Table 3.1, Figure 3.1).

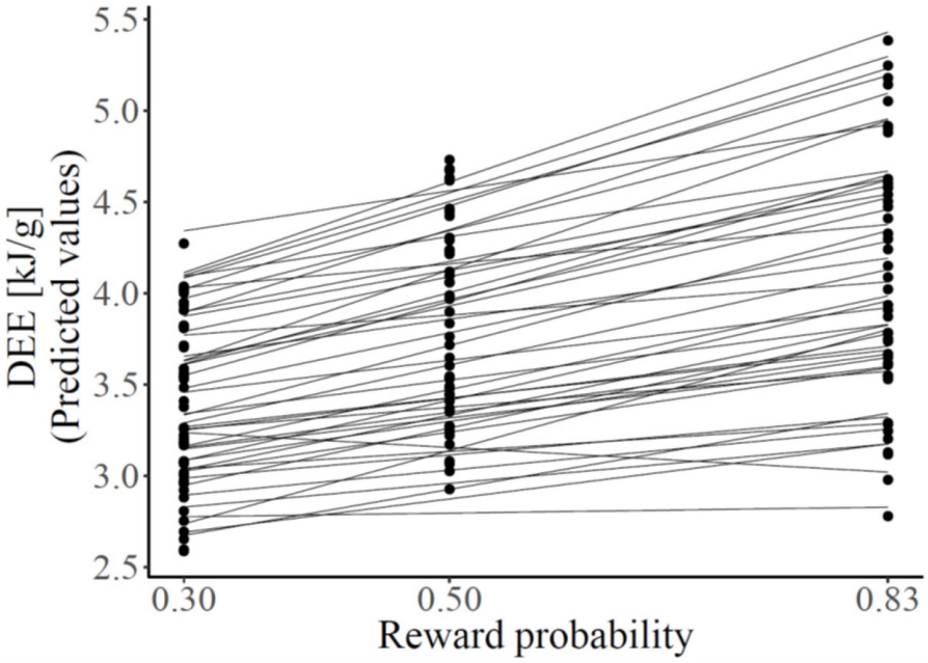

Figure 3.1: Data points represent the individual values predicted by the random regression model used to analyze the change of individual DEE over a gradient of different reward probabilities. Every line represents the individual regression derived from the random regression model and the steepness of the slope is a measure of individual plasticity. Individual daily energy expenditure increased significantly ($\beta = 1.38$, 95% CI: 0.95, 1.76) with increasing reward probability and individuals also differed in their plasticity to adapt their daily energy expenditure (Between-individual slope variance: 1.12, 95% CI: 0.25, 2.18).

The energy that is available to an individual can either be derived from food assimilation or from mobilizing energy from body reserves (= 24h body mass change). The mean proportion of energy from body stores was 16.3% (sd ± 10%) during 30%, 6.3% (sd±5%) during 50% and 10.9% (sd ± 6%) during 83% reward probability. This shows that most of the energy an individual spent per day was derived from food assimilation and therefore individual DEE was mostly determined by individual nectar intake. The proportion of energy that was mobilized from body reserves changed depending on reward probability (Figure 3.2). At the lowest reward probability and therefore during the condition with the highest foraging costs, the proportion was significantly higher than at both 50% ($t = 7.99$, $p < 0.001$) and 83% ($t = 4.10$, $p < 0.001$) reward probability. However, the proportion of energy derived from body

reserves during 83% reward probability was also significantly higher than from 50% reward probability ($t = 3.89$, $p < 0.001$).

Like DEE, individual nectar intake increased significantly with decreasing foraging costs due to increasing reward probability from 6.42ml (sd ± 1.80) during 30% to 8.79ml (sd ± 2.19) during 83% reward probability ($t = 6.42$ $p < 0.001$). Individual nectar intake was influenced by both foraging activity and sampling rate.

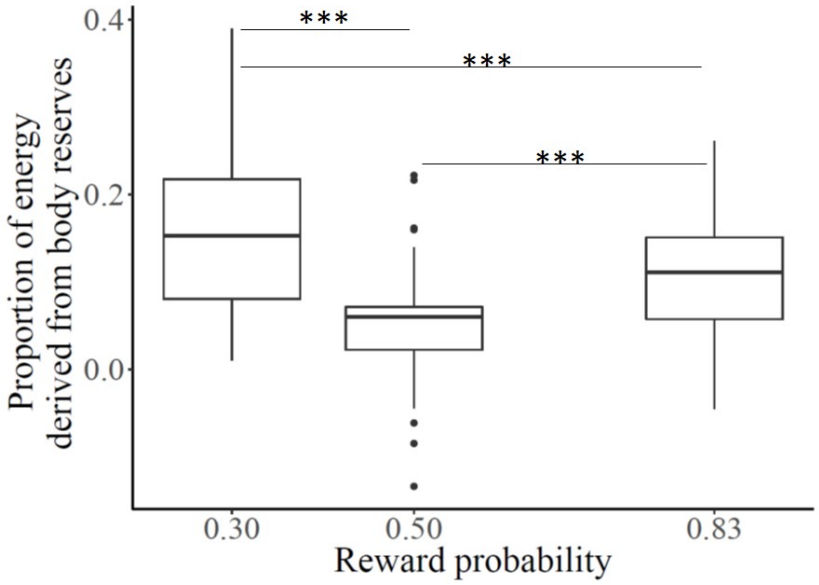

Figure 3.2: The energy an individual spends per day can be gained either through consumption of nectar or through mobilizing body reserves (measured as weight loss per day). The proportion of energy that originated from mobilizing body stores was calculated for each reward probability and pairwise comparisons show that during the lowest reward probability (30%) the proportion is significantly higher than during 50% and 83% reward probability. Notably, the proportion of energy derived from body reserves during 83% reward probability is also higher than during 50% reward probability.

Nectar intake was strongly positively correlated with foraging activity (number of flower visits) during all three reward probabilities (30%: $t = 14.88$, $p < 0.001$, 50%: 13.92, $p < 0.001$ and 83%: $t = 20.16$, $p < 0.001$) (Figure 3.3 A) and thus individual DEE is mainly determined by individual differences in foraging activity. Only during the lower reward probabilities, the amount of sampling had a negative impact on individual nectar intake (30%: $t = -9.75$, $p < 0.001$, 50%: -4.19, $p < 0.001$ and 83%: $t = -1.80$, $p = 0.08$). Although individuals spent less energy at the lowest reward probability, foraging activity increased significantly with decreasing reward probability ($\beta = -902.13$, 95% CI: -1004.42, -795.64). Independently from the sequence of reward probabilities, foraging activity decreased with day ($\beta = -5.28$, 95% CI: -9.30, -0.43) (Table 3.1). Similar to DEE, individuals differed consistently in foraging

activity during 30% (R = 0.42, 95% CI: 0.26, 0.59), 50% (R = 0.22, 95% CI: 0.08, 0.46) and 83% (R = 0.37, 95% CI: 0.19, 0.53) reward probability.

Furthermore, individuals differed significantly in their plasticity to adapt their foraging activity to changes in reward probability (between-individual slope variance = 73986 (95% CI: 27851, 127137) (Table 3.1).

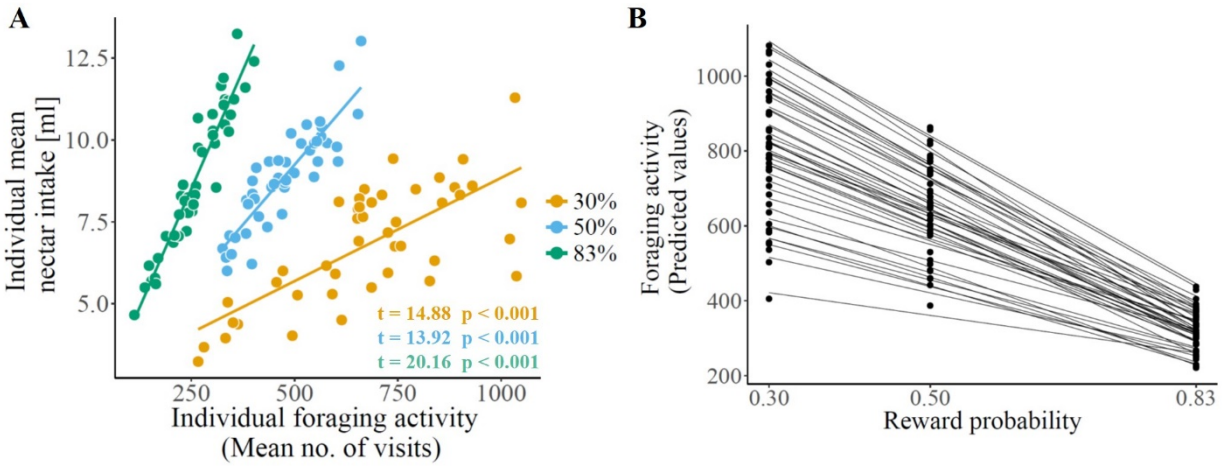

Figure 3.3: (A) Correlation of individual foraging activity, measured as the total number of flower visits and individual mean nectar intake during all three reward probabilities. (B) Furthermore, bats increased their foraging activity with decreasing reward probability (β = -901.91, 95% CI: -1005.43, -797.13) and individuals differed significantly in their plasticity of foraging activity (Slope variance: 72179, 95% CI: 22748, 125436). Shown are the values predicted by the random regression model used to analyze the individual foraging activity.

The results of Chapter 2 showed that individuals also differed in their plasticity to adapt their sampling rate to changes in reward probability. Individual plasticity of foraging activity correlated significantly with individual plasticity in sampling behavior (t = 3.36 p = 0.002) (Figure 3.4 A) and the plasticity of DEE (t = 3.49 p = 0.001). This means individuals that showed the highest increase of foraging activity during 30% reward probability also showed the highest increase in their sampling rate. Furthermore, individuals with a higher increase in foraging activity were able to maintain a higher DEE during the lowest reward probability. This is shown by the significant positive correlation of the steepness of the individual slope of foraging activity and the DEE during 30% reward probability (t = 6.77, p < 0.001) (Figure 3.4 B).

During the lowest reward probability and therefore during the condition with the highest foraging costs, foraging activity correlated positively with DEE (t = 9.96 p < 0.001) and sampling rate (t = 7.53 p <

0.001) and negatively with weight loss (t = -6.30 p < 0.001). This means individuals with high foraging activity during the condition with the highest foraging costs had high sampling rates, lost less weight and were able to maintain higher DEE.

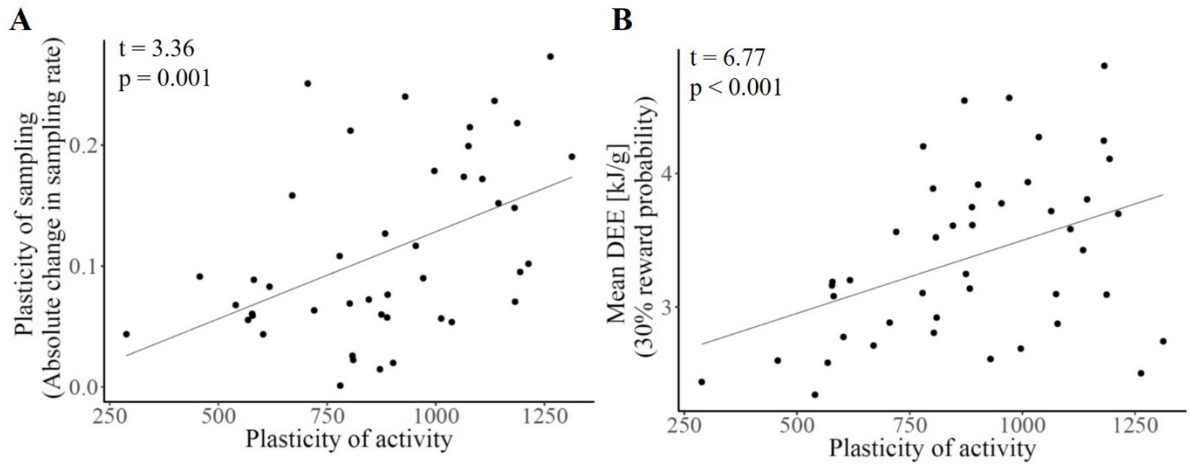

Figure 3.4: The random regression model used to analyze individual foraging behavior showed that individual regression lines varied in the steepness of their slopes. The absolute value of the steepness is a measure for individual plasticity and quantifies how much an individual increased its foraging activity during 30% reward probability compared with 83%. (A) Individual plasticity in foraging activity correlated significantly with individual plasticity in sampling behavior. Thus, individuals with higher increase in foraging behavior also showed a higher increase in their sampling behavior during the lowest reward probability (30%). (B) Individual plasticity in foraging behavior also correlated positively with individual DEE during the condition with the highest foraging costs (30% reward probability).

3.4. Discussion

3.4.1. Influence of foraging costs on DEE

During the present experiment foraging costs of the nectar-feeding bat *G. commissarisi* were manipulated by changing the reward probability at artificial flowers. The lower the reward probability, the more flower visits a bat had to make in order to receive the same amount of nectar. Previous studies that investigated the relationship between changes in DEE and changes in foraging costs provided inconsistent results. The results of the present study show that despite of increasing their foraging effort by increasing the number of flower visits during conditions with higher foraging costs, the DEE of *G. commissarisi* decreased significantly from 4.09kJ/g (sd ± 0.86) to 3.37kJ/g (sd ±0.63). Therefore, these results are in accordance with the observed changes in DEE of zebra finches (Wiersma and Verhulst 2005) in response to changes in foraging costs regardless of the variable reward schedule used in this experiment.

Although most of the DEE was covered by energy gained from food assimilation, the mean proportion of energy derived from mobilizing body reserves changed with changing foraging costs and the proportion was the highest during the lowest reward probability of 30%. Therefore, the challenge of covering energetic demands solely through nectar intake was the highest during the condition with the highest foraging costs. Together with the significant decrease of nectar consumption, these results indicate that the increase in foraging activity was not sufficient to compensate the lower reward probability and higher foraging costs. However, the mean proportion of energy derived from mobilizing body stores during 83% reward probability was also significantly higher than during 50%. This could be an indication that bats lost more weight with time independent of the reward probability sequence. Hereafter, I discuss possible explanations as to why bats did not increase their foraging effort sufficiently to maintain energetic intake and body mass.

Physiological constraints

During a previous experiment with the closely related species *G. soricina* the sugar content of rewards provided by artificial flowers was manipulated by changing either the sugar concentration or the reward volume (Von Helversen and Winter 2005). In this experiment bats increased their flower visitation rate as a result of decreasing sugar content per reward. By changing the number of flower visits bats were able to maintain their energy intake over a large range of different sugar contents (1mg/reward to 10mg/reward). Interestingly, the flight activity was constant throughout the experiment. Instead of decreasing their flight activity during conditions with rewards of high sugar content, bats only decreased the number of visits to the artificial flowers but the amount of time bats spend flying remained the same (Von Helversen and Winter 2005). In the present experiment, the average amount of sugar bats received per reward was 7.18mg/reward during 83%, 4.33mg/reward during 50% and 2.60mg/reward during 30% reward probability. According to the results of the experiment described by von Helversen and Winter, bats should have been able to maintain their level of energy intake and as a consequence also their individual DEE. However, other studies have found that *G. soricina* were unable to perform compensatory feeding if sugar concentrations were too low and the water intake very high, potentially due to digestive and osmoregulatory constraints (Ramirez et al. 2005, Ayala-Berdon et al. 2008). Ayala-Berdon and colleagues argued that these different results could be due to differences in the experimental designs (space availability and control of climatic conditions). However, physiological and osmoregulatory constraints provide no explanation for the failed compensatory feeding of bats in this experiment, since the flower profitability was manipulated by changing the reward probability without changing the sugar content per reward. In order to achieve the same energetic intake, the same number of rewards and therefore the same amount of water had to be consumed during all three reward probabilities. Only the number of flower visits had to be adapted accordingly.

Energetic cost of flight

Since flight and especially hovering flight are energetically costly, another possible explanation for the failure to compensate the lower flower profitability in this experiment could be that foraging costs during the lowest reward probability were higher than the energetic gain. During 30% reward probability bats received a mean reward of 12µl which is equivalent to 42J. Furthermore, the mean body weight of bats during 30% reward probability was 8.3±0.6g and the mean hovering duration was 1.05±0.5s. The cost of hovering flight for the closely related species *G. soricina* has been estimated as 158W/g (Voigt and Winter 1999) which means the mean cost of a flower visit was 1.5J. The mean cost of forward flight of *Glossophaga commissarisi* at intermediate speed can be calculated as 1.25W using the formula established by Winter and von Helversen (Winter and Von Helversen 1998) and therefore even 20s of flight, which is more than sufficient in regard to the limited space of the flight cage, would require around 25 J. These calculations show that even during the lowest reward probability the nectar reward at artificial flowers whose average energetic value was 42 J, exceeded the energetic requirement of foraging by far and therefore they provide no explanation why bats had lower energetic intakes and lost significantly more weight when experiencing elevated foraging costs.

Together, these considerations indicate that the reason for the failure of compensatory feeding of *G. commissarisi* in this experiment might lie in the probabilistic reward schedule and the behavioral response to this elevated uncertainty of getting a reward at the known feeders.

In free-living *G. commissarisi* the daily energy expenditure has been estimated as 5.3kJ/g (sd ±0.6) (Voigt et al. 2006). During the highest reward probability of 83%, the average DEE of bats in this experiment was 4.09kJ/d (sd ±0.86) and therefore lower than in free-living individuals. However, this difference might be explained by the smaller flight distances due to the spatial limitations of the flight cage.

Bats increased their average foraging activity from 260 (sd±72) to 680 (sd±206) flower visits per night, and therefore spent more energy on foraging during the condition with 30% reward probability. However, the average DEE decreased 4.09 kJ/g to 3.37 kJ/g. This implies that bats had to reduce their energy expenditure elsewhere. In accordance with this proposition, it has been shown that male mice facing elevated foraging costs save energy in a variety of other physiological aspects for example by reducing pelage, the size of metabolic organs and muscles. Notably, these effects were less severe if animals were only food restricted without the increase of foraging costs (Schubert et al. 2008).

In addition to foraging costs, DEE was also influenced by experimental day (Table 3.1). Foraging activity also decreased over time and therefore bats might have spent less energy later in the experiment independently from the sequence of reward probabilities. However, the effect size of the influence of experimental day on foraging activity and individual DEE was very small. Moreover, bats with smaller

forearm lengths, an indicator of size, showed higher individual DEE. A possible reason for this counterintuitive result might be that although the absolute cost of flight is higher in larger animals, the energy required for each gram of body mass decreases with increasing body mass (Winter 1999). Since we standardized DEE for body mass the finding that bats with lower forearm length had higher DEE per gram is in accordance with this finding.

3.4.2. Individual differences in DEE

Depending on reward probability, 31% to 52% of the variance of DEE was explained by differences between individuals which is slightly lower than the reported repeatability of DEE in wild chipmunks (R = 0.59 (95% CI: 0.25, 0.72)) (Careau et al. 2015). So far, the majority of empirical studies interested in individual differences in metabolic rates measured resting metabolic rates although only DEE incorporates energy spent on actual behavior. The results of this study provide further evidence that individuals also differ consistently in their DEE. Furthermore, bats differed in how much their DEE changed in response to decreasing reward probability and consequently increasing foraging costs (Figure 3.1). Individual differences in the plasticity of metabolic rates have been reported rarely. One study found that individual brown trout differed in how much they changed their standard metabolic rate in response to changes in food availability and showed that more plastic individuals benefited in terms of growths during high food availability whereas less plastic individual benefited during low food availabilities (Auer et al. 2015). Thus, individual differences in the plasticity of DEE can have different consequences for survival under different environmental conditions but further studies are necessary to determine the consequences of individual differences in the plasticity of DEE in these bats.

At the individual level, the DEE of bats was largely determined by the energetic intake through nectar consumption and nectar intake was tightly correlated with foraging activity (Figure 3.3). Therefore, individual differences in DEE and in the plasticity of DEE was largely determined by individual differences in foraging activity and plasticity to adapt foraging activity to changes in reward probability. Individuals with the highest plasticity of foraging activity were able to maintain a higher DEE and lost less weight during the condition with the lowest reward probability and the highest foraging costs. Additionally, plasticity in foraging activity correlated positively with individual plasticity in sampling of unrewarding flowers. This means individuals with the highest foraging activity and DEE during 30% reward probability also invested the most in sampling.

According to the framework of the pace-of-life syndrome, aggressive, bold and active individuals are thought to have a “fast” lifestyle and therefore are also supposed to have higher metabolic rates. High levels of aggressiveness, boldness and activity are also characteristic of a proactive coping style (Koolhaas et al. 1999). Proactive individuals are known to explore quickly and superficially. In the

present experiment, I quantified how much individuals invested in sampling of unrewarding flowers. Individuals with high sampling rates keep track of the environment more closely and should be considered as slow explorers and therefore are more likely to express a reactive coping style. Contrary to expectations, these individuals showed higher foraging activity and also higher individual DEE. However, these results are in line with a recent study with wild chipmunks which also found the counterintuitive result that slow explorers in an open-field test had higher individual DEE (Careau et al. 2015).

Together, the results of this experiment with bats show that individual differences in behavior can indeed be linked to individual differences in DEE, but the direction of this correlation is in contradiction with expectations from theoretical predictions. Further studies are necessary to shed more light on the relationship between individual behavior, energy metabolism and life-history traits.

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CHAPTER 4
Resource defense in the nectar-feeding bat *Glossophaga soricina*

SUMMARY

Aggressive resource defense is frequently observed throughout the animal kingdom and although it is a widespread phenomenon in nectar-feeding birds, reports of interference competition in nectarivorous bats are extremely rare. *Glossophaga soricina*, has been observed to defend flowers of *Agave desmettiana* but not much is known about the social structure during resource defense and how the nectar intake of individual bats is influenced by interference competition. Here, I further investigated the resource defense behavior of *G. soricina* in the controlled environment of a laboratory setting. By using an experimental setup consisting of two patches of computer-controlled artificial flowers it was possible to track the nectar intake of every group member. Furthermore, I was able to establish a method to record aggressive interactions directly at artificial flowers fully automatically. Theoretical models of interference competition predict that aggressive interactions increase the more resources are spatially clumped. Within each experimental night the resource distribution changed from being clumped in one patch to being distributed across two patches in order to assess how changes in the distribution of resources influence the amount of aggressive interactions. Resource defense behavior was assessed in 36 individuals marked with radio-frequency identification (RFID) tags divided into one male and one female group, and four mixed-sex groups. Throughout the experiment males engaged in aggressive interactions significantly more often than females. Only males were successful in defending artificial flowers and they defended the flowers mainly against other males. Subordinate males experienced a substantially decrease their nectar intake. However, females were only marginally affected by male aggression and were able to maintain their level of nectar intake throughout the experiment. These results suggest that the amount of aggressive interactions and the influence of aggressive resource defense on individual nectar intake are sex-dependent in *G. soricina*. Furthermore, as expected, the amount of aggressive interactions was higher and resource defense was only successful during the first part of the night when resources were clumped in one patch, indicating that territoriality in *G. soricina* is more likely to lead to transient monopolization of flowers instead of long term feeding territories with permanent exclusion of intruders.

4.1. Introduction

Competition for limited resources like food or mates is a ubiquitous phenomenon throughout the animal kingdom. Such competition can be indirect by exploiting a common resource and hence prevent others to benefit from it or it can be direct by aggressively defending a profitable location. The latter is known as interference competition (Amarasekare 2002). Aggressive resource defense establishes dominance and by exclusion of competitors leads to priority access to those resources. In the extreme, aggressive resource behavior can lead to exclusive territoriality. Territoriality is a concept belonging to a continuum ranging from the transient monopolization of a preferred feeding opportunity to the long-term defense of an area as exclusive territory (Maher and Lott 2000). The rules of economic defendability state that the benefits of resource defense have to outweigh the costs (Brown 1964) and therefore the species-specific costs of resource defense determine the intensity of interference competition and where its resulting territoriality aligns itself along this continuum.

In nectar-feeding birds resource defense and territoriality have been described commonly around the world. For example, males of the American hummingbird species *Calypte anna* hold feeding territories during the non-breeding season (Stiles 1971), the Australian red wattlebird defends flowers of *Eucalyptus cosmophylla* against other nectar-feeding birds (Ford 1981) and the African Golden-Winged sunbird establishes feeding territories which contain flowers of the montane weed *Leonotis nepetitolia* (Gill and Wolf 1975). In contrast to nectar-feeding birds, not much is known about territoriality and resource defense in nectarivorous bats and reports are extremely rare.

Although both use nectar as their main food source, the costs of resource defense might be higher for nocturnal, echolocating bats than for diurnal, visually oriented birds. Especially nectarivorous bats of the neotropical family Phyllostomidae are known to have echolocation calls at very low intensity which gave them the descriptive name “whispering bats” (Howell 1974). Thus, detecting intruders at a feeding territory’s boundary might require extensive and energetically costly patrolling flights. In addition to generally low intensity echolocation calls, nectar-feeding bats trying to intrude a defended space have another advantage if they know their feeding area. Compared to an insect-hunting bat that continually scans for elusive prey by active echolocation, an intruding flower visitor can approach a target with minimal echolocation when seeking nectar at known locations. Thus, the potentially higher costs for patrolling flights and the possibility of “sneaking” into a defended territory might affect interference competition of nectar-feeding bats.

Some nectar-feeding bat species forage in flocks like for example *Leptonycteris yerbabuenae* (Howell 1979) whereas other species seem to forage solitary like *G. soricina* (Heithaus et al. 1974). In accordance with these different foraging strategies, the few accounts of resource defense come from species that have been described to forage solitary.

In these reports, resource defense does not cover the area of a typical feeding range but is restricted to a single or a few flowering plants. Costa Rican *Glossophaga commissarisi* have been observed to

occasionally defend and temporally monopolize single inflorescences of the understory palm *Calypterogyne ghiesbreghtania* against other hovering bats, perching bats and katydids (Tschapka 2003). Another report of flower defense in nectar-feeding bats comes from behavioral observations of the species *Glossophaga soricina* in Colombia (Lemke 1984, 1985). Individuals of this species were observed to defend inflorescences of *Agave desmettiana* against conspecifics by chasing intruders away and sometimes even knocking them from their feeding perch (Lemke 1984, 1985). *Agave desmettiana* is especially profitable and therefore suitable to defend due to the large number of flowers per inflorescence and high nectar productivity (Lemke 1984). Furthermore, the exposed position of inflorescences and the close proximity of flowers facilitate resource defense. Both, males and females were observed in aggressive interactions but only six individuals were marked for individual identification and only for a short period of time.

Together these two studies show that glossophagine bats can defend resources aggressively in order to establish dominance and gain priority access to resources. However, the social structure and the extent to which resource defense influences the nectar intake of individual bats remains poorly understood. In order to assess the consequences of resource defense and to investigate potential differences between males and females, commonly observed in nectar feeding birds (Gill and Wolf 1975, Ford 1981, Rousseu et al. 2014), I conducted a naturalistic foraging study of interference competition in the laboratory with the nectar-feeding bat species *Glossophaga soricina.* By using artificial computer-controlled flowers and marking each individual with an electronic radio-frequency identification (RFID) tag I was able to track all flower visits and the total nectar consumption of every individual. The occurrence of resource defense is predicted to be highest at intermediate levels of food abundance (Grant et al. 2002). When food is scarce the defended territory does not provide enough resources to cover the energetic costs of aggressive defense behavior whereas during high levels food abundance individuals that do not defend resources can obtain the same amount of food as territorial individuals without paying the costs of defense. In order to limit the resource availability, artificial flowers were programmed to provide nectar with a fixed interval reward schedule. Once a nectar reward had been taken by any bat, the fixed interval had to pass before the next reward was available at that flower. This reward schedule mimics the natural situation of flowers with nectar reservoirs that get depleted by foraging bats and refill with time due to the steady rate of nectar secretion.

Theoretical models of interference competition predict that clumped resources lead to more agonistic behavior and resource defense than distributed resources (Grant 1993). To include a test of this prediction in the experimental design, the flower field was spatially subdivided into two patches and programmed to automatically change the spatial distribution of available nectar resources during the night.

By using mixed-sex, male and female groups of *G. soricina,* it was possible to further investigate the social structure and the consequences of the resource defense behavior on the nectar consumption of

other group members. Furthermore, the change of resource distribution allowed for testing the hypothesis that aggressive resource defense behavior increases at spatially concentrated resources.

4.2. Methods

4.2.1. Subjects and housing

Experiments were conducted with 36 individuals of the nectarivorous bat species *Glossophaga soricina* (Palla's long-tongued bat). Bats came from a captive colony and were older than one year as judged by finger joint ossification (Brunet-Rossinni and Wilkinson 2009). Bats carried Radio Frequency Identification (RFID) tags attached to cable tie collars (total weight of collar and RFID tag = 0.2g, max. 2.4% of the body weight) that were removed after the experiment. Additionally, bats had numbered plastic split rings (A C Hughes) for visual identification around the forearm. Temperature in the experimental and colony room was kept at 20-25°C and air humidity at 65-75%.

4.2.2. Experimental setup

In the experimental room ten artificial flowers with automated nectar delivery (Winter and Stich 2005) were mounted along a 4.2m bar at a height of 1.20m (Fig. 4.1A). The distance between flowers was 0.4 m. Flowers were divided into two groups of five to simulate two flower patches. Each patch was enclosed by a sheet-covered frame to separate the groups of flowers spatially (Fig. 4.1B). The only entrance to the patches was a 40cm gap between the ground and the bottom end of the enclosure (Fig. 4.1C, dashed line). From this entrance bats had to fly up vertically to reach the flowers.

A syringe pump delivered nectar via tubes and electronic valves to the artificial flowers. The interruption of an infrared light beam at the flower opening detected the visit of a bat and triggered the delivery of a nectar reward. The RFID reader below the flower head identified a bat's RFID code. Visits to all flowers including non-rewarding visits during every experimental night from 4pm to 4am were recorded. The reward schedule of the artificial flowers was configured using PhenoSoft Control (Phenosys GmbH, Berlin, Germany). Every detected event at a flower (including date, time, identity of the individual, duration of the event and amount of nectar delivered) was recorded for data analysis.

4.2.3. Experimental procedure

Six bats were randomly caught from the colony, and were tested as a group at the same time. Four experimental groups consisted of three males and three females (mixed groups) whereas one group consisted of six males and one of six females. Before the experiment bats were weighed and forearm length was measured.

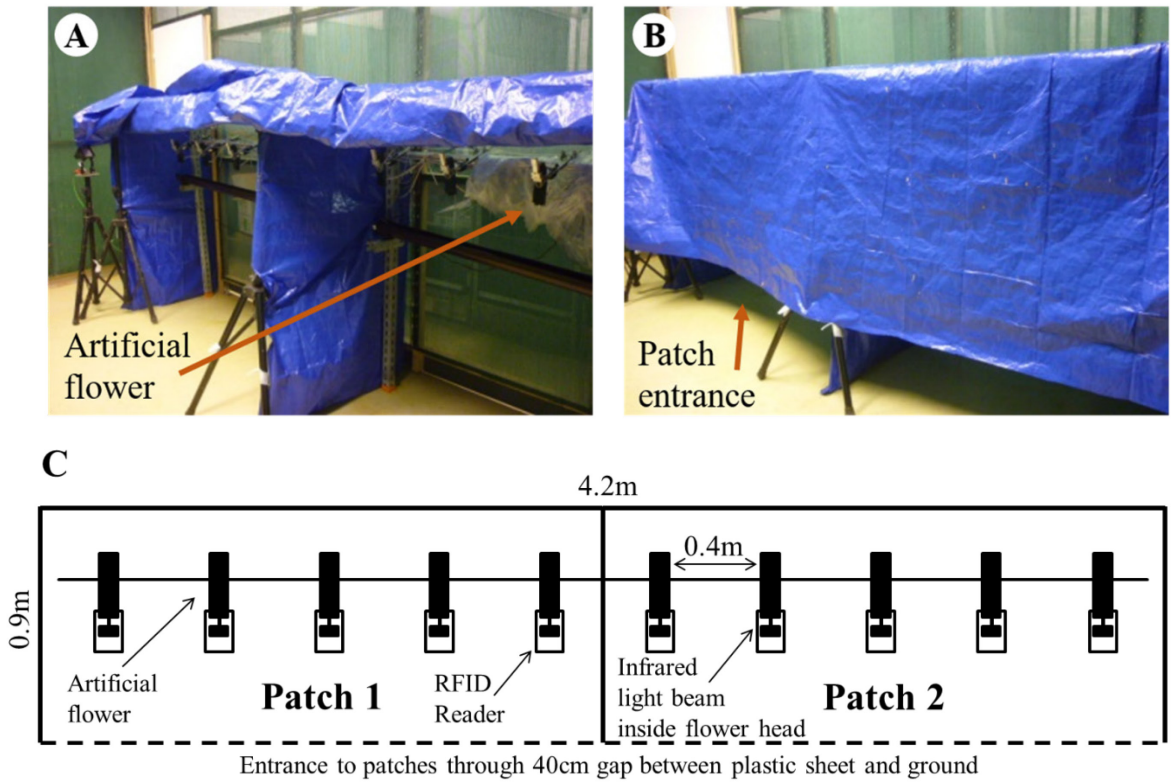

Figure 4.1: Experimental setup consisting of two spatially separated flower patches. (A) The flowers where mounted 1.2m above ground. These ten flowers were divided into two patches with 5 flowers each. (B) By using a wooden frame covered with plastic foil the patches were spatially separated. To make it more demanding for bats to enter the patch, the only entrance was close to the ground in front of the flowers through a gap of 0.4m from the ground to the lower rim of the plastic foil. (C) Schematic drawing of the experimental setup from above, the dashed line indicates the side with access to the two flower patches.

During the nightly experiments, in addition to the nectar provided by artificial flowers, bats had access to pollen and water ad libitum and to 6ml of additional food containing 200mg NektarPlus and 300mg milk powder resolved in water. Rewards at flowers consisted always of 30µl nectar (15% w/w sugar concentration, sucrose: fructose 1:2). Before the experimental schedule started, individuals were allowed to familiarize themselves with the setup and the artificial flowers. Since during this phase the cover was removed, the two flower patches were not spatially separated and every flower visit was rewarded. This phase lasted for one to four days until each bat visited the flowers regularly. One female of the first mixed group did not visit any artificial flower during the first night and was replaced by another female.

During the experimental schedule, the two flower patches were covered and spatially separated. Experimental nights were divided into two phases. During the first phase of the night only one of the

two flower patches was rewarding, and therefore the resources were spatially clumped. The fixed time interval between rewards was 60 seconds. During the second phase of the night both patches gave rewards, resources were evenly distributed across the patches, and the fixed time interval between two rewards at a flower was increased to 120s. Therefore, the amount of food available per unit time remained the same during the whole night; only the spatial distribution of food changed from the clumped resource condition with one patch rewarding (five flowers) during the first phase of the night to the distributed resource condition with two patches rewarding (ten flowers) during the second phase of the night. With this experimental schedule, the maximal amount of nectar the bats could collect was 108ml, which corresponds to 18ml nectar per individual and night. The rewarding patch during the first phase of the night was chosen pseudo-randomly and the same patch was never chosen in more than two consecutive nights. Details about the sequence of the first rewarding patch during the clumped resource condition is provided in Table 4.1. For the mixed groups, the duration of the clumped resource condition was 6 hours and the experiment lasted 9 days (7 days for the first mixed group). The duration of the first part of the night was more variable (4-8 hours) in the same-sex groups and the experiment lasted 8 days for the male group and 9 days for the female group. For more details about the experimental schedule for each group see Table 4.1.

Table 1: Experimental schedule for all six social groups. Patch1 contained flowers 1-5 and Patch 2 contained flowers 6-10.

	Four mixed groups		**Male group**		**Female group**	
Night	Duration of clumped resource condition [h]	Active patch during clumped resource condition	Duration of clumped resource condition [h]	Active patch during clumped resource condition	Duration of clumped resource condition [h]	Active patch during clumped resource condition
1	6	1	6	1	6	1
2	6	2	8	2	8	2
3	6	2	6	1	5	1
4	6	1	6	1	7	1
5	6	1	7	2	4	2
6	6	2	6	1	6	1
7	6	1	7	2	8	2
8	6	1	6	2		
9	6	2				

4.2.4. Chasing behavior

The chasing frequency of individuals in front of artificial flowers was quantified as an estimate of the amount of aggressive interactions between group members. I developed a method to automatically detect and score chasing events using the computer collected data from the RFID sensors and feeder sensors. Initially, three mixed groups were video recorded for 24h spread out over 14 days, and the

video data was synchronized to the computer collected data. From the analysis of the combined video and computer recorded data I was able to identify the following pattern of events in the computer collected data that reliably indicated a chasing event between two identified individuals: (i) an identified bat collected a reward at a flower, (ii) its visit ended and (iii) was immediately followed by a very brief detection (<200ms) of a second bat, the chaser, but through the RFID sensor only. Importantly, this second bat never attempted to drink and therefore did not interrupt the light barrier inside the flower head. This distinguishes a chase from the occasional quick succession of two drinking visits by two bats at the same flower (for details see Table A 2.4). The advantage of detecting chasing events from a pattern of automatically recorded data was not only a highly time-efficient procedure for the experimenter but also avoided the risk of human observer bias as is common for video analysis. For the 24-hours of combined video and automatically logged data, all 89 chasing events detected in the computer-logged data could be confirmed by video. Therefore, the algorithm for detecting chasing events in the logged data was considered to be highly reliable. Of course, chasing took also place outside of flower visits. Thus, our numbers are only an index of chasing intensity between pairs of bats. In one hour of video 61 chasing events were observed of which only 5 were during flower visits and recorded in the data. But since a total of 1757 such chasing events were detected (see below) for 36 participating bats, the automated approach was considered sufficient for quantifying the within-group dominance relationships. Since the total number of flower visits per night affects the number of possibilities that a bat can be chased by another individual, we corrected our counts of chasing events by dividing observed chases by the number of all visits made by a bat per night.

4.2.5. Statistical Analysis

To investigate the difference in chasing behavior between males and females and between the resource conditions (one versus two rewarding patches) a general linear mixed model (GLMM) with a binomial error structure was used. Forearm length as an approximation of size, the duration of the experiment (number of days) and the interaction of resource condition and sex were included as fixed effects and the influence of these fixed effects on the proportion of chasing events was assessed. Experimental group and individual were included as random effects. The same model structure was used to address the question if the proportion of being chased was influenced by these independent variables. If one or more individuals start to defend flowers and thus exclude others from drinking, nectar consumption should increasingly differ between individuals since the successful chaser should gain a higher nectar intake whereas the chased individual should receive less. Therefore, the between-individual difference in nectar consumption during the first two days was compared with the difference in nectar consumption during the last two days for each experimental group and for each resource condition. First, each individual's mean nectar consumption during the clumped (one rewarding patch) and distributed (two rewarding patches) resource condition was determined for the first and the last two days respectively.

Then this data was used to calculate group means and their standard deviation. However, the group means were calculated separately for the males and females of each group. By definition, standard deviation is a measure of the spread within a set of data points. The higher the standard deviation the larger are the differences between individuals. In order to assess the influence of resource defense on the individual differences in nectar consumption a linear model was used with the following fixed effects: sex, the beginning/end of the experiment (first two days vs. last two days), the duration of the experiment (number of days) and the interaction of sex and beginning/end of the experiment. The influence of these fixed effects on the standard deviation of mean nectar consumption was assessed during the clumped and distributed resource condition respectively. To compare the standard deviations of males and females at the end of the experiment during the clumped resource condition, a Post Hoc test was performed using Tukey HSD (honest significant difference) provided by the lsmeans package in R (Lenth 2016).

By plotting individual nectar consumption during the last two days of the experiment against the frequency of chasing other individuals two non-overlapping groups of males were obtained, which were labelled dominant and subdordinate males respectively. Such a pattern was not observed in females. Therefore, each mixed group contained individuals belonging to one of three different types of social status: female, dominant male, and subordinate male. To address the question how nectar consumption changed during the course of the experiment depending on the social status I merged the factors social status and part of the experiment (first two days vs. last two days) into a combined factor with six levels. With a linear model, I assessed the effect of that factor on individual nectar consumption. To further investigate which levels of that factor were different from each other, simultaneous tests for general linear hypotheses using Tukey contrasts for multiple comparisons of means were performed. The multcomp package in R (Hothorn et al. 2008) was used to conduct these tests. Since the number of data points in the three groups of social status differed greatly I controlled for heteroscedasticity by incorporating sandwich estimators which provide a heteroscedasticity-consistent estimate of the covariance matrix (Zeileis 2006). This procedure was applied for the first part of the night during which resources were spatially clumped at one patch and for the second part of the night during which resources were spatially distributed across two patches.

All statistical tests were performed using R version 3.3.1 (R Core Team 2015).

4.3. Results

The goal of these experiments was to investigate the social structure of resource defense in *Glossophaga soricina* and how interference competition influences individual nectar intake. An example of the progression of nectar consumption throughout the experiment within one mixed group is shown in Figure 4.2. After only two days the nectar consumption of two males reached a level close to zero

whereas the third male increased consumption substantially (Fig. 4.2A). This pattern, however, only occurred during the condition when resources were clumped. Nectar consumption of females did not change even during the clumped condition. On the same days but during the second half of the night with resources distributed over two patches, nectar consumption of males and females equalized at the end of the experiment (Fig. 4.2B).

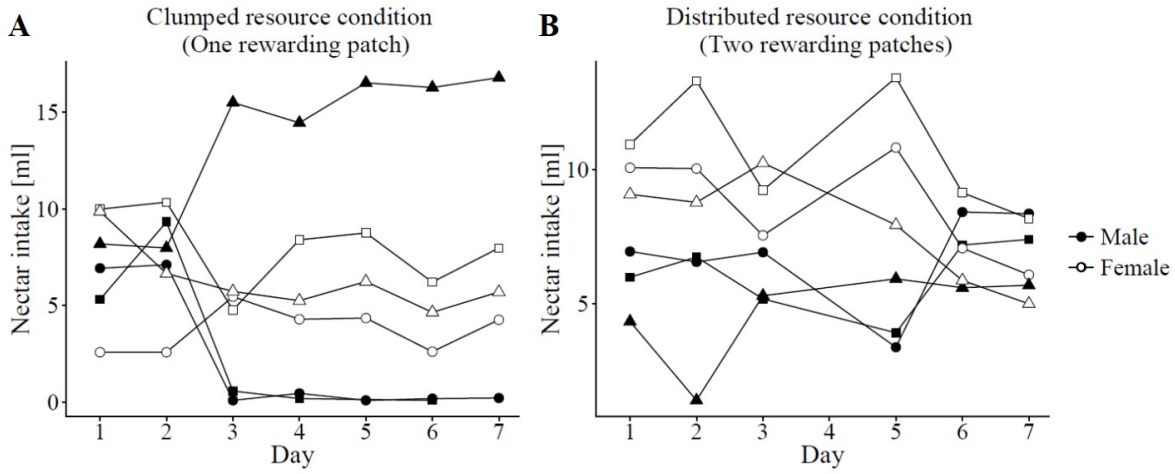

Figure 4.2: Exemplary nectar consumption during the course of the experiment by individuals belonging to a mixed-sex group consisting of three males and three females. Black symbols represent male individuals, white symbols females. (A) Clumped resource condition (first part of the experimental night) with rewards being concentrated at one patch. (B) Distributed resource condition (second part of the experimental night) with rewards being available at both patches. (The second part of the night of day 4 had to be excluded due to technical problems.)

In total 1757 chasing events were identified within the data. In all mixed groups males chased other bats in front of flowers significantly more often than females ($z = -3.57$, $p < 0.001$) (Fig. 4.3A, Table 4.2). Notably, the frequency of females as active chasers in female-only groups was much higher than chasing by females in the mixed groups. Although the rate of nectar availability remained constant throughout the night and only the spatial distribution of the resources changed, the number of chasing events was significantly lower during the distributed resource condition when rewards were available at both patches (chasing: $z = -11.45$, $p < 0.001$). The significant interaction between sex and resource condition (clumped vs. distributed) shows that females decreased their chasing frequency more than 1 males ($z = -0.34$, $p = 0.007$). There was no significant difference between the sexes in how often a bat was chased by another individual ($z = 1.97$, $p = 0.05$) but individuals were chased less during the distributed resource condition ($z = 13.39$, $p<0.001$) (Fig. 4.3B). Forearm length as an indicator of size had no significant effect on chasing frequency or the frequency of being chased ($z= -1.87$, $p = 0.07$).

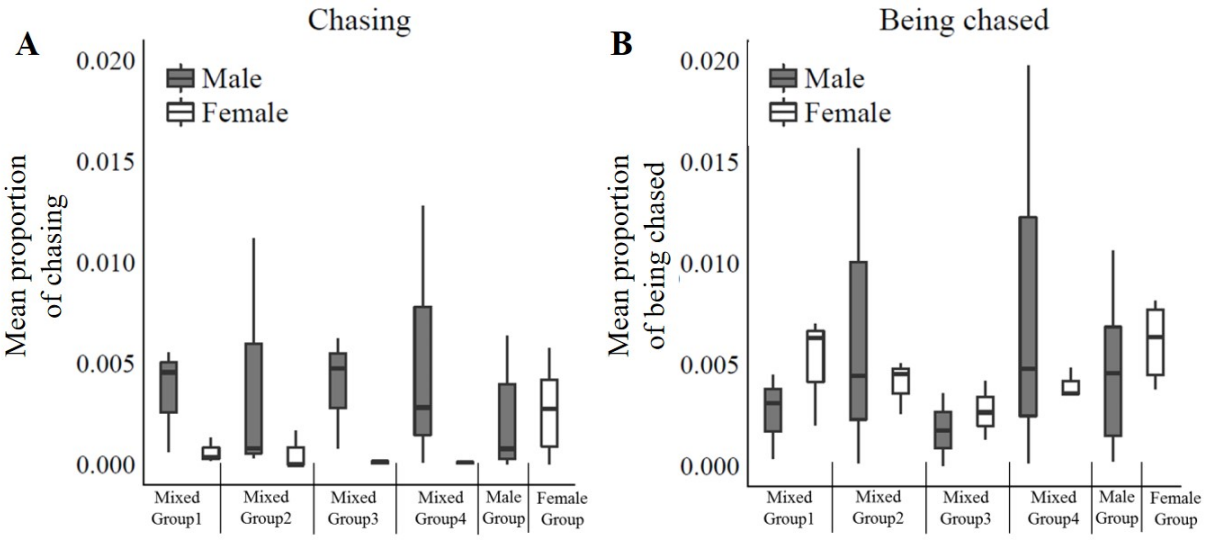

Figure 4.3: (A) During the clumped resource condition in mixed groups of three females and three males, females chased other individuals significantly less often than males did ($z = -3.57$, $p < 0.001$). Shown are the mean proportions of chasing events for each individual over the course of the experiment. Notably, in the females-only group the proportion of chasing events is higher than of females in any of the mixed groups. (B) The proportion of being chased by other bats did not differ significantly between the two sexes ($z = 1.97$, $p = 0.05$), but the variance was much higher for males. Shown are boxplots with medians, quantiles, whiskers and outliers.

Resource defense is expected to increase the differences in nectar consumption between individuals. Between-individual differences in nectar consumption was quantified as the standard deviation of group means. However, group means were calculated for males and females separately. During the clumped resource condition, the standard deviation increased significantly ($t = 4.04$, $p = 0.001$) from the first two to the last two days of the experiment (Fig. 4A, Table 2). This increase was significantly higher in males as compared to the increase in females ($t = -2.63$, $p = 0.02$) and the post hoc comparison (Tukey HSD correction of p-values) of male and female standard deviation of nectar consumption at the end of the experiment showed that the between individual differences in the male subgroups was significantly higher than in the female subgroups ($t = 3.9$, $p = 0.007$). However, during the distributed resource condition the standard deviation of mean nectar consumption neither differed between the subgroups of the two sexes ($t = -1.12$, $p = 0.25$) nor did it change from the first two days to the last two days of the experiment ($t = -1.53$, $p = 0.15$) (Fig. 4.4B).

Table 4.2: Fixed effect estimates derived from the generalized linear mixed model used to assess differences in proportion of chasing (1) and being chased (2) between the sexes and between clumped and distributed resource conditions. Groups and individuals were included as random effects.

Model		Estimate	z-value	p
1)	**Difference in chasing behavior between males and females (n=581)**			
	Intercept	-5.70	-13.36	
	Sex (female)	-1.80	-3.57	**<0.001**
	Part of experimental night (clumped resources)	-0.64	-11.45	**<0.001**
	Sex (female) * Part of experimental night (clumped resources)	-0.34	-2.69	**0.007**
	Forearm length	-0.02	-1.87	0.07
2)	**Difference in being chased between males and females (n=581)**			
	Intercept	-2.24	-0.64	0.52
	Sex (female)	0.76	1.97	0.05
	Part of experimental night (clumped resources)	-1.06	-13,39	**<0.001**
	Sex (female) * Part of experimental night (clumped resources)	-0.07	-0.64	0.52
	Forearm length	-0.42	-1.17	0.24

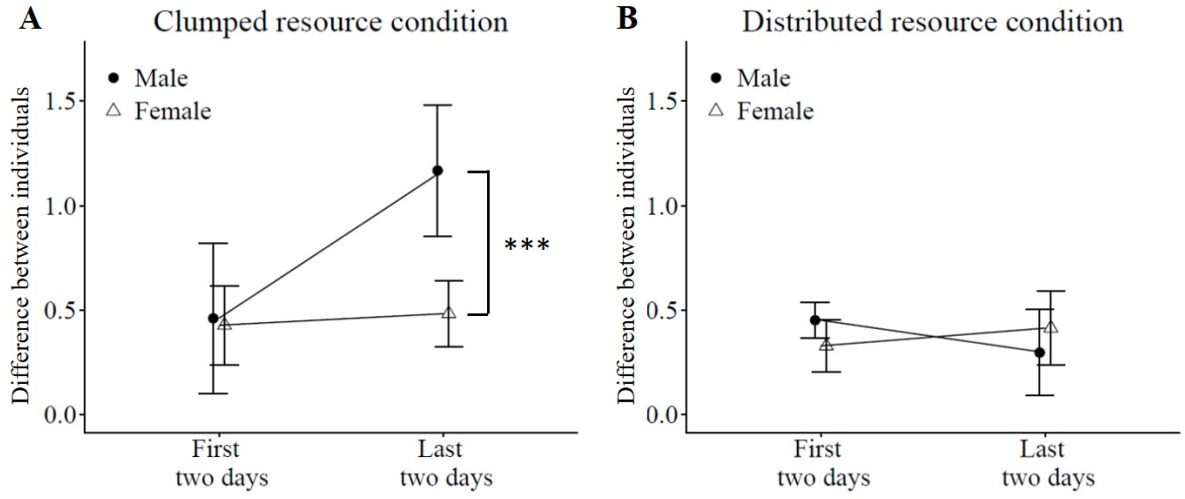

Figure 4.4: The standard deviation of group mean nectar consumption was used to measure the between individual differences in nectar intake. It was calculated for each experimental group and for males and females of each group separately. Every data point represents the average between individual difference across all experimental groups and whiskers illustrate the standard deviation of the mean between individual difference. (A) During the clumped resource condition with only one patch being rewarding the difference between individuals in nectar consumption increased significantly during the experiment ($t = 4.04$, $p = 0.001$) but Post hoc comparison of standard deviations show that individuals in male subgroups differed significantly more in their nectar consumption than individuals in female subgroups ($t = 3.9$, $p = 0.007$). (B) During the distributed resource condition, between individual differences in nectar consumption did not change during the experiment ($t = -1.53$, $p = 0.15$) and there was no difference between the sexes ($t = -1.12$, $p = 24\ 0.25$).

Table 4.3: Fixed effect estimates from the linear model used to assess the effect of sex and clumped (1) and distributed (2) resource condition on the standard deviation of mean nectar consumption of males and females throughout the experiment (first two days vs. last two days).

Model		Estimate	t-value	p
1)	**Standard deviation of mean nectar consumption of males and females (clumped resource condition) (n=20)**	0.68	1.14	0.27
	Intercept	-0.04	-0.22	0.83
	Sex (female)	0.71	4.04	**0.001**
	Part of the experiment (last two days)	-0.65	-2.63	**0.02**
	Sex(female) : part of the experiment (last two days)	-0.03	-0.37	0.72
	Duration of the experiment (no. of days)			
2)	**Standard deviation of mean nectar consumption of males and females (distributed resource condition) (n=20)**	0.34	0.93	0.37
	Intercept	-0.12	-1.12	0.25
	Sex (female)	-0.15	-1.53	0.15
	Part of the experiment (last two days)	0.24	1.67	0.11
	Sex(female): part of the experiment (last two days)	0.01	0.32	0.75
	Duration of the experiment (no. of days)			

When plotting chasing events against nectar consumption the data for males fall into two non-overlapping groups. The males of one cluster (Fig.4.5A, inside circle) chased (chasing frequency > 0.0045) and consumed more nectar (> 1.5ml/h) than the other males. This cluster always included only one male for the mixed groups but two males for the males- only group. These males were categorized as “dominant”. The second cluster of males (Fig.4.5A, outside dashed circle) was characterized by a low amount of active chasing and by low nectar consumption. These males were categorized as “subordinate”. In females, such a pattern did not emerge (Fig. 4.5B). In the group of only females four females chased other females more often but these four females did not fall into a non-overlapping cluster.

During the last two days of the experiment, the three groups clearly differed, with the highest nectar intake in dominant males, a median intake in females, and lowest nectar intake in subdominant males (Fig. 4.6A, Table 4.4). Females maintained their level of nectar intake during the clumped resource condition throughout the experiment ($t = -0.76$, $p = 0.97$). Overall, nectar consumption did not change for any social group during the distributed resource condition (Fig. 4.6B).

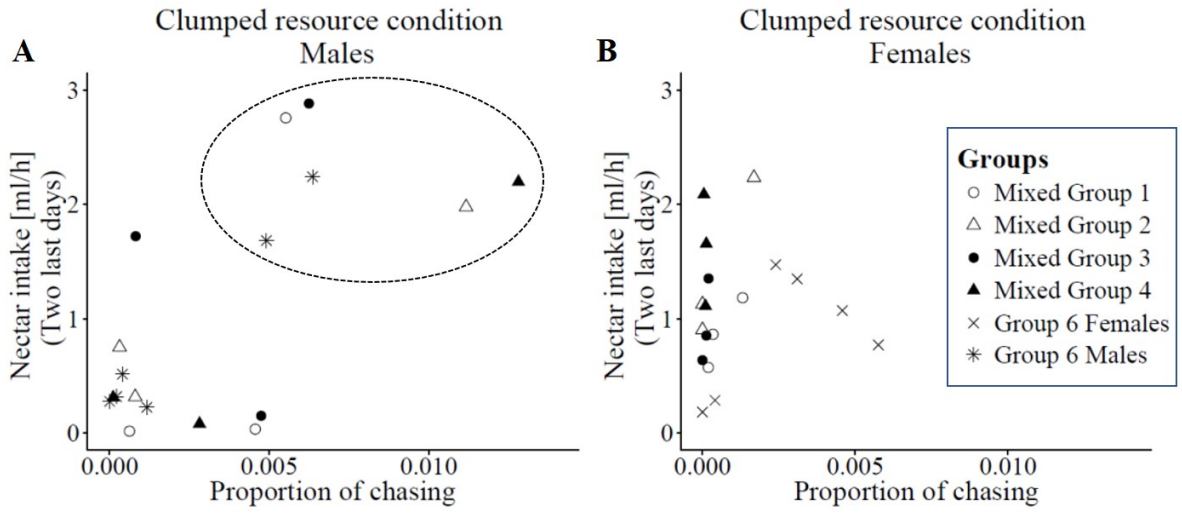

Figure 4.5: Influence of chasing frequency on nectar intake during the last two days of the clumped resource condition. (A) Males with a high proportion of chasing events also consumed more nectar at the end of the experiment. By considering the amount of chasing and the amount of nectar an individual received at the end of the experiment, males were divided into two non-overlapping groups. Dominant males (inside dashed line oval) met both conditions; they chased other individuals in front of flowers more (>0.0045) and received more nectar (>1.5ml/h). Individuals outside the dashed line oval were considered as subdominant males. (B) Nectar consumption of females in mixed groups did not depend on chasing frequency during the clumped resource condition and non-overlapping groups did not emerge. However, in the females-only group four females clearly received more nectar and chased other individuals more than the two remaining females.

Table 4.4: Post-hoc multiple pairwise comparisons of factor levels influencing the nectar consumption 23 of individuals during the clumped (1) and distributed (2) resource conditions throughout the experiment. 24 Significance levels were corrected using Tukey Honest Significant Differences. Only significant 25 comparisons are shown.

Model		Estimate	z-value	p
1)	**Change of nectar consumption during the experiment depending on social status (clumped resource condition)** (Multiple comparisons of means, only relevant significant interactions listed below) (n=72)			
		-0.81	-5.25	**<0.001**
	First two days (subdominant) – First two days (dominant)	0.69	3.20	**0.02**
	Last two days(dominant) – First two days (dominant)	-0.43	-3.38	**0.01**
	First two days (subdominant) – First two days (female)	-1.20	-5.64	**<0.001**
	Last two days(female) – Last two days (dominant)	-1.90	-8.88	**<0.001**
	Last two days(subdominant) – Last two days (dominant)	-0.70	-3.90	**0.003**
	Last two days(subdominant) – Last two days (female)			
2)	**Change of nectar consumption during the experiment depending on social status (distributed resource condition)** (Multiple comparisons of means, only relevant significant interactions listed below) (n=72)			
	First two days (subdominant) – First two days (female)	-0.36	-3.39	**0.01**

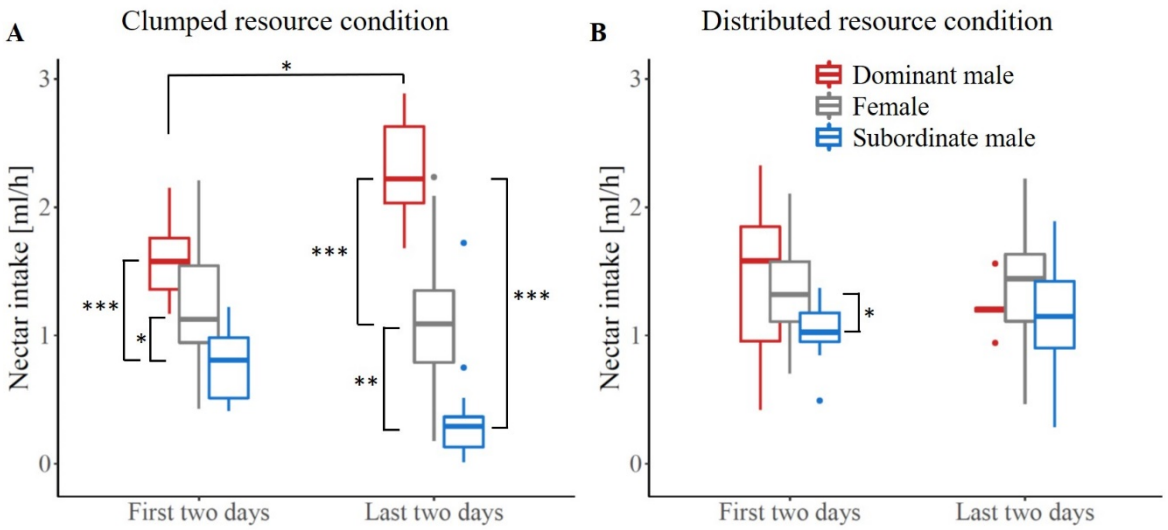

Figure 4.6: Comparison of nectar consumption during the first and last two days of the experiment depending on sex and social status. (A) During the clumped resource distribution, already at the beginning of the experiment subdominant males received significantly less nectar than dominant males ($t = -5.3$, $p<0.001$) and females ($t = -3.4$, $p = 0.01$). Dominant males further increased their nectar intake significantly during the course of the experiment ($t = 3.2$, $p = 0.02$). At the end of the experiment females, dominant and subdominant males differed to a large extent in their nectar consumption (Dominant-Female: $t = -5.6$, $p<0.001$, Dominant-Subdominant: $t = -8.9$, $p<0.001$, Female-Subdominant: $t = -3.9$, $p = 0.003$). (B) During the distributed resource condition at the beginning of the experiment subdominant males received less nectar than females ($t=3.4$, $p = 0.01$) but these differences disappeared by the end of the experiment.

Behavioral observations

Qualitative behavioral observations of four hours of video recordings revealed several behaviors that seem to be characteristic for dominant males. Instead of just visiting the flowers and leaving the patch as the other individuals did, dominant males remained hanging between the flowers within the patch for a significant amount of time. When other individuals came close due to visits of directly adjacent flowers, dominant males often spread one wing in the direction of the other individual which could be interpreted as a threatening posture.

Some individuals were attacked and chased away by dominant males while visiting artificial flowers. In this case, dominant males mostly attacked from above with their mouth wide open, and followed the intruder for a short distance. Sometimes the chasing escalated into fighting with both bats falling towards the ground and resuming their flight only shortly before they hit the floor. In rare cases, these fights might have led to small injuries. One subordinate male had various new scratches on its wing that were not present before the experiment and that were possibly caused by claws (Fig. A 2.2). After a successful flower defense, the dominant male normally visited most of the flowers within the patch before returning to its position between the flowers.

4.4. Discussion

Similar to observations in free-living populations, in this experiment *Glossophaga soricina* competed for nectar not only by exploitation but also by interference competition. However, the results show that the predisposition to defend resources and the influence of interference competition on individual nectar intake differed significantly between the sexes. Only a subset of males successfully defended flower patches. These males were characterized by the highest frequency of chasing other individuals away from profitable flowers and by a substantial increase in their nectar intake by the end of the experimental run. Although these dominant males chased females and other males equally often, only the nectar intake of subordinate males was affected by this behavior whereas females were able to maintain their level of nectar consumption throughout the experiment. Thus, interference competition increased the difference in nectar intake between males but not between females. The amount of aggressive interactions was higher and males only defended resources successfully at the beginning of the night when the available nectar was concentrated at only one flower patch thereby confirming the hypothesis that clumped resources lead to an increase in aggressive interactions (Grant 1993).

To the best of my knowledge, this study is the first report of sex-dependent differences in the resource defense behavior of nectar-feeding bats. In mixed sex groups, females seemed to be unaffected by the behavior of dominant males whereas subordinate males were excluded at least partially from the defended flower patch. There are two possible explanations for this differential effect on subordinate males and females. One the one hand, dominant males might be just not capable to exclude females. On the other hand, dominant males could tolerate females in their defended patch because they might receive additional benefits, for example tolerating females could lead to an increase in mating opportunities. Similar social dynamics have been described in the insectivorous bat species *Myotis daubentoniid*. Dominant males of this species temporarily exclude other males from profitable habitats whereas females are tolerated and in addition to securing access to resources, the successful exclusion of other males has been shown to increase the reproductive success of dominant males (Senior et al. 2005). Also in the hummingbird species *Eulampis jugularis* it has been observed, that males which successfully defend highly profitable feeding-territories against other males while they share the available resources with females, experienced an increase in their mating success (Temeles and Kress 2010).

However, the results of this study show that dominant males chase females as often as other males. If females are able to feed in the defended patch because dominant males tolerate them due to potential additional benefits, it would be likely that the observed chasing behavior of dominant males itself differs depending on the sex of the intruder. In this experiment, the frequency of chasing events was extracted from data automatically recorded at artificial flowers (interruption of infrared light beam and individual identification through RFID reader). Therefore, it was not possible to determine if males showed

behavioral differences between chasing other males and chasing females. However, the recorded video revealed that individuals chased each other not only directly at the artificial flowers but also in other areas of the flower patch. Since individuals could only be identified by their RFID tag directly at the RFID reader attached to artificial flowers the sex of individuals chasing each other in other areas remains unknown. However, some individuals showed marks from small injuries at their wings after the experiment (see example Fig. A 2.2) and such marks were only observed in males. This could be an indication that dominant males could have been additionally aggressive towards subordinate males besides the interactions observed directly at the flowers and this could explain why subordinate males were more affected than females by the aggressive resource defense behavior of dominant males.
Generally, the chasing frequency of females was always low and although the amount of agonistic behavior increased within the female group compared to in mixed groups, female bats never succeeded in defending a flower patch neither against other males nor against other females. These findings are similar to the social structure of resource defense found in some nectar-feeding bird species. For example, in a study on resource defense of free-living ruby throated hummingbirds Rousseu and colleagues also found only low levels of defense in females (Rousseu et al. 2014) and although in the hummingbird species *Eulampis jugolaris* both, males and females, defend feeding territories during the non-breeding season, males were always dominant over females (Wolf and Hainsworth 1971, Temeles et al. 2005).
Further studies are necessary in order to better understand why females are less affected by the aggressive resource defense behavior of dominant males compared to subordinate males and also why females themselves were not able to monopolize the profitable patch.

In all mixed sex groups, only one male per group became dominant and successfully defended flowers, whereas in the male group two males succeeded in excluding other males from a flower patch (Fig 4.5). However, a closer look at the nectar consumption at each flower revealed that these two males did not share all flowers of the patch but divided the flowers between the two of them (Appendix Fig. A 2.3). Therefore, food resources were defended by at most one male at the same time. Additionally, the successful resource defense of two individuals in the male-only group showed that resource defense can occur independent of the presence of females.
Although the position of the rewarding patch during the clumped resource condition changed between the nights, always the same male continued to successfully defend the patch. This means males defended the resources themselves and not the space. Furthermore, it shows that even after changing the location of the defended patch the same individuals were able to succeed in monopolizing the resources indicating a stable hierarchy at least for the duration of the experiment.

The ability of an individual to successfully defend and monopolize resources is often correlated with distinct physical characteristics for example size (Searcy 1979). However, the results show that forearm length as an approximation of size did not correlate significantly with the chasing frequency of individuals (Table 4.2) and therefore did not predict which male succeeded to defend a flower patch during this study. Another factor that could influence the success in defending flowers is age and therefore experience (Yasukawa 1979, Arcese 1987). Since I could only discriminate between young and adult animals, age and consequently experience cannot be dismissed as a predictor of successful flower defense.

In this study, subordinate males received considerably less nectar than dominant males and females. However, except in one mixed-sex group, subordinate males were rarely excluded completely from the flower patch and their average nectar intake during the clumped resource condition was still 0.39 ml/h (sd ±0.47). This result is in accordance with observations of free-living *G. soricina* in Colombia. There, subordinate bats exploited the flowers defended by other individuals as soon as they had the opportunity preventing the dominant bat from having an exclusive access to the defended flowers (Lemke 1984). Furthermore, the frequency of chasing events decreased significantly during the distributed resource condition during the second part of the night thereby confirming the theoretical prediction that aggressive defense behavior increases when resources are spatially concentrated (Grant and Guha 1993). Resource defense should only occur when the energy gain outweighs the cost of aggressive interactions (Brown 1964). Therefore, a possible explanation for this observation could be the decrease in quality of the defended patch once the nectar was divided between the two patches. Together, these results suggest that in the continuum of territoriality, resource defense observed in *G. soricina* seems to represent rather a transient monopolization of resources than a long term permanent exclusion of intruders.

In summary, although flower defense behavior of *G. soricina* was investigated in a laboratory setting, similar behavior was observed as in free-living populations. In addition, these results revealed a sexual dimorphism in flower defense behavior. Only males successfully defended flower patches and excluded other males from their defended resource, whereas females remained unaffected by this behavior and continued to visit the flowers guarded by a male. This observed pattern is similar to resource defense behavior observed in other nectar-feeding vertebrates. Furthermore, I could show that the amount of aggressive interactions was, as predicted, higher when resources were clumped in one patch. Future studies with free-living populations have to be conducted to assess how frequent and important resource defense in these nectar- feeding bats is and if males that are successful in defending resources have additional advantages for example an increase in mating opportunities.

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CHAPTER 5
Changes in social group composition do not disrupt individual behavioral differences in the nectar-feeding bat *Glossophaga soricina*

SUMMARY

Even in highly dynamic social environments, individuals can differ consistently in their behavior. There are at least two hypotheses that propose mechanisms that can explain how consistent individual differences emerge and persist despite of the unpredictability of social interactions. The behavioral type hypothesis states that consistent individual differences in behavior in a social environment reflect individual differences in other contexts and thus, social group composition should have no influence on individual behavior. The social niche construction hypothesis on the other hand predicts that repeated social interactions and competition avoidance promote individual differences in behavior and as a consequence individual behavior should change depending on the social environment. However, these two hypotheses are non-mutually exclusive and different behavioral traits could be influenced to different degrees by changes in the social environment. In this chapter I investigated the influence of social group composition on consistent individual differences in multiple behavioral traits in the nectar-feeding bat species *Glossophaga soricina*. Four social groups, of six female bats each, were confronted with a foraging context consisting of two flower patches. The short-term and long-term consistency of individual differences in five behavioral traits were quantified: Foraging activity, spread evenness of flower visits, agonistic behavior, sampling of the unrewarding patch and the latency to switch to a newly available patch. After assigning individuals to new social groups, individual behavior was reassessed in order to quantify the influence of social group composition on the consistency of individual differences in behavior. All three repeatable behavioral traits were consistent across social groups and differences between individuals also did not increase with time spent in the same social group. However, social group composition had an effect on individual foraging performance. These results indicate that social niche construction plays only a minor role in shaping consistent individual differences in the behavior of *G. soricina*.

5.1. Introduction

Animals show consistent individual differences in a wide variety of behaviors. In recent years, great attention has been given to research showing that individuals differ in animal personality traits like activity, exploration and aggressiveness (Bell 2007, Wolf and Weissing 2012, Dall and Griffith 2014). In general, these individual differences in behavior are consistent across contexts and/or time (Bell et al. 2009). Moreover, personality traits can be correlated and thereby form behavioral syndromes (Sih et al. 2004a), for example delicate skinks (*Lampropholis delicata*) that are more active tend to be faster explorers and are also more social (Michelangeli et al. 2016). However, individuals of a given species can also consistently differ in other aspects of behavior like foraging (Alcalay et al. 2015), dispersal behavior (Hogan et al. 2014) or habitat selection (Ehlinger 1990).

Finding consistent individual differences in social environments is interesting because social interactions are thought to be highly dynamic. There are at least two non-mutually-exclusive hypotheses that can explain why consistent individual differences in behavior in a social environment emerge and persist (Laskowski and Bell 2014). The behavioral type hypothesis predicts that individuals differ in their behavior in a social environment because differences reflect individual behavior in other contexts due to common underlying mechanisms (e.g. pleiotropic genes) that reduce behavioral plasticity (Sih et al. 2004a). In accordance with the behavioral type hypothesis, changes in the social environment should not influence individual behavior. However, since individuals behave according to their behavioral type, the behavioral composition of the group could influence individual and group performance (Pruitt and Riechert 2011). For example, a study with sticklebacks showed that individual differences in behavior were not influenced by group familiarity whereas the average social foraging behavior of group members was predicted by the behavioral composition of the group (Laskowski and Bell 2014).

Nevertheless, the social environment itself might play a role in shaping consistent individual differences in behavior. The social niche specialization hypothesis states that individual differences in behavior can emerge within a social context through repeated social interactions (Bergmüller and Taborsky 2010, Montiglio et al. 2013). In order to avoid competition, individuals might develop different behavioral strategies and as a consequence settle in different social niches. These individual differences in behavior could be maintained through negative-frequency dependent pay-offs, which means that the benefit of inhabiting a certain social niche is higher the less individuals occupy it (game-theoretic dynamics). Individual differences in behavior that have been established through social niche construction should be strongly influenced by changes of the social environment. For example, nutmeg mannikins differ consistently in their tactic use in a producer-scrounger foraging game but these individual differences were not stable across social groups (Morand-Ferron et al. 2011). Furthermore, social niche specialization also implies that initial differences between individuals in non-familiar groups should increase the longer individuals remain in the same social environment. An experiment conducted with

the social spider *Stegodyphus mimosarum* provides an example. The longer individuals lived within the same social group the greater the between-individual variation and the lower the within-individual variation in boldness (Laskowski and Pruitt 2014). Additionally, it has been shown that changing the social group composition of *S. mimosarum* negatively impacted individual and group foraging performance due to the disruption of social niches (Laskowski et al. 2016).

However, social niche construction and innate behavioral differences could interact in order to shape consistent individual differences in behavior within a social environment and different behaviors might be affected to different extents by changes in the social group composition. For example, recently it has been shown that the amount of between-individual variation in agonistic behavior depends on how sociable a shrew species is whereas individual differences in activity did not change between social and less social species (von Merten et al. 2017).

In this study, I investigated the role of repeated social interactions and the influence of changes in the social environment on individual differences in multiple behavioral traits in the nectar-feeding bat species *Glossophaga soricina*. Consistent individual differences in behavior were assessed in a social foraging regime similar to the simultaneous patch regime introduced in a study that investigated the influence of repeated social interactions on individual behavioral differences in sticklebacks (Laskowski and Bell 2013). In this experimental schedule only one out of two food patches is rewarding at the beginning of a trial. After a certain time, a second food patch becomes rewarding without increasing the total amount of available food. Rewards are now distributed equally across the two patches. As a consequence, as soon as the second patch becomes active, the amount of food available in the first patch drops. Therefore, the competition increases in this patch and individuals start to distribute themselves across the two patches.

In the current experiment, each patch consisted of five artificial flowers that delivered nectar rewards with a fixed time interval schedule. In order to keep the amount of available food constant, the time interval between rewards doubled without increasing the reward volume as soon as the second flower patch became active. In this experimental regime, multiple behavioral traits were investigated in four social groups of six female bats and the consistency of individual differences was assessed short-term (across seven nights) and long-term (across three months). Thereafter, bats were reassigned to new social groups in order to investigate the influence of the social environment on individual behavior.

The following five behavioral traits were assessed in this experiment: Foraging activity, spread evenness of flower visits, agonistic behavior, sampling of the unrewarding patch and the latency to switch to a newly available patch. I expected that individuals which sample the unrewarding patch more often might also switch faster to the newly rewarding patch because they should be more likely to detect changes in the status of the second patch. Furthermore, similar to the results of the experiment with sticklebacks, individual differences in the latency to switch to the newly rewarding patch should increase the longer individuals remain in the same social group. With increasing time spent within the same social group, individuals might become familiar with the individual strategies of their groupmates

and therefore some individuals might not switch to the new patch at all and others might switch faster. Thereby individual differences would increase with time and individual latency to switch should dependent on social group composition.

Glossophagine bats have been shown to aggressively chase other bats away from artificial flowers (see Chapter 4). Although, females rarely chased other individuals in mixed sexed groups, agonistic behavior in female-only groups has been shown to be as high as in male groups. Since agonistic behavior inherently has a social component, I expected that individual differences in agonistic behavior change depending on social group composition. Additionally, more aggressive individuals might be more likely to concentrate their flower visits on a smaller subset of flowers (Milinski 1984). Therefore, the distribution of individual visits across the flowers should also depend on social group composition. On the other hand, foraging activity, which is related to energy intake, is expected to be independent of repeated social interactions and social group composition. In addition to assess how the social group composition influences individual behavior, I explored how the behavioral composition of a social group affects foraging performance.

5.2. Methods

5.2.1. Subjects and housing

Experiments were conducted from March to October 2015 with 24 females of the nectarivorous bat species *Glossophaga soricina* (Palla's long-tongued bat). All bats were caught from the same colony reared at Humboldt-University of Berlin (Germany) and were older than one year according to the ossification status of their finger joints (Brunet-Rossinni and Wilkinson 2009). In the colony housing room and in the experimental room, temperature was kept at 20-25°C and air humidity at 65-75%. While living in the colony, bats had access ad libitum to 20% honey water, 20% honey water mixed with Nektar Plus (Nekton®, Günter Enderle, Pforzheim, Germany) and 20% honey water mixed with milk powder (Alete2 Folgemilch, Nestle). Additionally, bee-collected pollen was provided. Once a week, five drops of Multi-Mulgat® (BioWeyxin, Veyx-Pharma GmbH, Schwarzenborn, Germany) were added to the honey water and once a month, some live flies were released into the housing room. For the experiment, all bats were marked with unique Radio Frequency Identification (RFID) tags attached to self-made thin beaded cable tie collars (total weight of collar and RFID tag = 0.20g, 2.4% of the body weight of the lightest bat). After the experiment, the RFID collars were removed. Additionally, bats were permanently marked with numbered plastic split rings (A C Hughes Ltd., Middlesex, UK) around the forearm, before they took part in the experiment.

5.2.2. Experimental setup

In the experimental room ten artificial flowers with automated nectar delivery (Winter and Stich 2005) were mounted along a 4.2 m bar at the height of 1.20 m. The distance between flowers was 0.4 m. Flowers were divided into two groups of five to simulate two flower patches. Each patch was surrounded with a wooden frame covered with plastic sheets to separate the two patches spatially. The entrance to the patches was a 40-cm gap between the ground and the plastic sheet in front of the flowers. The low position of the entrance forced the bats to fly up vertically to reach the flowers, which made it energetically more demanding to enter a patch. A schematic illustration of the experimental setup can be found in Chapter 4 Figure 4.1. Nectar was delivered at the artificial flowers via tubes connected to a stepper-motor syringe pump. Hovering visits by a bat were detected through the interruption of an infrared light beam at the flower opening and the RFID reader mounted underneath the flower head received the RFID signal for identification. We recorded visits to all flowers including non-rewarding visits during every experimental night from 4 pm to 4 am. The reward schedule of the flowers was configured using PhenoSoft Control (Phenosys GmbH, Berlin, Germany).

5.2.3. General procedure

Each experimental group consisted of six female bats. Before participating in the experiment all bats were weighted around 2-4 h before the start of the dark phase. During the experiment bats had access to pollen and water ad libitum and every night 6ml of additional food was provided containing 200 mg NektarPlus and 300 mg milk powder dissolved in water. Rewards at artificial flowers were 30 µl nectar consisting of 15 % w/w sugar concentration (sucrose: fructose 1:2). Before their first participation in the experiment, individuals were allowed to familiarize themselves with the setup and the artificial flowers. Since during this phase the wooden frame was not covered with the plastic sheet the two flower patches were not spatially separated and every visit at the artificial flowers was rewarded. This phase lasted until every bat visited the flowers regularly, which took 1-4 days. In total six individuals (belonging to three different experimental groups) did not visit the artificial flowers at all and were therefore replaced by six new females from the colony after the first night of habituation. Once the experimental schedule started, the wooden frame was covered with the plastic sheet and therefore the two flower patches were spatially separated.

Every experimental night was divided into two parts. During the first part of the night only one of the two flower patches was rewarding and therefore the resources were spatially clumped. The fixed time interval between rewards was 60 seconds. During the second part of the night both patches gave rewards and the resources were evenly distributed across the two patches. The fixed time interval between two rewards at a flower was 120s. Therefore, the amount of food available remained the same during the

whole night only the spatial distribution of food changed from the clumped resource condition with one patch rewarding (five flowers) during the first part of the night to the uniformly distributed resource condition with two rewarding patches (10 flowers) during the second part of the night. The maximal amount of nectar the bats could collect per night was 108 ml which corresponds to 18 ml nectar per individual and per night.

Experimental schedule

Each experimental run lasted for seven nights. The rewarding patch during the first part of the night (clumped resource condition) was chosen pseudo-randomly so that the same first rewarding patch was never repeated on more than two consecutive nights. The duration of the clumped resource condition varied between four and eight hours to avoid habituation to a specific time. The sequence of the first active patch and the duration of the clumped resource condition were determined for the first experimental run and then kept constant for all subsequent runs (details see Table 5.1).

Table 5.1: Sequence of the first active patch and the duration of the clumped resource condition during each experimental run. Every experimental run lasted for seven nights.

Night	Duration of clumped resource condition [h]	Active patch* during clumped resource condition
1	6	1
2	8	2
3	5	1
4	7	1
5	4	2
6	6	2
7	8	1

* Patch 1 includes flowers 1-5, Patch 2 includes flowers 6-10

Every individual experienced this experimental schedule three times (Fig. 5.1). During the first and the second experimental run ("Test" and "Retest") individuals remained in the same social group consisting of six females. The time between test and retest was three months. These two experimental runs were conducted to assess the long-term repeatability of individual differences in behavior. Directly after the retest individuals were assigned to new experimental groups ("New social group") to assess the influence of the social environment on the consistency of behavior. Between experimental runs, all individuals returned to the housing colony.

One female became pregnant after the retest and could therefore not participate in the last experimental run during which individuals were assigned to new social groups. For this reason, another female was assigned to two new social groups. The individual data of this female during this last experimental run

was only included once (data of the experimental group that was tested first) whereas group means were calculated using the data of this individual in both groups, so that every 1group consisted of six individuals.

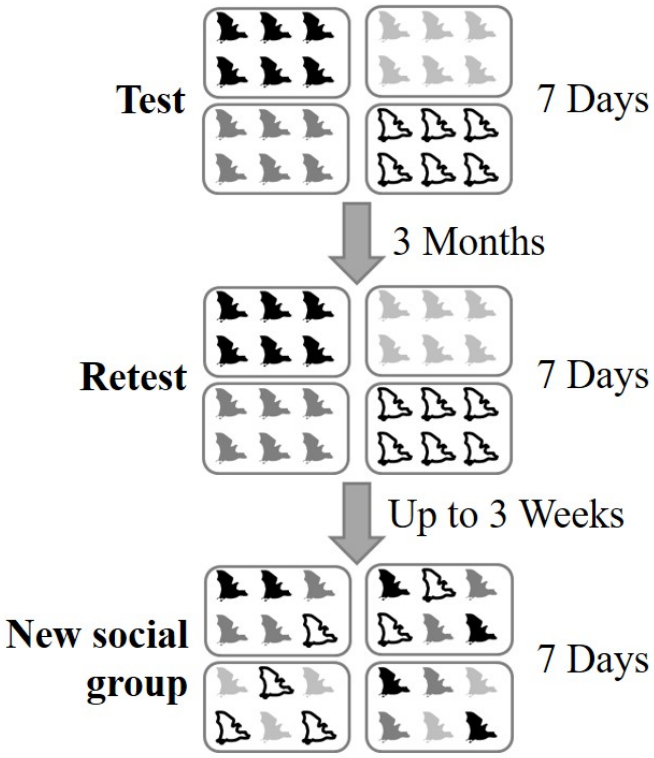

Figure 5.1: Each experimental run with four groups of six female bats lasted for seven days. During the first experimental run ("*Test*"), individual differences in multiple behavioral traits were assessed in a foraging context with two patches of five artificial flowers. The long-term consistency of individual behavior within the same social group was assessed by repeating the same experimental procedure three months later ("*Retest*"). Thereafter, individuals were reassigned pseudo-randomly into new social groups and during the last experimental run ("*New social group*") the consistency of individual differences in behavior across different social groups was assessed.

5.2.4. Behavioral traits

Agonistic behavior

Individual agonistic behavior was assessed by quantifying how often an individual was chasing other individuals away from artificial flowers. These chasing events were indicated by a special sequence of events automatically recorded by the experimental system. For more details see Chapter 4. Furthermore, as described in Chapter 4, females rarely chased other bats in the presence of males. However, the amount of chasing in a group that only consisting of females was as high as in mixed sex groups. Notably, in contrast to males, females never succeeded in successfully monopolizing a flower patch by excluding other individuals. Since individuals that make more visits to artificial flowers also have more

possibilities to chase other individuals away from artificial flowers we used the proportion of chasing events from the total number of visits as a measure for agonistic behavior.

Foraging activity

Foraging activity was quantified as the number of visits at artificial flowers. Activity specifically during the clumped or uniformly distributed resource condition was calculated as the number of visits per hour since the duration of each condition differed between nights.

Spread evenness index

The calculation of the spread evenness index was based on Simpson's Equitability (E_D) (Simpson 1949) which can be used as a measure of how well an animal distributes its visits across the available artificial flowers (Ohashi and Thomson 2009, Nachev 2014). The following equation was used to calculate the spread evenness index:

$$E_D = \frac{1}{\sum_i^S \left(\frac{n_i}{N}\right)^2} \times \frac{1}{S}$$

n_i is the number of visits a bat made at feeder i, N is the total number of visits and S is the number of available flowers. This index can take values between 0 and 1. The higher the spread evenness index the more evenly a bat distributes its visits across the available flowers.

The daily individual spread evenness index was only calculated during the uniformly distributed resource condition when both patches and therefore ten flowers were rewarding.

Latency to utilize new food patch

To assess if individuals differ in how fast they switch to the newly available patch during the uniformly distributed resource condition, the latency to utilize the new food patch was quantified as the number of seconds between the activation of the second patch and the first visit an individual made at the newly available patch.

Sampling behavior

Sampling was defined as the proportion of visits to the non-rewarding patch during the clumped resource condition. Since the position of the rewarding patch changed between nights, bats had to learn the new position of the rewarding patch at the beginning of each night. Sampling was only quantified after a bat reached the asymptotic phase of its performance curve. Details about the calculation of the individual sampling rate can be found in Chapter 2.

5.2.5. Foraging traits

Efficiency

One way to quantify individual foraging performance is efficiency. Here I measured efficiency by dividing the total amount of nectar intake by the number of visits during the uniformly distributed resource condition. With this calculation efficiency is a measure of the average amount of nectar a bat received per visit.

Nectar intake rate

Nectar intake during the uniformly distributed resource condition was calculated as milliliters of nectar obtained per hour since the duration of conditions differed between nights.

Mean spatial overlap

As previously proposed (Ohashi and Thomson 2009, Nachev 2014), spatial overlap between foraging bats was calculated using Pianka's symmetrical index of niche overlap (Pianka 1973). This index quantifies the spatial overlap of two individuals. In order to get one daily measurement for each bat, the spatial overlap between one bat and the five other bats of the respective group was calculated and then the mean of these five values was determined. The following equation was used to calculate Pianka's index between for example bat 1 and 2:

$$Pianka's\ index = \frac{\sum_i^{10} p_{i1} p_{i2}}{\sqrt{\sum_i^{10} p_{i1} \sum_i^{10} p_{i2}}}$$

p is the proportion of visits to the *i*th flower made by bat 1 and 2 respectively. The value of Pianka's index lies between 1 and 0. The higher the value of Pinaka's index calculated for two bats, the higher the spatial overlap between them.

5.2.6. Statistical Analysis

Individual differences in five behavioral traits were analyzed: agonistic behavior, foraging activity, sampling behavior, spread evenness index and latency to utilize a new a food patch. Since foraging conditions changed between the clumped (one rewarding patch) and the distributed resource condition (two active patches), individuals might have adjusted their level of foraging activity and agonistic behavior accordingly. Thus, repeatability estimates might change between these two parts of the night. However, initial analyses failed to show an effect of resource condition on individual differences in foraging activity (Table A3.1; Figure A3.3) and proportion of chasing events (Table A 3.1; Figure A

3.4). Thus, foraging activity and agonistic behavior were estimated over whole nights in all further analyses, without discriminating between the clumped and distributed resource condition.

A Bayesian Markov chain Monte Carlo generalized linear-mixed models (MCMCglmm package version 2.24, (Hadfield 2010)) was used to calculate repeatability of the five behavioral traits. In each model, the individual behavioral trait was used as a dependent variable and individual and experimental group were included as random effects. Day was initially included as a fixed effect to assess if behavioral traits were changing with time within each experimental run. However, estimates of repeatability only changed marginally with day as fixed effect and therefore I calculated repeatability estimates without any fixed effects which usually leads to a more conservative measure of repeatability. Sampling and agonistic behavior were quantified as proportions and therefore the multinomial2 family was used in these models. Latency to utilize a new food patch was assumed to have a Poisson error structure and Gaussian error structure was assumed for foraging activity. The spread evenness index takes values between 0 and 1 and can be considered as a rate, but not a proportion. Usually a beta regression can be used to analyze rates. However, beta regressions are not yet implemented in the MCMCglmm package and to my best knowledge packages that include beta regressions with random effects are not available. Thus, in order to be able to assume a Gaussian error structure, I used the z-transformation on the spread evenness index. The z-score quantifies the distance in terms of standard deviation of an individual value from the group mean and it was calculated daily and within each experimental group. The repeatability of individual z-scores would therefore provide information of how consistently individuals remained at their relative position within an experimental group.

As priors, I used an inverse-Wishart distribution for the residual variance and a parameter expanded prior for random effects. Analysis of autocorrelation, effective sample size and visual inspection of trace plots were used to assess the models.

Repeatability, the proportion of variance that can be explained by between-individual differences, was calculated following Nakagawa and Schielzeth (Nakagawa and Schielzeth 2010). Since each experimental run lasted for seven days, behavioral traits were measured seven times for each individual for each run and repeatability of behavioral traits was calculated for each experimental run ("*Test*", "*Retest after three months*", "*New social group*"). Additionally, to assess the potential change of between-individual differences, repeatability estimates were also calculated for the first three days and last three days of each experimental run. Spearman rank correlation coefficients were calculated to explore possible between-individual correlations of behavioral traits which would indicate a behavioral syndrome.

To investigate the long-term consistency of individual differences in behavioral traits and the consistency of individual behavior across social groups the individual mean behavior was calculated for each experimental run. Linear (foraging activity and agonistic behavior) and beta regressions (spread evenness index) were used to assess the correlations of individual mean behavior.

To explore the influence of consistent individual differences on individual and group foraging performance I calculated the mean nectar intake rate, Pianka's index and foraging efficiency during the uniformly distributed resource condition. However, the mean spatial overlap of foraging individuals was highly collinear with the measure of individual spread evenness index (Spearman's $\rho = 0.95$, $p < 0.001$) and therefore was dropped from further analysis. Linear models were used to explore potential correlations.

All analysis was performed in R version 3.3.2 (Team R Core 2016). All linear mixed-effects models were performed using the R package "nlme" version 3.1-128 (Pinheiro et al. 2007), all linear models were performed using the R package "lme4" version 1.1-12 (Bates et al. 2014) and beta regressions were performed using the R package "betareg" version 3.1-0 (Cribari-Neto and Zeileis 2009).

5.3. RESULTS

5.3.1. Effects of resource distribution

Bats increased their flower visitation rate significantly from the clumped to the distributed resource condition during all three experimental runs (Test: $t = 8.07$ $p < 0.001$, Retest: $t = 12.10$ $p < 0.001$, New social group: $t = 8.69$ $p < 0.001$) whereas the amount of agonistic behavior decreased significantly during the distributed resource condition (Test: $t = -14.24$ $p < 0.001$, Retest: $t = -13.92$ $p < 0.001$, New social group: $t = -11.53$ $p < 0.001$). However, individual behavior correlated significantly between the two conditions and repeatability estimates for both, foraging activity and agonistic behavior, did not differ between the two different resource distributions (Appendix: Table A3.1; Fig. A3.3, Fig. A 3.4). Therefore, foraging activity and agonistic behavior estimated over whole nights were included in all further analysis, without discriminating between the clumped and distributed resource condition.

5.3.2. Repeatability of behavioral traits

During the seven days of each experimental run, multiple behavioral traits were quantified daily in every individual in order to assess the consistent individual differences in these traits. Three behavioral traits were highly repeatable which indicates that individuals behave consistently different from each other: Foraging activity, agonistic behavior and spread evenness index (Figure 5.1, for actual values see Appendix Table A3.2). Mean spread evenness index, a measure of how evenly bats distribute their visits across flowers during the uniformly distributed resource condition, ranged from 0.51 (SD ± 0.32) to 0.94 (SD ± 0.03). Individual average foraging activity ranged from 597 (SD ±378) to 5694 (SD ± 1158) visits per night. Individuals also differed to a great extent in their aggressive behavior. Some individuals never chased other bats away from artificial flowers whereas for other individuals up to 94 chasing

events per night were recorded. However, the repeatability of individual differences in sampling of the unrewarding patch during the clumped resource condition was very low (Test: R = 0.07, 95% CI [0.01, 0.15], Retest: R = 0.08, 95% CI [0, 0.26], New social group: R = 0, 95% CI [0, 0.11]) and individual differences in the latency to switch to the newly rewarding patch did only differ significantly from zero during the experimental run with new social group composition (Test: R = 0, 95% CI [0, 0.16], Retest: R = 0, 95% CI [0, 0.21], New social group: R = 0.23, 95% CI [008, 0.43]). Thus, only the three behavioral traits with high repeatability estimates were included in the further analysis: foraging activity, agonistic behavior and spread evenness index.

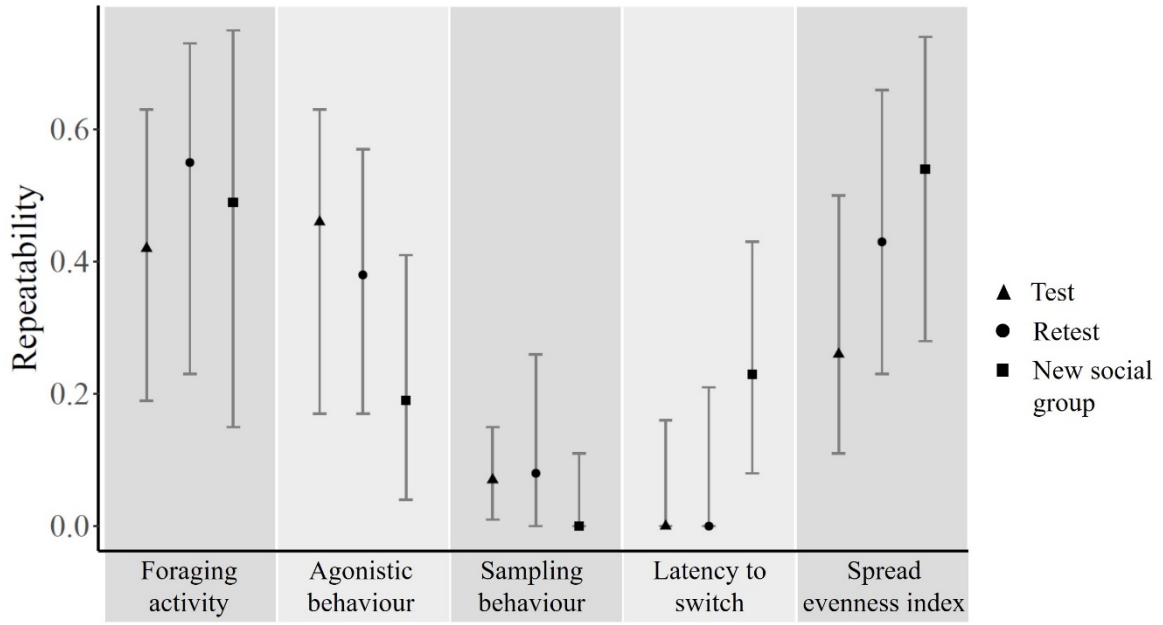

Figure 5.2: Repeatability estimates for each behavioral trait during all three experimental runs. Error bars represent the 95% confidence interval. Three behavioral traits were highly repeatable: Foraging activity, agonistic behavior and spread evenness index.

The long-term consistency of these behavioral traits was assessed by correlating the individual mean behavior during the test and the retest after three months. Individual mean values of all three behavioral traits were highly consistent across three months (Foraging activity: $t = 4.96$ $p < 0.001$, spread evenness index: $t = 5.29$ $p < 0.001$ and agonistic behavior: $t = 4.65$ $p < 0.001$).

Furthermore, Spearman's rank correlation coefficients were calculated to explore possible correlations of different behavioral traits which would indicate a behavioral syndrome (Table 5.2). Only individual mean agonistic behavior and mean spread evenness index correlated significantly across individuals in

all experimental runs (Spearman's ρ = -0.46 p = 0.03 (Test), ρ = -0.55 p = 0.006 (Retest), ρ = -0.64 p = 0.001 (New social group)).

Table 5.2: Spearman's rank correlation coefficients were calculated between individual mean behaviors for each experimental run. The spearman rank correlation coefficients for each experimental run are shown in the following order in each cell from top to bottom: Test, Retest after three months and experimental run with new social groups. Uncorrected significance values are reported in parentheses.

	Foraging activity	**Agonistic behaviour**	**Spread evenness index (Simpson's Equitability)**
Foraging activity	1		
Agonistic behaviour	0.30 (p = 0.16) -0.03 (p = 0.88) 0.36 (p = 0.09)	1	
Spread evenness index (Simpson's Equitability)	0.17 (p = 0.42) 0.43 (p = 0.04) 0.20 (p = 0.35)	-0.46 (p = 0.03) -0.55 (p = 0.006) -0.64 (p = 0.001)	1

5.3.3. Influence of social environment on individual differences in multiple behavioral traits

To investigate how social group composition influences the consistency of individual behavior, bats were reassigned to new experimental groups after the second experimental run. If the social environment influences individual behavior, it is expected that the individual mean behavior in the original groups does not predict the behavior in the new social groups (Figure 5.2). Individual mean foraging activity during both, the test and the retest after three months, correlated significantly with the individual mean foraging activity in new social groups (Test ~ New social group: t = 4.81 p < 0.001, Retest ~ New social group: t = 3.28 p = 0.004). In line with individual foraging activity, the mean spread evenness index during the test and retest also correlated significantly with the individual mean spread evenness index in new social groups (Test ~ New social group: t = 2.54 p = 0.01, Retest ~ New social group: t = 3.27 p = 0.001). However, the correlation between the retest and the experimental run with new social groups was only significant after excluding one outlier (Figure 5.2D, t = 0.92 p = 0.36 with outlier).

Contrary to foraging activity and spread evenness index, individual mean agonistic behavior during the test and the retest did not correlate with the mean agonistic behavior in new social groups (Test ~ New social group: t = 1.11 p = 0.28, Retest ~ New social group: t = 0.29 p = 0.78). However, the mean of individual daily z-scores was significantly correlated across social groups (Test ~ New social group: t = 2.28 p = 0.03, Retest ~ New social group: t = 3.61 p = 0.002). The z-score measures the distance of the individual value from the group mean agonistic behavior and therefore a strong positive correlation implies that the relative position of group members is stable across social groups.

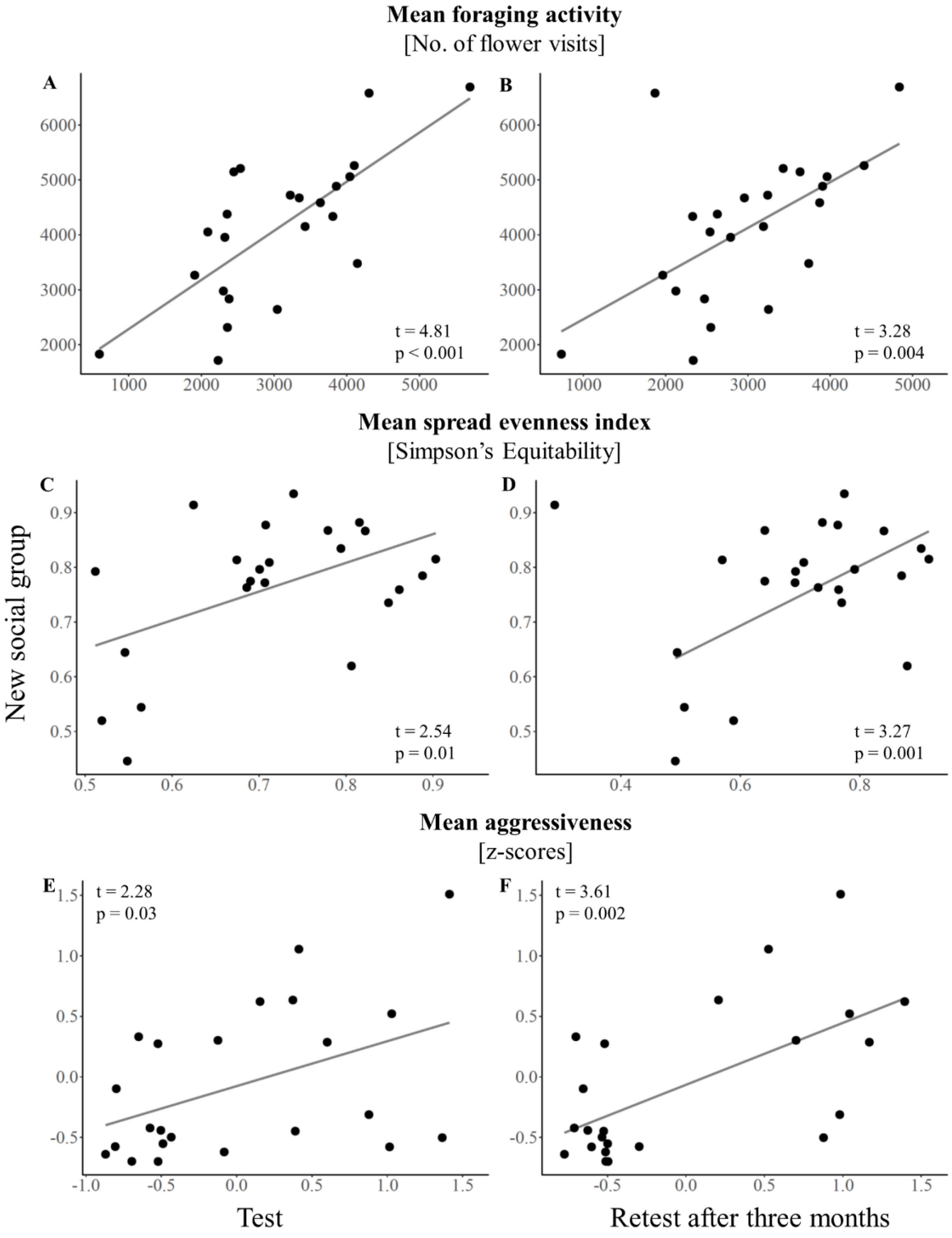

Figure 5.3: Correlations of individual mean behavior during the test (A, C, E) and retest (B, D, F), respectively, with individual mean behavior during the experimental run with new social groups. The consistency of individual differences in three behavioral traits across social groups was assessed: mean foraging activity (A, B), mean spread evenness index (C, D) and mean agonistic behavior (E, F). Since absolute values of individual mean agonistic behavior were not correlated significantly across social groups, individual mean z-scores were calculated as a rank measure of an individual within the experimental group. (* = outlier, further explanation, see text)

Another prediction of how social group composition could influence individual behavior is that differences between individuals might increase the longer bats remain in the same social group. By dividing the seven days of each experimental run into two section of three days each, I calculated the repeatability of individual behavior for the beginning and the end of each run. However, the repeatability of individual behavior was already significant during the first three days of each experimental run and repeatability estimates did not increase the longer individuals remained in the same social group (Table 5.3).

Table 5.3: The seven days of each experimental run were divided into two sections: First three days and last three days. Repeatability estimates were calculated for each section and each experimental run in order to assess if differences between individuals changed within an experimental run. Values represent repeatability estimates with 95% confidence intervals in parenthesis.

	Test		**Retest**		**New social group**	
	First three days	Last three days	First three days	Last three days	First three days	Last three days
Foraging activity	0.38 [0.09, 0.67]	0.36 [0.11, 0.74]	0.51 [0.24, 0.80]	0.36 [0.11, 0.68]	0.48 [0.12, 0.74]	0.34 [0.07, 0.67]
Spread evenness index	0.41 [0, 0.55]	0.41 [0.03, 0.57]	0.36 [0.14, 0.67]	0.43 [0.12, 0.65]	0.52 [0.18, 0.74]	0.56 [0.17, 0.73]
Agonistic behavior	0.32 [0.11, 0.60]	0.29 [0.10, 0.63]	0.38 [0.09, 0.61]	0.47 [0.20, 0.72]	0 [0, 0.24]	0.15 [0.03, 0.39]

5.3.4. Influence of individual differences in behavior on foraging performance

The previous results show that the three behavioral traits were consistent across social groups which means that individuals did not adapt their behavior to the new social group composition. This observation leads then to the question if the average behavior of group members influences foraging performance. In this section I explore will explore this relationship.

The following two parameters were used to describe the individual foraging performance of bats in this experiment: nectar intake rate and efficiency. Nectar intake rate was measured as the amount of nectar an individual received per hour and efficiency was quantified as the average amount of nectar received per flower visit. Foraging performance was only assessed during the uniformly distributed resource condition when both patches were rewarding.

The mean foraging efficiency of individuals was significantly negatively correlated with the spread evenness of group members (Figure 5.4, Spearman's ρ = -0.73, p = 0.009). Thus, individuals in groups with low mean spread evenness index and high mean agonistic behavior (individual spread evenness and agonistic behavior are negatively correlated) were more efficient.

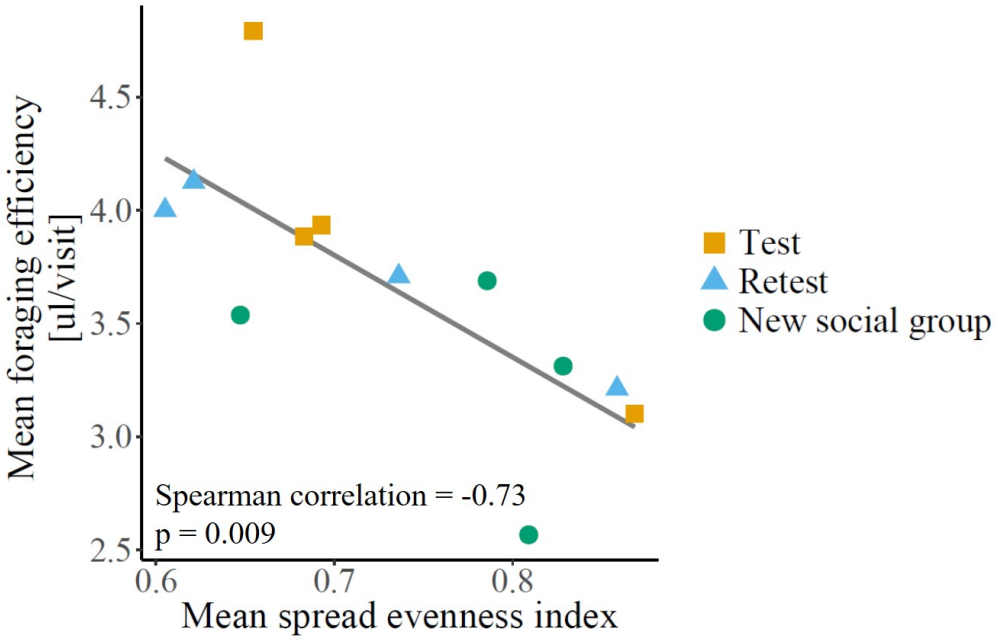

Figure 5.4: Correlation of group mean spread evenness index and mean group foraging efficiency during all three experimental runs.

In this experiment rewards at artificial flowers were delivered with a fixed time interval schedule. Therefore, for individual bats, the probability of getting a reward at an artificial flower should decrease the higher the average foraging activity of the social group. As a consequence, individual nectar intake should change depending on the behavioral composition of the group and therefore the individual nectar intake should not be consistent across social groups.

The correlation between the nectar intake during the test and the retest shows that the individual nectar intake is, as expected, stable across social groups (Fig 5.5 A) (t = 2.87, p = 0.009) whereas the individual nectar intake during the test did not correlate with the individual nectar intake in the new social group (Fig. 5.5 B) (t = 1.00, p = 0.33). However, the nectar intake during the retest tends to predict the individual nectar intake during the experimental run with new social group composition, although the correlation was not significant (Fig. 5.5 C) (t = 1.99, p = 0.06).

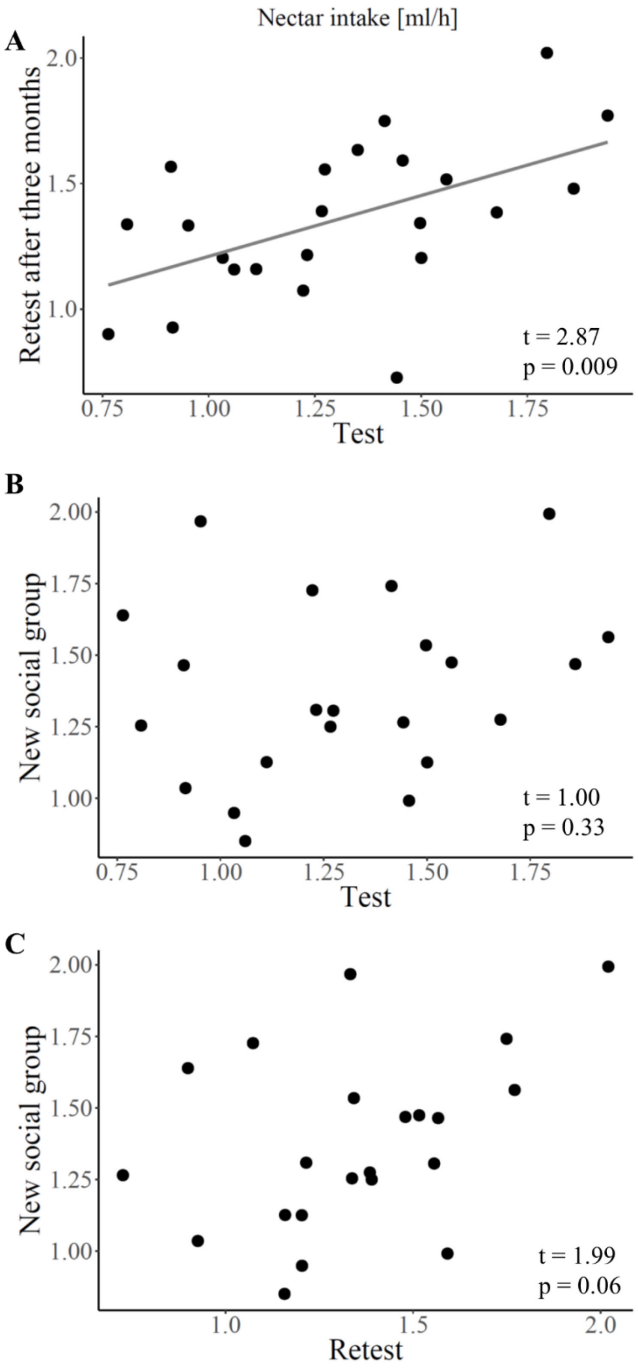

Figure 5.5: The individual mean nectar intake rate [ml/h] during the first test correlated significantly with the individual mean nectar intake rate during the retest after three months ($t = 2.87$, $p = 0.009$) (A). However, the individual mean nectar intake rate during the test (B) ($t = 1.00$, $p = 0.33$) and the retest (C) ($t = 1.99$, $p = 0.06$) were only weak predictors for individual nectar intake during the experimental run with new social groups.

5.4. Discussion

Both the behavioral type and the social niche construction hypothesis propose mechanisms that can explain how individual behavioral differences emerge and persist in a highly dynamic social environment (Sih et al. 2004b, Bergmuller and Taborsky 2010). The social niche construction hypothesis predicts that repeated social interactions and competition avoidance can promote individual differences in behavior. On the other hand, the behavioral type hypothesis states that individual differences in behavior in a highly dynamic social environment reflect individual behavior in other contexts and therefore individual differences in behavior should not be affected by changes in the social environment. These two hypotheses are non-mutually exclusive and therefore they could influence different behavioral traits to different degrees. In this chapter, I assessed the consistency of individual behavior in a social foraging context across different social groups in order to investigate the role of the social environment in shaping individual behavioral differences in the nectar-feeding bat species *Glossophaga soricina*. Three behavioral traits, agonistic behavior, foraging activity and spread evenness of flower visits, were highly repeatable short-term within experimental runs and long-term over three months. In accordance with the behavioral type hypothesis, individual behavior was consistent across social group. Therefore, I explored how the behavioral composition of the group could influence the performance during foraging. I could show that the average spread evenness index (which is negatively correlated with agonistic behavior across individuals) influenced the average foraging efficiency of individuals and also individual nectar intake was influenced to some extent by changes in the social group composition.

Influence of social environment on foraging activity

As predicted, individual foraging activity was independent of social group composition. This result is in line with a previous finding showing that the level of individual differences in activity did not differ between social and non-social shrew species (von Merten et al. 2017). Foraging activity measured as the number of flower visits is related to individual nectar consumption and food intake determines how much energy an individual can spend. In Chapter 3, I could show that individuals of the closely related species *G. commissarisi* differ consistently in their daily energy expenditure and individual differences in energy metabolism have been proposed to correlate with different life-history strategies (Careau et al. 2008, Réale et al. 2010). Therefore, in line with the behavioral type hypothesis, individual differences in foraging activity and consequently energy intake might reflect individual differences in other contexts independent of the social environment.

Influence of social environment on agonistic behavior

Contrary to foraging activity, absolute values of individual agonistic behavior did not correlate across social groups. However, individuals that showed high agonistic behavior relative to the group average were also more likely to show higher than average agonistic behavior in new social groups as shown by the correlation of z-scores (Figure 5.2 E and F). Individual agonistic behavior was quantified as the proportion of chasing events at artificial flowers on the total number of visits. However, the number of chasing possibilities does not only depend on the individual aggressive tendency but also on the behavior of other group members. For example, other individuals might have avoided proximity to aggressive individuals and therefore diminished their amount of chasing opportunities independent of their individual aggressive tendency. Since the relative amount of agonistic behavior was consistent across social groups, social niche construction seems to play also only a minor role in shaping individual differences in aggressiveness.

Influence of social environment on spread evenness index

Agonistic behavior and spread evenness index of flower visits were correlated across individuals (Table 5.2) In accordance with the result that the social environment had only a minor effect on individual agonistic behavior, individual spread evenness index was also correlated across social groups. How individuals distribute their visits across flowers could have been a consequence of differences in aggressiveness associated with competitive ability. However, individuals differed in their spread evenness index already during the first three days of the first experimental run (Table 5.3) indicating that aggressive individuals might have a tendency to visit less flowers even before they could demonstrate their competitive ability. This is in line with results from a previous study that has shown that *G. commissarisi* differ consistently in the number of flowers they visit in an flower array independent of aggressive interactions (Nachev 2014).

The role of repeated social interactions

Although individual behavior was consistent across social groups in all three repeatable behavioral traits (agonistic behavior, foraging activity and spread evenness index), repeated social interactions could have still played a role in shaping differences between individuals by reducing within-individual variation as has been shown in the social spider *Stegodyphus mimosarum* (Laskowski and Pruitt 2014). However, repeatability estimates did not change with time (Table 5.3) in any of the three behavioral traits, supporting the hypothesis that differences in behavior of bats in a social foraging context were the result of individual differences in other contexts rather than the result of repeated social interactions. However, since the confidence intervals of these repeatability estimates were very wide, the failure of

showing an increase could also be due to low statistical power. Nevertheless, repeatability estimates during the first three days are already significantly greater than zero showing that individuals already differed consistently at the beginning of each experimental run.

Latency to switch to a newly availably flower patch

Contrary to results of a study with sticklebacks that used the same experimental design, individual bats did not differ in their latency to switch to a newly rewarding patch. The experiment with sticklebacks showed that individual fish differed consistently in their latency to switch to a newly available patch and that these differences increased the longer individuals remained in the same social group (Laskowski and Bell 2013). However, a subsequent experiment showed that switch latency was predicted by individual differences in other behaviors measured in different contexts like the tendency to shoal with other individuals. This showed that not only repeated social interactions played a role in shaping individual differences in switch delay (Laskowski and Bell 2014). In the present experiment with nectarivorous bats, the main reason for the lack of individual differences in switch delay might have been the close proximity of the two patches and consequently the very low costs of switching to the newly available patch. Additionally, every time an artificial flower delivered a reward, the valve controlling the nectar flow was audible which could have served as a signal indicating the availability of the new patch as soon as one bat started to exploit it. In this case leaving the first flower patch might have not been due to competition avoidance but due to an audible signal and the benefit of being the first to switch was therefore very low.

Sampling of unrewarding patch

Although, individuals of the closely related species *G. commissarisi* have been shown to differ in how much they sample unrewarding flowers, in the present experiment the repeatability of sampling behavior was very low and did not even differ from zero during the last experimental trial with new social groups (Fig. 5.2). Contrary to the previous experiment with *G. commissarisi* (Chapter 2), in this experiment the same five spatially concentrated artificial flowers were rewarding for all bats instead of two single rewarding flowers per individual distributed among ten unrewarding flowers. This indicates that individual differences in sampling might be only present in more challenging situations. In line with this proposition, individual differences in learning in great tits have been shown to be also only present in difficult tasks (Titulaer et al. 2012).

Influence of behavioral group composition on foraging performance

In accordance with the behavioral type hypothesis, individual differences in all three repeatable behavioral traits were consistent across social groups. Since individuals did not change their behavior in response to changes in social environment, individual foraging performance might be influenced by the behavior of its group members (Bleakley et al. 2007). An exploratory analysis showed that average

group spread evenness index indeed influenced the mean spatial overlap of group members and the lower the mean spatial overlap the higher the mean individual foraging efficiency was. Since the individual spread evenness index correlated significantly with agonistic behavior, individual efficiency was higher in groups consisting of individuals with higher mean agonistic behavior.

Individuals did not adapt their foraging activity to changes in social group composition, individual nectar intake could be influenced by changes in the social environment. The analysis showed that individual mean nectar intake rate during the uniformly distributed resource condition, was significantly correlated between the test and the retest after three months ($t = 2.87$, $p = 0.009$, Figure 4.5A). This indicates that the individual nectar intake rate was stable within the same social group. However, individual mean nectar intake during both the first test and the retest after three months were only poor predictors of the individual mean nectar intake rate during the experimental run with new social groups (Figure 5.5 B and C) which indicates that the foraging performance measured as nectar intake might indeed be influenced by social group composition.

In the present experiment, I confronted *G. soricina* with a social foraging design in which individual foraging decisions influenced the experience of other members of the group. In their natural environment however, *Glossophaga soricina* been observed to forage mainly independently instead of in social flocks (Heithaus et al. 1974). The lack of evidence for social niche construction in the behavioral traits measured in these bats is therefore in line with the hypothesis that the importance of the social niche construction on shaping consistent individual differences in behavior should depend on the sociability of the species (von Merten et al. 2017). However, recently it has been shown that *G. soricina* are capable of using social information to learn new profitable flower positions (Rose et al. 2016) indicating that there is a social component in their foraging behavior. Additionally, even though individual differences in behaviors might be innate they can still be influenced by group composition and habitat, like it has been shown for individual differences in boldness of perches (Magnhagen and Staffan 2005).

Further studies that investigate the role of social niche construction in shaping between-individual variation of behavioral traits in various species can contribute to the understanding of the ecological conditions that favor consistent individual differences due to social niche construction.

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CHAPTER 6
Conclusions and future directions

The results from a large number of animal personality studies in a range of different species indicate that consistent individual differences in behavior are ubiquitous throughout the animal kingdom (Sih et al. 2004, Reale et al. 2007, Bell et al. 2009). However, several ecological and evolutionary questions have not yet been fully answered. Why do consistent individual differences in behavior evolve, how are these consistent individual differences maintained within populations of a given species and what are the consequences for ecology and evolution? In order to further investigate these questions, various conceptual frameworks have been developed in the field of animal personality research and these theoretical and verbal models have generated predictions that can be tested empirically. Therefore, besides quantifying consistent individual differences in a new species, it is also important to design and conduct experiments that test these predictions in various species in order to assess the general validity of these theoretical frameworks (Dall and Griffith 2014).

Throughout this study, I investigated consistent individual differences in the foraging behavior of glossophagine bats and I could show that individuals differed consistently in several behavioral traits, namely foraging activity, exploration and aggressiveness. Furthermore, individuals also differed in their behavioral plasticity of exploration and in their flexibility to leave a previously rewarding flower. In addition to the quantification of individual behavioral differences, the experiments of this study were also designed to investigate theoretical assumptions and predictions stated in the field of animal personality research.

For example, behavioral plasticity is often assumed to be a single trait in which individuals differ because some animals might be generally more responsive to environmental stimuli than others. The results of the experiments presented in the second chapter show that in flower bats, contrary to the expectation, two different types of behavioral plasticity namely flexibility and contextual plasticity, are independent traits (Chapter 2). Additionally, in accordance with the life-history framework I was able to provide further evidence that not only resting metabolic rates but also the daily energy expenditure can differ consistently between individuals and that these differences correlate with differences in behavioral traits, in this case with exploration (Chapter 3). In addition to ecological factors, social factors are also thought to play an important role in shaping consistent individual differences in behavior by facilitating social niche construction. However, the results show that in *G. soricina* social group composition affected individual behavior only marginally (Chapter 5). This result illustrates that the importance of social interactions in shaping consistent individual differences in behavior differs and

likely depends on the ecological context of a given species. The fourth chapter was concerned with the investigation of behavioral differences depending on social status. I could show that in *G. soricina*, only males defended resources successfully and contrary to subordinate males, females were apparently unaffected by these aggressive interactions. These results show, for the first time, sex-dependent differences in the resource defense behavior in nectar-feeding bats (Chapter 4).

The results in chapter two show that different types of behavioral plasticity can be independent traits which contradicts the prediction that some individuals are generally more responsive to environmental stimuli than others (Coppens et al. 2010). Studies that investigate more than one type of plasticity within the same individuals are surprisingly rare (Stamps 2015) despite of the importance to better understand the underlying mechanisms that lead to individual differences in plasticity and how or if different types of plasticity are correlated. The lack of these studies might be also due to the lack of clear classifications and definitions of different types of behavioral plasticity (Stamps 2015). However, the problem of incoherent terminology is not only related to behavioral plasticity but it is a general issue within the field of animal personality research (David and Dall 2016). As a consequence, it is difficult to compare results across studies and to draw general conclusions from empirical data collected in various species. Therefore, one future goal in the field of animal personality research should be to achieve a consensus of terminology and definitions.

The life-history framework proposes an exciting wholistic approach that links individual differences in behavior with individual differences in various other physiological and life-history traits. Individual differences in metabolic rates seem to be overall an important underlying mechanism in generating consistent individual differences in behavior (Holtmann et al. 2016). In glossophagine bats the link between behavior and energy metabolism is especially interesting because of their high energetic demands. Further studies could focus on investigating the relationship between different metabolic rates within individuals. For example, it could be tested if individual differences in the daily energy expenditure correlate with differences in resting metabolic rates. Another interesting question is whether these individual differences in metabolic rates are heritable in bats and if individual metabolic rates and exploratory behavior are genetically linked as it has been shown in mice (Careau et al. 2011).

In nectar-feeding bats, aggressive resource defense has rarely been studied. The results of Chapter 4 indicate that flower defense behavior might not only be performed in order to receive more resources but it might also have other social functions due to the different consequences for males and females. The experiment presented in this thesis was performed in the controlled environment of a laboratory setting and future studies have to be conducted in order to confirm these findings in the natural environment. Furthermore, it could be assessed if males that defend resources successfully against other

males possibly receive additional benefits like an increasing chance for mating which could lead to a larger number of offspring.

In this study, the description of consistent individual differences in behavior in a new species was combined with the investigation of some theoretical predictions and assumptions stated in the field of animal personality research. However, the experiments presented in this thesis are only a first step towards understanding the underlying mechanisms that shape consistent individual differences in the behavior of these nectar-feeding bats. Furthermore, testing the predictions derived from conceptual and verbal models in different species will help to better understand which of the proposed mechanisms are important in different ecological conditions and how animal personality structure varies as a function of ecology.

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APPENDIX

A 1. Chapter 2: Supplementary material

A 1.1. Individual differences in acquisition rates

Every experimental night the bats had to learn the position of the rewarding flowers. In order to test if there are consistent individual differences in how fast the bats reach the performance criterion, the repeatability of the number of visits until the start of the asymptotic phase have been quantified daily and for every individual. Repeatability for each reward probability was calculated using MCMCglmms with Poison error distribution and individual as random effect. No fixed effects were included.

Results:

No significant consistent individual differences in acquisition rates could be detected during the experimental part of measuring plasticity in adapting the sampling rates.

Repeatability:

30% reward probability: R = 0.001 CI 0, 0.24
50% reward probability: R = 0.001 CI 0, 0.29
83% reward probability: R = 0.001 CI 0, 0.23

A 1.2. Change of individual sampling rates throughout the night (Chapter 2)

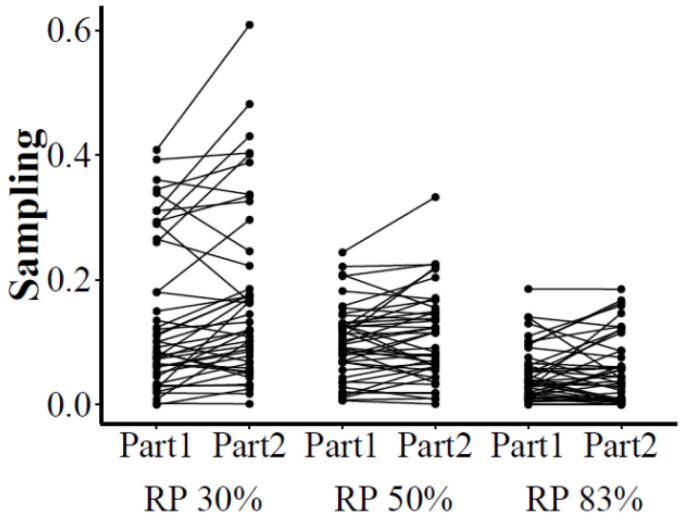

Figure A 1.2: To explore the possibility that the sampling rate of an individual changes during the night, the mean sampling rate during the first and the second half of the asymptotic phase of the performance curve was calculated.

Table A 1.2: The results of the generalized linear mixed model (GLMM) with reward probability and part of the asymptotic phase as interacting fixed effects showed that there was no significant change in sampling rate during the night between the first and the second part of the asymptotic phase of the performance curve.

Fixed effects	**β (95% CI)**
Intercept	-2.46 (-2.77, -2.11)
Reward probability 50% (Part1)	-0.22 (-0.54, 0.56)
Reward probability 83% (Part1)	**-1.41 (-1.75, -1.06)**
Reward probability 30% (Part2)	0.22 (-0.08, 0.51)
Reward probability 50% (Part2)	-0.28 (-0.71, 0.14)
Reward probability 83% (Part2)	-0.36 (-0.85, 0.06)
Variances	**σ (95% CI)**
Between-individual	**0.81 (0.46, 1.25)**
Within-individual	1.71 (1.53, 1.93)

A 2. Chapter 4: Supplementary material

A 2.1. Scratch marks on wings of Glossophaga soricina

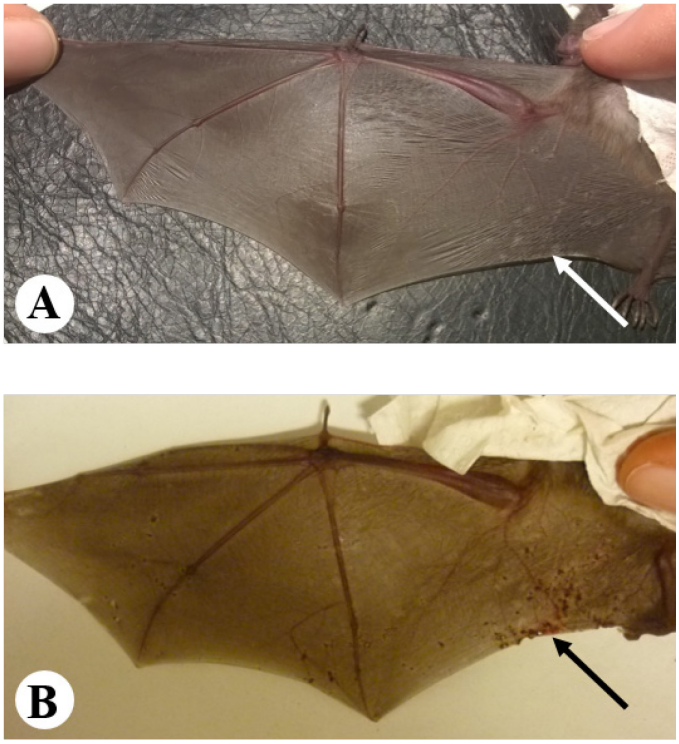

Figure A 2.1: Example of marks of small wing injuries before (A) and after the experiment (B) of a subordinate male that was part of a mixed group.

A 2.2. Proportion of visits at flowers depending on social status

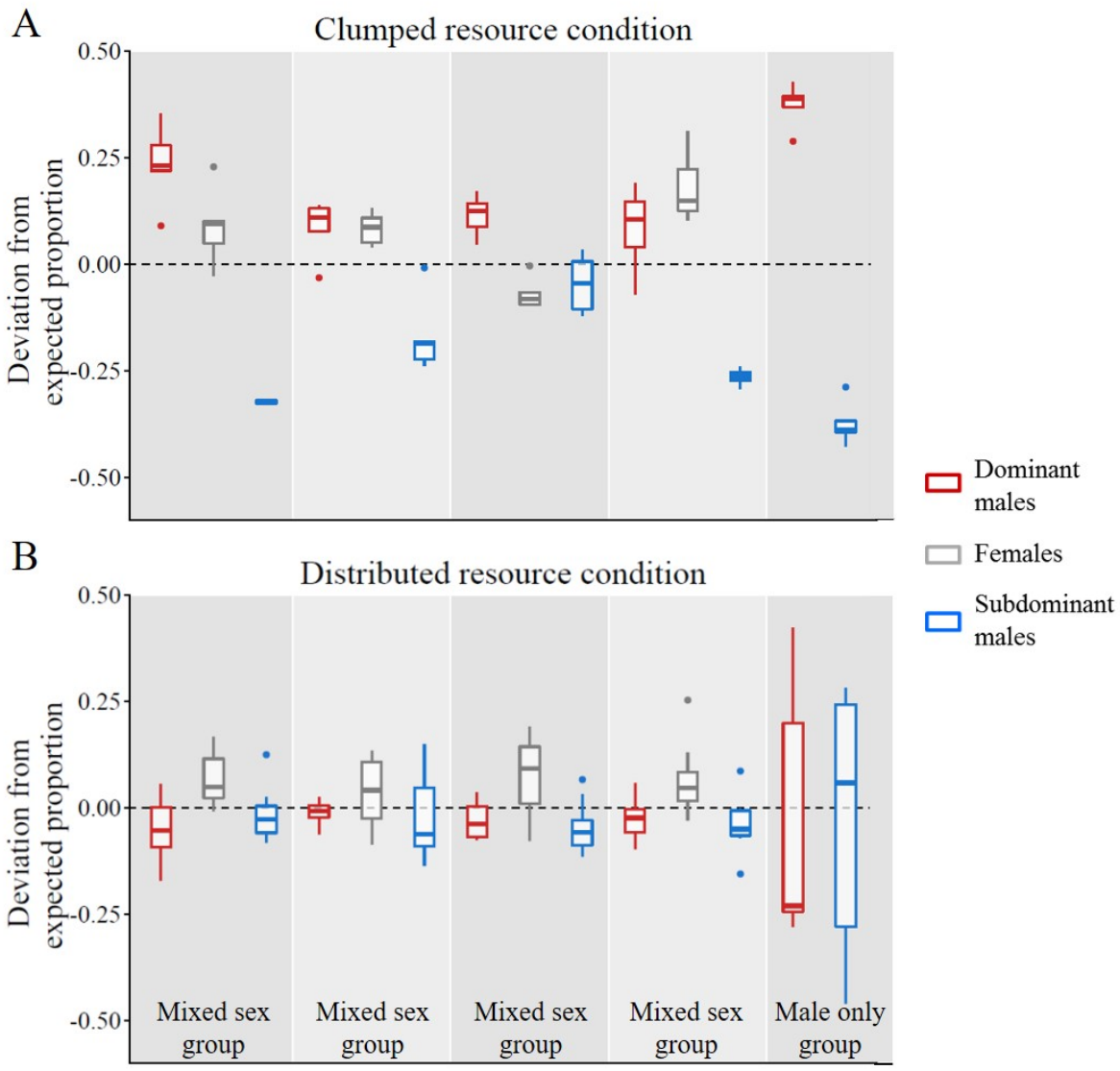

Figure A 2.2: Every mixed sex group included three females, one dominant and two subordinate males, whereas the male-only group consisted of two dominant and four subordinate males. If these social categories would have any influence on the access to resources, the proportion of visits at each flower should reflect the propotion of individuals in each category. For example in mixed sex groups 50% of visits at each flower should be made by females, 16.67% by the dominant male and 33.33% by subdominant females. Every bar in the figure above represents the mean difference in the proportion of visits made by individuals of the respective social category across all flowers. During the clumped resource condition only the five flowers of the active patch were taken into accound and only the data from the last two days of the experiment were included. (A) During the clumped resource condition the difference to the expected proportion of visits depended stronly on the social category. Dominant males had higher and subordinate males had lower propotions of visits at flowers than expected. The proportion of visits at flowers of females depended on the experimental group. (B) However, during the distributed resource condition the proportion of visits at flowers reflected the proportion of individuals in each category.

A 2.3. Distribution of visits across flowers in the male-only group

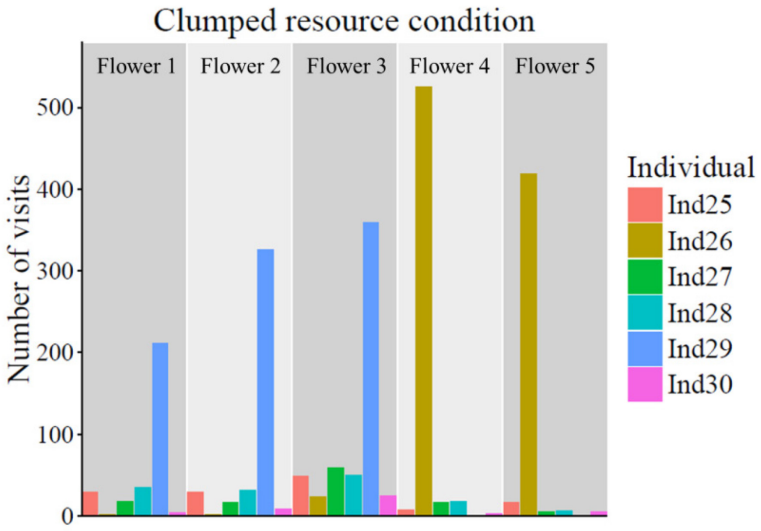

Figure A 2.3: Two males in the male-only group successfully defended flowers against competitors. However, rather than sharing all flowers within the defended patch, these two males distributed the flowers between them. Number of visits of each individual at the five flowers of the rewarding patch during the clumped resource condition during the last day of the experiment (day 8) are shown.

A 2.4. Pattern in automatically collected data indicating chasing

Table A 2.4: The two examples below illustrate the sequence of recorded events that identify a chasing event and one example that illustrates two drinking bats in quick succession.

	RFID identification	**Individual label**	**Module**	**Duration of RFID label detection [ms]**	**Duration of light barrier interruption [ms]**
Example for chasing event					
Mixed Group 4 day 2					
25.11.2015 17:45:55,666	04185D008F	Ind21	CondMod7	503	209
25.11.2015 17:45:55,967	04185D12B9	Ind22	Reader7	0	
25.11.2015 17:45:55,754			LS7		231
25.11.2015 17:47:23,915	04185CE762	Ind20	CondMod8	1611	767
25.11.2015 17:47:23,994			LS8		540
25.11.2015 17:47:24,766	04185D12B9	Ind22	Reader8	0	
Example of two drinking bats following each other closely					
Mixed Group 4 day 2					
25.11.2015 16:27:18,334	04185CE762	Ind20	CondMod7	703	610
25.11.2015 16:27:19,861	04185D12B9	Ind20	CondMod7	757	470

CondMod = Indicates a complete visit at artificial flowers with identification of RFID label and interruption of infrared light beam within the flower head. Such a visit can be rewarded or not depending on the fixed interval reward schedule of flowers.
Reader = Detection of RFID label without interruption of infrared light beam.
LS = interruption of infrared light beam without detection of RFID label.

A 3. Chapter 5: Supplementary material

A 3.1. Repeatability estimates of foraging activity and agonistic behavior depending on the experimental regime

Table A 3.1: Repeatability estimates for foraging activity and aggressiveness during the clumped and the uniformly distributed resource condition respectively. Values are presented with 95% confidence interval in parenthesis.

	One patch rewarding	**Two patches rewarding**
Foraging activity	0.36 [0.10, 0.53] (Test) 0.33 [0.04, 0.53] (Retest) 0.52 [0.13, 0.73] (New social group)	0.20 [0.02, 0.48] (Test) 0.39 [0.11, 0.62] (Retest) 0.20 [0.05, 0.56] (New social group)
Agonistic behaviour	0.39 [0.15, 0.60] (Test) 0.36 [0.12, 0.54] (Retest) 0.21 [0.04, 0.41] (New social group)	0.54 [0.06, 0.70] (Test) 0.31 [0.16, 0.62] (Retest) 0.10 [0.001, 0.26] (New social group)

A 3.2. Repeatability estimates of behavioral traits

Table A3.2: Bayesian Markov chain Monte Carlo generalized linear mixed models were used to quantify different variance components of behavioral traits within the seven days of each experimental run. All models included individual and experimental groups as random effects and no fixed effects. Repeatability is the proportion of variance of behavioral traits that is explained by differences between individuals and it is a measure for the consistency of individual differences. Values below represent repeatability estimates and their 95% confidence intervals in brackets.

Behavioral trait	**Test**	**Retest (after 3 months)**	**New social group**
Foraging activity	0.42 [0.19, 0.63]	0.55 [0.23, 0.73]	0.49 [0.15, 0.75]
Agonistic behavior	0.46 [0.17, 0.63]	0.38 [0.17, 0.57]	0.19 [0.04, 0.41]
Sampling of unrewarding patch	0.07 [0.01, 0.15]	0.08 [0, 0.26]	0 [0, 0.11]
Latency to switch to newly rewarding patch	0 [0, 0.16]	0 [0, 0.21]	0.23 [0.08, 0.43]
Spread evenness index (Simpson's Equitability)	0.26 [0.11, 0.50]	0.43 0.23, 0.66]	0.54 [0.28, 0.74]

A.3.3. Correlation of individual activity between the clumped and distributed resource condition

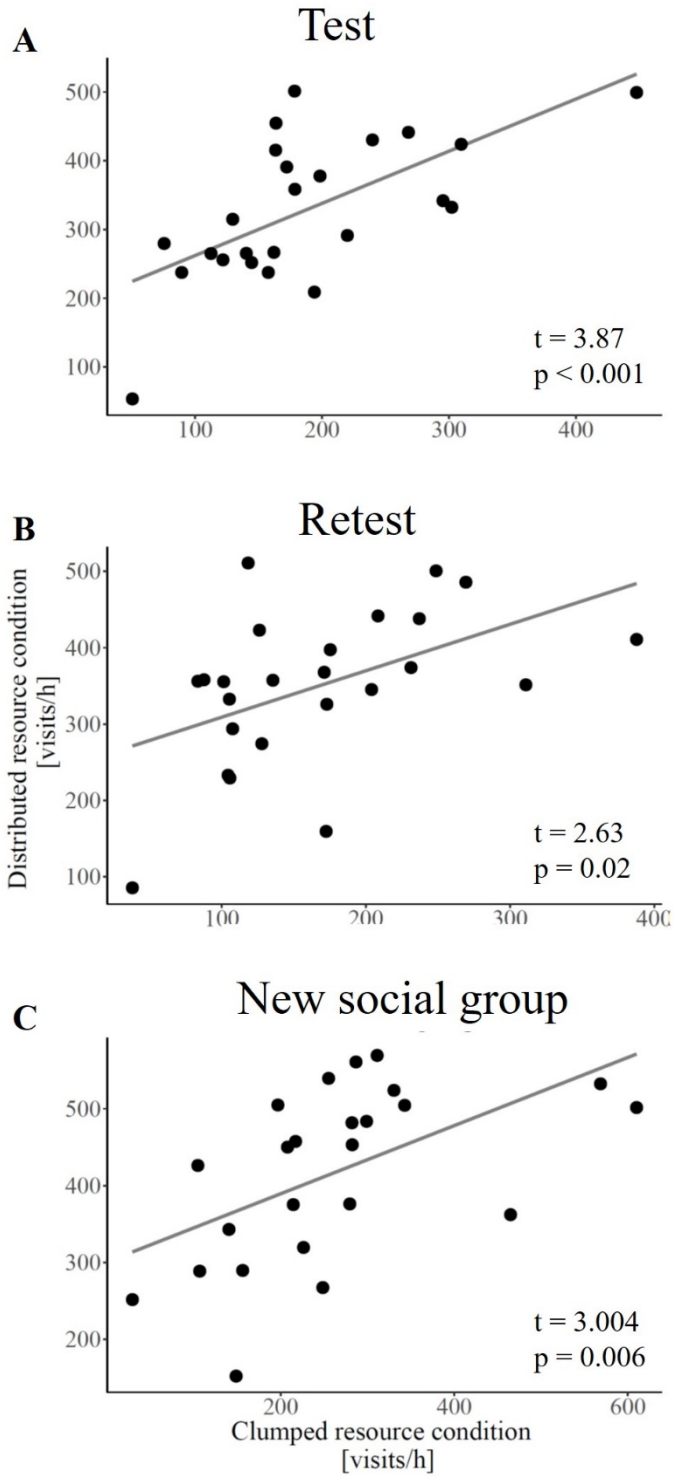

Figure A 3.3: Correlation of individual mean flower visitation rate (foraging activity) during the distributed and the clumped resource distribution.

A.3.4. Correlation of individual aggressiveness between the clumped and distributed resource condition

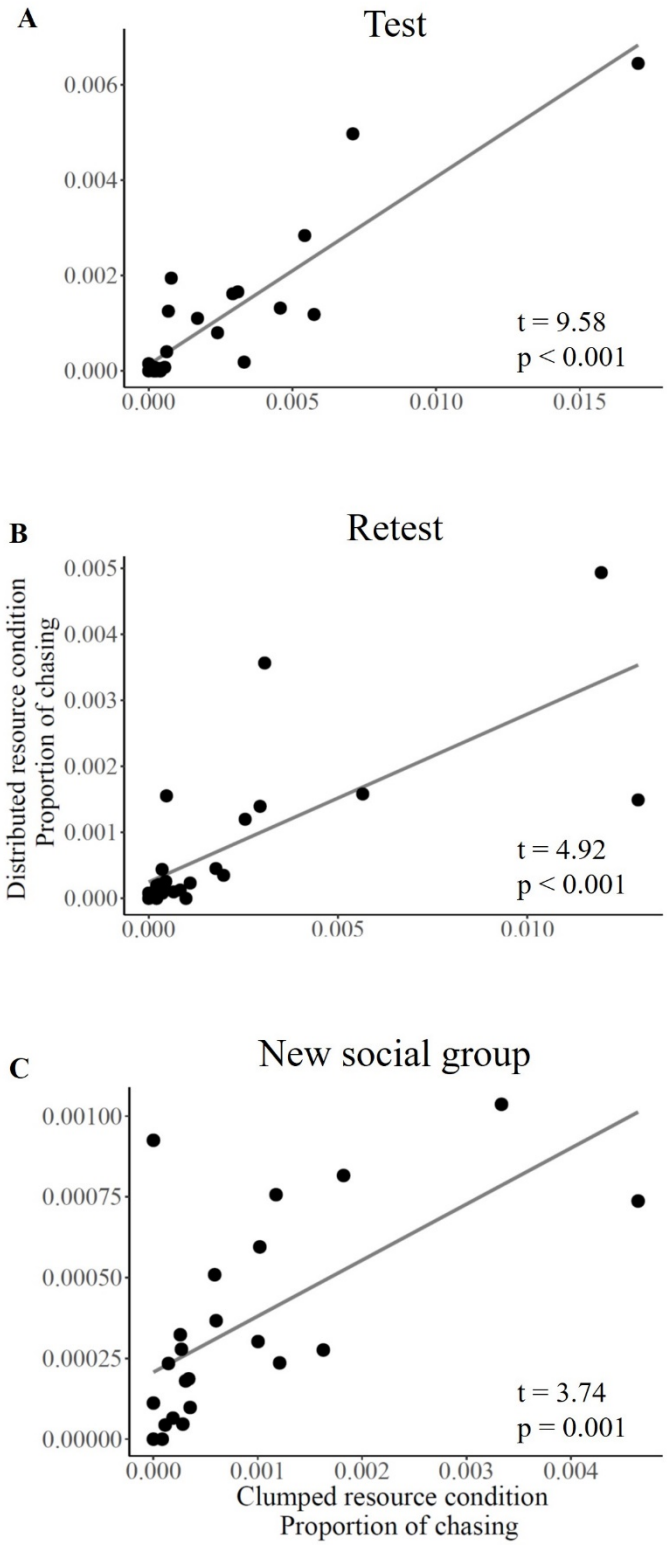

Figure A 3.4: Correlation of individual mean proportion of following (agonistic behaviour) during the distributed and the clumped resource distribution.

List of contributions

Chapter 2: This study was designed with the help of York Winter and Vladislav Nachev. Experimental software was programmed by Alexej Schatz.

Chapter 3: This study was designed with the help of York Winter and Vladislav Nachev. Experimental software was programmed by Alexej Schatz.

Chapter 4: This study was designed with the help of York Winter and Vladislav Nachev. Experimental software was programmed by Alexej Schatz.

Chapter 5: This study was designed with the help of York Winter and Vladislav Nachev. Experimental software was programmed by Alexej Schatz.

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